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OPEN SESSION

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OBSERVATIONAL STUDIES AND SAMPLING

AN INTRODUCTION TO VETERINARY OBSERVATIONAL STUDIES

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Observational studies investigate disease aetiology using observations of naturally occurring disease in groups of animals, rather than by experiment. The techniques were developed in human medicine in the 1950's, a famous example being Doll and Hill's (1964a,b) study of the association between smoking and lung cancer. Expansion of these studies was an indicator of a shift in emphasis in European and North American human epidemiology from the public health approach - tracing cases of, and controlling, infectious diseases - to the investigation of the causes of non-infectious diseases. This shift partly resulted from the development of vaccines and antimicrobial agents, during the first half of this century, that were responsible for a reduction in infectious diseases in the western world. Over the past 20 years, observational studies also have been applied to diseases of livestock and companion animals. The techniques, however, are unfamiliar to many veterinarians. This paper is designed as an introduction to these studies. A basic statistical knowledge is essential to a proper understanding of the topic. An appendix of appropriate statistical concepts and techniques therefore is included for the benefit of readers who are unfamiliar with them.

SOME RELEVANT CONCEPTS

Observational studies involve the making of inferences about the cause of disease, based on the frequency of occurrence of disease in groups of animals. Therefore, a knowledge of methods of expressing disease frequency and of contemporary concepts of cause are prerequisite to an understanding of these studies.

Measures of the frequency of occurrence of disease

Prevalence

Prevalence is the number of cases of disease in a population, without distinction between old and new cases. It is usually expressed as point prevalence, that is, the number of cases of disease in a population at a particular point in time. Prevalence thus gives no indication of the time at which disease develops during an animal's life.

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Incidence

Incidence is an expression of the number of new cases that occur in a population. It is a dynamic measure of the amount of disease that occurs in a population (c.f. prevalence, which is a static measure). The two essential components of an incidence value are:

1. the number of new cases,
2. the period of time over which the new cases occur.

For instance, an epidemic of feline viral rhinotracheitis may occur in a cattery, and 20 animals may develop the disease during a week. The incidence is therefore '20 cases per week'.

The relationship between prevalence and incidence

Prevalence (P) depends on the duration (D) and incidence (I) of a disease. A disease with a long duration and high incidence will result in many cases in the population at a single point in time, contributing to a high prevalence at that time. Conversely, a disease of short duration and high incidence will result in fewer cases at a particular point in time. Expressed symbolically:

$$P \propto I \times D.$$

This means that a change in prevalence can be due to:

1. a change in incidence,
2. a change in the average duration of the disease,
3. a change in both incidence and duration.

Application of prevalence and incidence values

Epidemiological investigations of causes ideally require a knowledge of incidence. Causes operate before a disease is identified. Therefore, an incidence value, recorded as near as possible to the time of onset of a disease, can associate the disease with the action of the causes.

Prevalence values are less useful than incidence values in investigating causal associations because the former values are associated not only with incidence (and therefore time of onset) but also with duration; the prevalence of a disease therefore may be recorded a long time after the causal factors have operated. The main use of prevalence measurements is in indicating the extent of disease problems for administrative purposes and for defining research priorities and long-term disease control strategies.

Prevalence rate

It is usual to relate the number of diseased animals to the total population "at risk" of developing the disease, to gain some idea of the magnitude of the disease problem. The ratio of the number of diseased animals to the population at risk is the prevalence rate. (To be correct, this is not a true rate which, in conventional scientific usage, is defined as a change in one quantity with respect to another [e.g. velocity: change in distance with respect to time]. However, 'prevalence rate' is now well-established in epidemiology). This can be expressed as a fraction, but is usually expressed as a percentage:

$$\text{Prevalence rate} = \frac{\text{Number of cases of disease}}{\text{Population at risk}} \times 100.$$

For example, if 60 cats from a population of 200 were found to be infected with Microsporum canis, then the prevalence rate of infection would be 30%.

A prevalence rate describes the probability of a disease being present in a typical member of the population. A prevalence rate of 33% (i.e. out of 100 animals, 33 have a disease) represents a probability (p) of any one animal having the disease of 0.33. A prevalence rate of 100% represents a probability of 1 (i.e. certainty that every animal has the disease).

Incidence rate

An incidence rate expresses the number of new cases of disease, per defined unit of time, as a proportion of the population at risk:

$$\text{Incidence rate per unit time} = \frac{\text{Number of new cases}}{\text{Population at risk}} \Big/ \left(\text{Number of units of time of observation period.}\right)$$

For example, if, in a cattery containing 100 cats, 3 cats develop infectious enteritis in a week, and then 5 further cats develop the disease during the following week, then the incidence rate of infectious enteritis is 4/100 per week = 4% per week.

Rates are expressed in three main forms: crude, specific and adjusted.

Crude rates

Crude rates are an expression of the total amount of a disease in a population. The numerator in the rate formula is the number of diseased animals; the denominator is the total number of the population at risk. Although crude rates may express the prevalence or incidence of a particular disease, they take no account of specific host characteristics such as age, sex, breed or method of husbandry, which can have a major effect on the occurrence of disease.

Specific rates

Specific rates of disease are those that describe the rates of disease in specific categories of the population relating to certain host attributes such as age, sex, breed and method of husbandry. Specific rates are calculated in a similar manner to crude rates, except that the numerator and denominator apply to one or more categories of a population with specific host attributes. For instance, a whole series of different age-specific prevalence or incidence rates could be calculated that would cover the entire life-span of a population.

Crude rates are the weighted summation of specific rates of various categories in a group of animals. If the proportions of various categories differ between groups, then crude rates can be misleading when comparing groups. Table 1 summarises information on testicular Leydig cell tumours in Channel Island and other breeds of dairy cattle, in two groups of bulls. Although the crude prevalence rates, 98 per 1000 and 88 per 1000, are similar for Channel Island and other breeds, the rates specific to the category of bull are much lower for Channel Island breeds in group B (76/1000) than in group A (198/1000). Conversely, the rates specific to other breeds are higher

in group B than in group A. These differences are masked by the crude rates because of the different proportions of Channel Island and other breeds in each population.

Table 1. Rates of Leydig cell tumours in two groups of bulls
[Data for Group A extracted from Sponenberg (1979).
Data for Group B are hypothetical.]

Group	Crude rate per 1000	Rate specific to breed of bull		Proportion of population	
		Channel Island	Other breeds	Channel Island	Other breeds
A	98	198	44	0.35	0.65
B	88	76	107	0.60	0.40

Group A: Crude rate per 1000 = (198 × 0.35) + (44 × 0.65)
= 69.3 + 28.6
= 97.9.

Group B: Crude rate per 1000 = (76 × 0.60) + (107 × 0.40)
= 45.6 + 42.8
= 88.4.

In most circumstances, specific rates are the most useful type of rate to calculate when attempting to identify causes.

Adjusted (standardised) rates

If, in the example above, morbidity due to Leydig cell tumours were being compared between the two groups of bulls, irrespective of breed of bull, then the crude rates would be misleading owing to the difference in breed weighting, whereas the rates specific to breed give accurate information only about individual categories of the bull population. These difficulties can be overcome partially by using the technique known as adjustment (standardisation) of rate, by direct or indirect methods.

In direct adjustment of rate, a standard population is chosen and the rates of specific categories in the population under investigation are multiplied or weighted by the proportion of the similar specific category in the standard population. When these adjusted specific rates are summed, the crude rate in the population under study then becomes adjusted to the standard population. A comparison can then be made between the population under study and other populations, similarly adjusted to the standard population, without the comparison being distorted by different proportions of various categories of the population.

$$\text{Direct adjusted rate} = sr_1 \times \frac{S_1}{N} + sr_2 \times \frac{S_2}{N}$$

where sr = specific rate of study population,
S = number of specific category in the standard population,
N = total number in the standard population, ($S_1 + S_2 = N$).

For example, direct adjustment may be applied to the specific rates relating to the two classes of bull (Channel Island and other breeds) in Table 1, using information on bulls from breed societies as a standard in relation to the overall proportion of the Channel Island and other breeds in the total bull population. If there were 67000 bulls in the standard population, of which 5600 were Channel Island, then:

for group A:

sr_1 (Channel Island) = 198,
S = 5600,
N = 67000.

sr_2 (other breeds) = 44,
S = 61400 (i.e. 67000-5600),
N = 67000.

$$\text{Adjusted rate for group A} = 198 \times \frac{5600}{67000} + 44 \times \frac{61400}{67000}$$
$$= 16.6 + 40.3,$$
$$= 56.9.$$

Similarly, for group B:

sr_1 = 76,
S = 5600,
N = 67000,

sr_2 = 107,
S = 61400,
N = 67000.

$$\text{Adjusted rate for group B} = 76 \times \frac{5600}{67000} + 107 \times \frac{61400}{67000}$$
$$= 6.3 + 98.1$$
$$= 104.4.$$

If breed of bull were the factor responsible for the differences then the two adjusted rates would have been similar. However, if factors other than breed were responsible for the difference in rates between the two groups of bulls, then the adjusted rates would be different, as in this example, where age might account for the difference. The rates could be simultaneously adjusted for age by first calculating the specific rates for each age group and then again adjusting in relation to the overall standard population.

Direct adjustment of rate requires knowledge, for each category, of the numbers of animals and the disease rate in the population for which the adjusted rate is needed (but not knowledge of the population proportions). If either this information is not available or the numbers in each category are so small that large fluctuations in prevalence and incidence rates occur through presence or absence of a few cases, then the indirect method of adjustment can be used. Fleiss (1981) describes both methods of adjustment.

Causal concepts

'Necessary' and 'sufficient'causes

The relationship of causes to their effects allows classification of causes into two types: 'sufficient' and 'necessary' (Rothman, 1976).

A cause is sufficient if it inevitably produces an effect (assuming that nothing happens to interrupt the development of the effect, such as death or prophylaxis). A sufficient cause virtually always comprises a range of component causes; disease is therefore multifactorial. Frequently, however, one component is described, in general parlance, as the cause. For example, distemper virus is referred to as the 'cause' of distemper, although the sufficient cause actually involves exposure to the virus, lack of immunity and, possibly, other components. If component causes produce their effect by increasing susceptibility to disease then they sometimes are called 'predisposing factors'. Last (1983) lists other types of component cause. Component causes also are termed determinants. It is not mandatory to identify all components of a sufficient cause to prevent disease, because removal of one component may render the cause insufficient. For example, an improvement in floor design can prevent foot abscesses in pigs even though the main pyogenic bacteria are not identified.

A particular disease may be produced by different sufficient causes. The different sufficient causes may have certain component causes in common. If a cause is a component of every sufficient cause then it is necessary. Therefore, a necessary cause always must be present to produce an effect. For example, distemper virus is a necessary cause of distemper.

Some multifactorial diseases, such as pneumonia, can have many sufficient causes, although none may be necessary. Part of the reason is taxonomic: pneumonia is a loosely connected group of diseases whose classification is based on lesions (inflammation of the lungs), rather than particular component causes; the lesions can be produced by different sufficient causes. When a disease is classified according to aetiology, there is, by definition, usually only one major cause, which is therefore likely to be necessary. Examples include lead poisoning and many 'simple' infectious diseases, such as tuberculosis and brucellosis.

The object of epidemiological investigations of cause is the identification and quantification of the effects of sufficient causes and their component causes. Removal of one or more components from a sufficient cause will then prevent disease produced by that sufficient cause.

Koch's postulates

The increased understanding of microbial diseases in the late nineteenth century led Robert Koch to formulate his postulates to determine the cause of infectious disease. These postulates state that an organism is causal if:

1. it is present in all cases of the disease;
2. it does not occur in another disease as a fortuitous and non-pathogenic parasite;
3. it is isolated in pure culture from an animal, is repeatedly passaged, and induces the same disease in other animals.

These postulates emphasise the importance of microbial determinants, but attenuate the importance of other determinants relating to the host and environment. Similarly, the postulates are unsuited to non-infectious diseases.

Evans' postulates

Evans (1976) has produced a set of postulates that accommodate non-infectious diseases and the 'multifactorial' diseases that have many component causes, relating to agent, host and environment, in their sufficient causes. These postulates are:

1. the prevalence of the disease should be significantly higher in those exposed to the supposed cause than in those who are not;

2. exposure to the supposed cause should be present more commonly in those with than those without the disease, when all other risk factors are held constant;

3. the incidence of the disease should be significantly higher in those exposed to the supposed cause than in those not so exposed;

4. temporally, the disease should follow exposure to the supposed causal agent with a distribution of incubation periods on a bell-shaped curve;*

5. a spectrum of host responses, from mild to severe, should follow exposure to the supposed cause along a logical biological gradient;

6. a measurable host response (e.g. antibody, cancer cells) should appear regularly following exposure to the supposed cause in those lacking this before exposure, or should increase in magnitude if present before exposure; this pattern should not occur in individuals not so exposed;

7. experimental reproduction of the disease should occur with greater frequency in animals or man appropriately exposed to the supposed cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the laboratory, or demonstrated in a controlled regulation of natural exposure;

8. elimination (e.g. removal of a specific infectious agent) or modification (e.g. alteration of a deficient diet) of the supposed cause should decrease the incidence of the disease;

9. prevention or modification of the host's response (e.g. by immunization, or use of specific lymphocyte transfer factor in cancer) should decrease or eliminate the disease that normally occurs on exposure to the supposed cause;

*The bell shape is usually obtained only when the horizontal "time" axis is mathematically transformed (Sartwell, 1950; 1966); if a linear time scale is used, then the curve is usually positively skewed, that is, there are few long incubation periods, relative to the number of short incubation periods.

10. all relationships and associations should be biologically and epidemiologically credible.

An important characteristic of Evans' postulates is that they require the association between an hypothesised causal factor and the disease in question to be statistically significant.

Demonstration of a statistically significant association does not prove that a factor is causal. The logical reduction of proof requires that the mechanism of induction of a disease by a cause needs to be explained by describing the chain of events, from cause to effect, at the molecular level. This requires experimental investigation. The goal of observational studies is the identification of factors, the removal or reduction of which decreases the incidence of disease. Such factors are described as causal in the sense that their presence increases the probability of disease developing.

Sensitivity and specificity

An animal may be incorrectly diagnosed as having a disease when, in fact, it does not; this is a 'false positive' result. Conversely, an animal may be recorded as not being diseased whereas, in reality, it is; this is a 'false negative' result. The likelihood of false positives and negatives depends on the nature of the disease that is being studied and the diagnostic criteria that are used. For example, a mid-shaft femoral fracture is unlikely to be misdiagnosed (i.e. there will be few false positives and negatives) using physical examination as the diagnostic criterion. However, diabetes mellitus is more likely to be misdiagnosed than the fracture, using physical examination; there will be more false positives and negatives. The diagnostic error in the second example can be reduced by using the fasting blood glucose level as the diagnostic criterion.

The validity of a method of diagnosis can be quantified by comparing results obtained by the diagnostic method with those obtained from an independent accurate criterion (Table 2). The sensitivity of a diagnostic method is the proportion of true positives that are detected by the method. The specificity of the method is the proportion of true negatives that are detected.

Table 2. Sensitivity and specificity of diagnostic methods

		Actual status	
		+	-
Status according to diagnostic method	+	w	x
	-	y	z

Sensitivity = $w/(w + y)$,

Specificity = $z/(x + z)$.

Causal models

Often, disease occurrence does not depend simply on the presence or absence of a factor; there may be continuous variation in the frequency of occurrence of disease associated with both the strength of a factor and the number of factors involved. There is often a 'background' frequency of occurrence associated with none of the factors under consideration. When two or more factors are associated with disease, the frequency of disease may be proportional to the occurrence of disease resulting from the separate frequencies attributable to each factor (i.e. the frequency when each factor is present singly minus the 'background' frequency). Alternatively, the frequency may be either in excess of or less than that expected from the combined effects of each factor, in which case statistical interaction occurs.

When several component causes are present simultaneously their joint effect can be explained quantitatively in terms of two causal models: additive and non-additive (Kupper and Hogan, 1978). The additive model interprets disease occurrence, when two or more factors are present, as the sum of the amount of disease attributable to each factor. If no interaction exists, then, for example:

when cause X is present alone, disease occurrence = 2,
when cause Y is present alone, disease occurrence = 5,
when X and Y are both present, disease occurrence = 7.

If positive interaction occurs, then the level of disease occurrence, when X and Y are present, will be greater than 7.

The commonest non-additive model is the multiplicative. This interprets disease occurrence, when two or more factors are present, as the product of the amount of disease attributable to each factor. If no interaction exists, then, for example:

when cause X is present alone, disease occurrence = 2,
when cause Y is present alone, disease occurrence = 5,
when X and Y are both present, disease occurrence = 10.

If positive interaction occurs, then the level of disease occurrence, when X and Y are present, will be greater than 10.

Disease occurrence can be measured in terms of incidence, risk of developing disease, or other measures. The type of model depends on the means of expressing disease occurrence; for example, a multiplicative model may become additive if log transformation of the measure of occurrence is conducted.

In epidemiology the additive model is the commoner of the two models. When there is evidence of positive interaction based on the additive model the model has sometimes been described as being synergistic. However there are arguable differences between interaction and synergism (Blot and Day, 1979). Evidence of a positive interaction does not imply a causal relationship. If it can be inferred that the factors are part of an aggregate of causes with a common causal pathway (MacMahon, 1972) then synergism is said to have occurred. Thus synergism may be thought of as a positive interaction where a causal pathway may be inferred.

The range of epidemiological studies

'Study' is a general term that refers to any type of investigation. However, in epidemiology, a study usually involves comparison of groups of animals, for example, a comparison of the milk yields of cows under different systems of husbandry. This contrasts with a survey which involves only description (e.g. recording prevalence by monitoring, surveillance or a specific survey). Epidemiological studies may be either observational or experimental.

In an experimental study the investigator has the ability to allocate animals to various groups, according to factors which the investigator can randomly assign to animals (e.g. treatment regimen, preventive technique). Examples are clinical trials and intervention studies. In a clinical trial, the investigator assigns animals either to a group that is treated with one or more drugs, or to an untreated control group. It is then possible to evaluate the efficacy of treatment. In an intervention study, the investigator 'intervenes' in the potential or actual development of a disease, by altering possible causes (e.g. changing diet).

An observational study is similar to an experimental study: animals are allocated to groups with respect to certain characteristics that they possess (trait, disease, usual diet etc.). However, in observational studies, it is not possible to randomly assign animals to groups, because the investigator has little control over the factors that are being studied.

Observational studies comprise the majority of epidemiological investigations. Observational and experimental science have their own strengths and weaknesses that are discussed in detail by Trotter (1930). A major advantage of an observational investigation is that it studies the natural occurrence of disease. Experimentation may separate factors associated with disease from other factors which may have important interactions with them in natural outbreaks.

OBSERVATIONAL STUDIES

The three types of observational study

There are three types of observational study: cross-sectional, case-control and cohort. Each classifies animals into those with and without disease and those with and without hypothesised causal factors. Therefore, they each generate a 2 × 2 contingency table for each disease/factor relationship (Table 3). However, the methods of generation differ between the studies.

Table 3. The 2 x 2 contingency table constructed in observational studies to determine the cause of disease.

	Diseased animals	Non-diseased animals	Totals
Hypothesised causal factor present	a	b	a + b
Hypothesised causal factor absent	c	d	c + d
Totals	a + c	b + d	a + b + c + d = N

In cross-sectional studies only N can be predetermined.
In case-control studies (a + c) and (b + d) are predetermined.
In cohort studies (a + b) and (c + d) are predetermined.

The cross-sectional study involves the selection of a total of N individuals from a larger population, and then the determination, for each individual, of the simultaneous presence or absence of attribute A and presence or absence of attribute B. In the context of a causal investigation, attribute A would represent a disease, and attribute B the hypothesised causal factor (inherent attribute, diet, etc.). At the beginning of a cross-sectional study, only the total number of animals (N in Table 3) is pre-determined. The numbers of animals with and without disease A, and possessing or not possessing attribute B, are not known initially.

In a case-control study, cases and controls are selected at the beginning of the study and traced back to determine whether they were exposed to an hypothesised cause. Therefore (a + c) and (b + d) are predetermined. In a cohort study a group (cohort) of animals exposed to an hypothesised causal factor, and a group not exposed to the factor, are selected, and observed, to record development of disease in each group. Therefore, (a + b) and (c + d) are predetermined. Cross-sectional and case-control studies categorize the number of existing cases and therefore are based on the measurement of prevalence; resolved cases and cases lost due to death can be missed. Cohort studies are based on the measurement of incidence; all cases can be detected.

These three types of study attempt to identify causes of disease by applying the first three of Evans' postulates:

1. the prevalence of the disease should be significantly higher in individuals exposed to the supposed cause than in those who are not (evidence supplied by a cross-sectional study),

2. exposure to the supposed cause should be present more commonly in those with than those without the disease, when all other risk factors are held constant (evidence supplied by a case-control study),

3. the incidence of the disease should be significantly higher in those exposed to the supposed cause than in those not so exposed (evidence supplied by a cohort study).

Nomenclature of observational studies

A variety of alternative names have been given to case-control and cohort studies. Both of these studies consider two events - exposure to an hypothesised causal factor or factors and development of disease - that are separated by a period of time. Because of this temporal separation of the two events, each of these studies is sometimes termed longitudinal.

The case-control study compares diseased animals (cases) with non-diseased animals (controls) and has therefore variously been called a case-comparison, case-referent and case history study. This study selects groups according to presence or absence of disease and looks back to possible causes; it has therefore sometimes been described as a retrospective study (looking back from effect to cause).

A cohort study selects groups according to presence or absence of exposure to hypothesised causal factors, and then looks forward to the development of disease. It has therefore sometimes been called a prospective study (looking forward, from cause to effect).

The groups may be selected as 'exposed' and 'unexposed' now, and then observed over a period of time, to identify cases; such a cohort study is 'concurrent'. Alternatively, if reliable records relating to exposure are available then groups may be selected according to presence or absence of previous exposure, and traced to the present, to determine disease status; this is a non-concurrent study.

Some investigators use 'retrospective' to refer to any study that records data from the past, and 'prospective' to refer to any study designed to collect future data. Therefore, a non-concurrent cohort (prospective, in the causal sense) study alternatively may be termed a retrospective (in the temporal sense) cohort study. Similarly a concurrent cohort study also can be called a prospective (in the temporal sense) cohort study.

Measures of association used in observational studies

The reader who is unfamiliar with basic statistics should read the Appendix before proceeding further.

The χ^2 test of association

The χ^2 test can be used to determine the significance of an association between a disease and an hypothesised causal factor. The value of χ^2 cannot be used as a measure of the degree of association. This is because χ^2 is a function of the proportions in the various cells and of the total sample size, whereas the degree of association is only really a function of the cell proportions; the sample size has a role to play in detecting significance but not in determining the extent of association. Two measures are commonly used to estimate the degree of association: 'relative risk' and 'odds ratio'.

Relative risk (R)

Consider the data in Table 4 relating to a disease (the feline urolithiasis syndrome: FUS) and an hypothesised cause (dry cat food), observed as the number of new cases over a defined period of time. Note that, when constructing the contingency table, disease is expressed horizontally, and the hypothesised causal factor is expressed vertically.

Table 4. Number of cases of the feline urological syndrome in relation to the feeding of dry cat food

DIET	FELINE UROLITHIASIS Present	Absent	Totals
Partial feeding of dry cat food	44 (a)	31 (b)	75 (a+b)
No feeding of dry cat food	9 (c)	55 (d)	64 (c+d)
Totals	53	86	139 (N)

[From Willeberg (1975b)]

The incidence rate among cats 'exposed' to dry cat food (p_1) is given by:

$$p_1 = a/(a+b).$$

The incidence rate among 'unexposed' cats (p_2) is given by:

$$p_2 = c/(c+d).$$

The ratio of the two incidence rates - the relative risk (R) - is given by:

$$R = p_1/p_2.$$

This alternatively may be expressed as:

$$R = \{a/(a+b)\}/\{c/(c+d)\}$$

which, using the data from Table 4:

$$= (44/75)/(9/64)$$
$$= 4.2.$$

A relative risk greater than 1 indicates a positive statistical association between a factor and disease. A relative risk less than 1 indicates a negative statistical association: possession of the factor may be said to have a protective effect against the disease. A relative risk of 1 suggests no association.

Odds ratio (ψ)

The odds ratio ψ (the Greek letter 'psi') is similar to the relative risk.

If an event occurs with probability p, then the ratio p/q is called the odds in favour of the event occurring, where $q=1-p$.

If the odds of disease among exposed animals is p_1/q_1 , and the odds of disease among unexposed animals is p_2/q_2 , then the ratio of the odds of disease among exposed animals to the odds of disease among unexposed animals, known as the odds ratio (ψ) , is given by

$$\psi = (p_1/q_1)/(p_2/q_2)$$

$$= p_1 q_2/q_1 p_2$$

$$= ad/bc \ ,$$

which, using the data from Table 4:

$$= (44\times55)/(31\times9)$$
$$= 8.7 \ .$$

For rare diseases, the risk of disease, p , is almost the same as the odds p/q , since q is approximately equal to 1, and so, for diseases of low incidence, the odds ratio provides a good approximation to the relative risk. When disease incidence is not low, the values of the relative risk and odds ratio diverge. 'Relative risk' and 'odds ratio' were once used interchangeably but their two distinct derivations are now well established.

These calculations produce point estimates of ψ and R . Interval estimates can be calculated although care has to be taken since the odds ratio and relative risk statistics are not Normally distributed and different formulae from those given in the Appendix have to be used. It is also possible to decide whether the values of ψ and R differ significantly between groups. The calculations are described in Fleiss (1981) and Schlesselman (1982), with more technical details in Plackett (1981).

A logical requirement of demonstration of cause is that an animal is exposed to a causal factor before disease develops (cause always precedes effect). The design of cohort studies ensures that this temporal sequence is detected. However, cross-sectional and case-control studies may not detect the sequence. For example, if the association between neutering and urinary incontinence in female dogs were being investigated using a cross-sectional study (neutering being the hypothesised risk factor), then neutered dogs with incontinence may be identified. However, incontinence may have developed before neutering in some of the cases, in which instance neutering could not have been a component cause in those animals. For this reason, and the reason that a cohort study measures incidence, this study is therefore theoretically a better technique for assessing risk and identifying causes than the other two types of study.

Interpreting results

If the value of the lower confidence limit of the measure of association, be it odds ratio or relative risk, is greater than one, at a defined level of significance, then the association between the hypothesised cause and the disease is statistically significant and it can be said that there is evidence of an association. Alternatively, significance can be demonstrated at a defined level using the χ^2 test. The conventional level of significance used in experimental investigation is 5%. This has the effect of restricting the probability of a Type 1 error - accepting an association when one does not

exist - to 0.05. A value for this probability as small as 0.05 is desirable when a particular causal association relating to one factor and a disease is being investigated. This is the so-called "searchlight" approach which focuses on one factor and is a common aim of cohort studies. However, if a study is undertaken to "trawl" for any possible causes, the so-called "bucket" approach to data collection, then the Type II error - the incorrect rejection of a true association - is more important. This is a common aim of case-control studies. In this latter case a higher level of significance, for example, 10% or 20%, with a corresponding lower value for the probability of a Type II error, would be more appropriate in order to reduce the risk of not recognizing true associations.

There is some debate as to whether significance levels or confidence intervals should be used (Jones and Rushton, 1982). A level of significance provides a clear-cut decision boundary which is somewhat artificial. It is now generally accepted that it is better to construct a confidence interval. The interval gives a range which will contain the true value of the measure of association with a certain pre-specified probability. The interval provides more than the significance test; it also provides, in an easily interpretable way, a measure of the variability of the data; the wider the interval the more imprecise the inferences to be drawn from the data, the narrower the interval the more precise the inferences are.

Demonstration of a statistically significant association using each of the three observational approaches can fulfil one of Evans' postulates. The credibility of cause is strengthened by fulfilling others of Evans' postulates. Jarrett's (1980) demonstration of an association between exposure to bracken and the development of intestinal cancer in cattle is more credible because a carcinogen has been isolated from bracken (Wang et al. 1976), (Evans' postulate 10).

Estimates of measures of association can be used to infer interaction. If relative risk is estimated, using the odds ratio approximation for rare diseases, then the 'background' risk (for 'unexposed' animals) is 1. If the relative risk for 'exposed' animals is R, then the risk attributable to the 'exposure' factor, A, is given by $R-1$. If two or more factors are considered, then, using the additive model, positive interaction occurs between factors when the attributable risk for the simultaneous occurrence of the factors is greater than the sum of the attributable risks for each independently occurring factor. For example, Willeberg (1976), in his study of the FUS, showed that castration and high levels of dry cat food intake, when present simultaneously, produced an attributable risk in excess of the sum of the attributable risks for each factor, indicating a positive interaction between the two factors. In this example, castration and high levels of dry cat food intake (usually associated with overfeeding) may both result in inactivity, thereby reducing blood flow to the kidneys, impairing kidney function, and therefore possibly promoting changes in the urine that are conducive to the formation of uroliths; this constitutes a possible common causal pathway, that is, synergism has occurred.

Bias in observational studies

Bias is any systematic (as opposed to random) error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease. There are several types of bias (Last, 1983; Sackett, 1979) of which three are particularly pertinent to observational studies:

1. selection bias,
2. misclassification,
3. confounding.

Selection bias

Selection bias results from differences between characteristics of the study population and the population from which it was drawn. Most observational studies use data gathered from convenient populations such as veterinary clinics, abattoirs and specific farms. Willeberg's investigation of the FUS in Denmark (Willeberg, 1977), for instance, utilised data collected at a veterinary school's clinic. Ideally, a sample should be selected from the population at risk (all cats in Denmark in this example), but this rarely is possible. Inferences from investigations that might be biased by selection need to be made with care if they are to be extrapolated to the general animal community. Consideration should be given to the likelihood of the study population being biased with respect to the disease and factors that are being investigated. Selection bias is unlikely if:

1. exposure to a factor does not increase the likelihood of an animal being present in the study population,
2. the likelihood of inclusion of cases and controls in the study population is the same.

For example, Darke et al. (1985) investigated the association between the presence of an entire tail (the factor) and tail injuries (the disease) in a veterinary clinic population to determine whether docking reduces the risk of tail damage. It is improbable that docking or otherwise affects attendance at a veterinary clinic and so selection bias was unlikely in this study.

Misclassification

Misclassification occurs when either diseased animals are classified as non-diseased, or animals without a particular disease are classified as possessing it. The likelihood of misclassification depends on the frequency of disease, the frequency of exposure to the hypothesised causal factor, and the sensitivity and specificity of the diagnostic criteria used in the study. Two types of misclassification can occur: non-differential and differential.

Non-differential misclassification occurs when the magnitude and direction of misclassification are similar in the two groups that are being compared (i.e. cases and controls, or exposed and unexposed individuals). Non-differential misclassification produces a shift in the estimated relative risk and odds ratio towards zero (Copeland et al., 1977). Specificity is more important than sensitivity in determining a biased estimate of the relative risk. However, sensitivity has increased importance in determining a biased estimate of the odds ratio.

Differential misclassification occurs when the magnitude or direction of misclassification is different between the two groups that are being compared. In this case, the relative risk and odds ratio can be biased in either direction. Therefore, differential misclassification can not only weaken an apparent association but also strengthen it.

If a simple, valid (i.e. highly specific and sensitive) test is not available, then there can be difficulty in defining a case in the absence of a rigorous definition. For example, in an investigation of the relationship

between enzootic bovine leukosis (EBL) and human leukaemia (Donham et al., 1980), cattle were defined as being exposed to EBL virus when post-mortem examination revealed alimentary lymphosarcoma, even though this lesion may develop without exposure to the virus, and exposure to the virus may not produce alimentary tumours.

Similarly, it may be difficult to define and quantify an hypothesised causal factor to which an animal is exposed. For example, if 'inadequate feeding' were the factor, then the investigator may have to rely on an opinion based on owners' descriptions of diet, rather than using the more rigorous results of an examination by a nutritionist.

Confounding

Confounding (from the Latin 'confundere': 'to mix together') is the effect of an extraneous factor that can wholly or partly account for an apparent association between other factors and disease. Confounding either can produce a spurious association between an hypothesised cause and disease, or can mask a real association. A factor that confounds is called a confounding variable or confounder.

A confounding variable is correlated (either positively or negatively) with the disease and hypothesised causes. A confounding variable must:

1. be a characteristic of the individual animal, population, or environment - not necessarily causal - that increases the probability of the disease developing (this characteristic is called a risk factor); and

2. be associated with the explanatory variable, but not be a consequence of exposure to the explanatory variable.

For example, sex is a confounding variable in relation to feline urolithiasis. If breed were being considered as the factor under study, then the results would be confounded (biased, confused, rendered unrepresentative) if the 'breed' group with the disease comprised all male neuters (which are likely to develop the disease), and the 'breed' non-diseased group comprised all females (which are much less likely to develop the disease). The uneven proportion of male neuters and females in each group will, therefore, confound the interpretation of the effect of breed on the disease. Confounding is particularly important in case-control studies because animals are chosen according to presence and absence of disease: therefore cases may have a whole range of factors in common, some of which may be causal and some of which may be statistically significant because of an association with a confounder.

Confounding is not an "all-or-none" event, but occurs to varying degrees. Tests for confounding are discussed by Schlesselman (1982); assessment of the extent of confounding is described by Ejigou and McHugh (1977) and Miettinen (1972). A pictorial representation of the effect that confounding has on the estimates of relative risk is given by Rothman (1975).

Controlling bias

It is not possible to effectively control selection bias; this results from inherent demographic characteristics of the study population.

Controlling of the effect of misclassification on the odds ratio is described by Keys and Kihlberg (1963); the control is essentially conducted during analysis.

There are two main methods of controlling confounding:

1. by adjusting for the confounder in the analysis, for example, by using adjusted rates specific to the confounder (e.g. age, sex and breed) or by producing a summary relative risk for the combined relative risks of each confounder (Mantel and Haenzel, 1959);

2. by 'matching' the two groups during the design of the study. Matching can be performed in two ways, by:

   a. Frequency matching in which the groups to be sampled are divided so as to contain the same proportion of the possible confounder, for example, if there are four times as many males as females in the 'case' group, then the 'control' group should also be selected to contain four times as many males as females;

   b. individual matching in which each case is matched with a control with respect to the potential confounder, for example, a six year old dog with bladder cancer is paired with a six year old dog without bladder cancer (matching for age).

It is usual to match for the main possible confounders: age, sex and breed. If the effect of a factor is in doubt, then it is best not to match but to control it in subsequent analysis; when a factor is matched it cannot be studied separately. It is important to note that when matched studies are conducted they should be analysed as such; the relevant methods are described in Schlesselman (1982).

Selection of sample size in cohort and case-control studies

Four values should be specified to determine optimum sample size:

1. the desired level of significance (α - the probability of Type I error - claiming that exposure to a factor is associated with a disease when, in fact, it is not);

2. the power of the test ($1-\beta$, the probability of claiming correctly that exposure to a factor is associated with disease, where β is the probability of a Type II error.);

3. the relative frequency of exposure among controls in the target population;

4. an hypothesised relative risk (odds ratio) that is considered important enough, from the point of view of the health of the animal population.

If a disease is rare, then a cohort study requires a considerable number of animals in the 'exposed' and 'unexposed' groups to detect a significant difference, especially when the relative risk is small. Table 5 illustrates this point. There are various formulae for the calculation of sample size (Snedecor and Cochran, 1980; Schlesselman, 1982) which involve different assumptions about the variance of disease incidence when the relative risk is

either 1 or a different value. The figures in Table 5 have been generated using a formula which assumes that-

(a) when the relative risk is 1 the variance is based on the incidence rate in the unexposed population; and

(b) when the relative risk is different from 1 the variance is based on the two incidence rates in the exposed and unexposed populations.

Table 5 The estimated number of individuals in each group (exposed and unexposed) for detecting a statistically significant relative risk in a cohort study by relative risk and the incidence rate of disease in the control (unexposed) group.* Figures are given to no more than three significant figures.

	Incidence rate in control group for period of study			
Relative Risk	1 per 10,000	1 per 1,000	1 per 500	1 per 100
2	143,000	14,300	7,140	1,410
3	40,200	4,010	2,000	396
4	19,700	1,960	980	193
5	12,000	1,200	599	117
10	3,230	322	160	31

* Based on the probability of detecting a difference between the two groups of 80% and a significance level of 5%.

Tables of the smallest and largest detectable relative risks, for different values of these parameters, are given by Walter (1977).

Multivariate techniques

In case-control studies, if matching is practised to adjust for confounding, then there may be many 2 × 2 contingency tables, for example, for different combinations of age, sex and breed. If the disease being investigated is rare then the number of animals in each cell may be very small, resulting in inestimable or large confidence intervals - possibly statistically insignificant - for R and ψ. Similarly, if R varies considerably between each contingency table, then the summary relative risk may be small, even though the individual table values are large: information is lost (Bender et al., 1983). These problems can be overcome by using multivariate techniques that consider many factors simultaneously. Common methods use a logistic model for discrete and continuous variables and a log linear model for discrete variables and grouped continuous data. An example is the study of the association between benign and malignant neoplasms in dogs (Bender et al., 1982). Multivariate techniques are not described in this introductory paper; details may be found in Kleinbaum et al. (1982) and Schlesselman (1982).

Conclusions

Observational studies offer a means of investigating cause 'in the field'. Therefore, component causes can be studied in their natural association with other components of sufficient causes, without the limitations induced by experimental conditions. Furthermore, the increasing concern for animal welfare, with the associated ethical implications of experimentation, may increase the role played by observational studies. However, design and analysis need to be planned carefully, to avoid erroneous inferences originating from bias.

This paper has introduced basic concepts and techniques. A detailed discussion is provided by Fleiss (1981), Feinstein (1977), Breslow and Day (1980) and Schlesselman (1982). The range of veterinary observational studies is exemplified in Table 6.

Table 6 Some veterinary observational studies

Species	Disease	Hypothesised causal factors	Source
Ox, horse, pig, dog, cat	Congenital, umbilical and inguinal hernias	Breed, sex	Hayes (1974a)
Ox, horse, dog, cat	Nervous tissue tumours	Breed, age, sex	Hayes et al. (1975)
Ox, horse, dog, cat	Various tumours	Breed, age, sex	Priester & Mantel (1971)
Ox, horse, dog, cat	Various tumours	Breed, sex, age	Priester & McKay (1980)
Ox, horse, dog, cat	Pancreatic carcinoma	For dogs: breed, sex, age	Priester (1974b)
Cat	Urolithiasis	Breed, sex, age, neutering, season of year, diet, weight, level of activity, time of diagnosis	Willeberg (1975a, b,c, 1976) Willeberg & Priester (1976)
Dog	Bladder cancer	Breed, sex	Hayes (1976)
Dog	Ectopic ureter	Breed	Hayes (1974b)
Dog	Elbow disease (mainly dysplasia)	Breed, sex	Hayes et al. (1979)

Table 6 (Continued)

Dog	Intervertebral disc disease	Breed, sex, age, site of involvement	Goggin et al. (1970)
Dog	Malignant neoplasms	Benign neoplasms	Bender et al. (1982)
Dog	Oral and pharyngeal neoplasms	Breed, age, sex	Cohen et al. (1964)
Dog	Progressive retinal atrophy	Breed, sex, age	Priester (1974a)
Dog	Renal tumours	Breed, age, sex	Hayes & Fraumeni (1977)
Dog	Tail injuries	Undocked tails	Darke et al. (1985)
Dog	Thyroid neoplasms	Breed, age, sex	Hayes & Fraumeni (1975)
Horse	Potomac Fever	Premises, husbandry and management variables previous history of the syndrome on premises	Perry et al. (1984)
Ox	Brucellosis	Herd size, stabling registration status history of previous reactors, time of exposure, vaccination level, farm density, herd type, insemination methods	Kellar et al. (1976)
Ox	Calf mortality	Various management and husbandry variables, e.g. corn silage feeding, penning, vaccination	Martin et al. (1982)
Ox	Respiratory disease	Immune status, antibody level to various infectious agents	Pritchard et al. (1983)

Table 6 (Continued)

Pig	Enzootic pneumonia	Age, sex, clinical disease, ventilation, herd size, replacement policy, diarrhoea	Aalund *et al.* (1976) Willeberg *et al.* (1978)
Sheep	Intestinal adenocarcinoma	Exposure to herbicides	Newell *et al.* (1984)

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APPENDIX

SOME BASIC STATISTICAL CONCEPTS

A major characteristic of biological data is their inherent variability. The weights of 100 pigs, for example, will not be identical. Table A1, listing the weights of two groups of piglets, illustrates this point. If many piglets were weighed, rather than the limited sample of 49 in each of the groups in Table A1, then the distribution of weights among the theoretically infinite population from which the piglets were drawn would appear like that depicted in Figure A1. This bell-shaped distribution, which relates to continuous variables (see below), is called the Normal distribution. The curve in Figure A1 is the Normal density curve and the area under the curve between two points on the horizontal axis is the relative frequency of the

Table A1. Specimen three-week weaning weights of two groups (A and B) of pigs (kg.)

Group A

4.2	5.3	5.6	6.0	6.4
4.6	5.3	5.7	6.0	6.4
4.7	5.4	5.7	6.1	6.4
4.8	5.4	5.7	6.1	6.5
4.9	5.4	5.9	6.1	6.5
5.1	5.4	5.9	6.1	6.5
5.2	5.4	5.9	6.1	6.8
5.2	5.5	5.9	6.2	6.8
5.2	5.5	6.0	6.3	6.8
5.3	5.5	6.0	6.4	

$n = 49$ $\bar{x} = 5.76$ kg. $s = 0.60$ kg.

$\Sigma x = 282.0$, $\Sigma x^2 = 1640.4$

Group B

2.6	4.3	4.6	4.8	5.3
3.4	4.3	4.6	5.0	5.5
3.6	4.3	4.6	5.0	5.5
3.8	4.4	4.6	5.0	5.5
3.9	4.4	4.7	5.0	5.6
4.0	4.4	4.7	5.1	5.6
4.0	4.4	4.7	5.1	5.6
4.1	4.5	4.8	5.2	5.7
4.1	4.5	4.8	5.2	6.3
4.2	4.5	4.8	5.2	

$n = 49$ $\bar{x} = 4.69$ kg. $s = 0.67$ kg.

$\Sigma x = 229.9$, $\Sigma x^2 = 1100.27$.

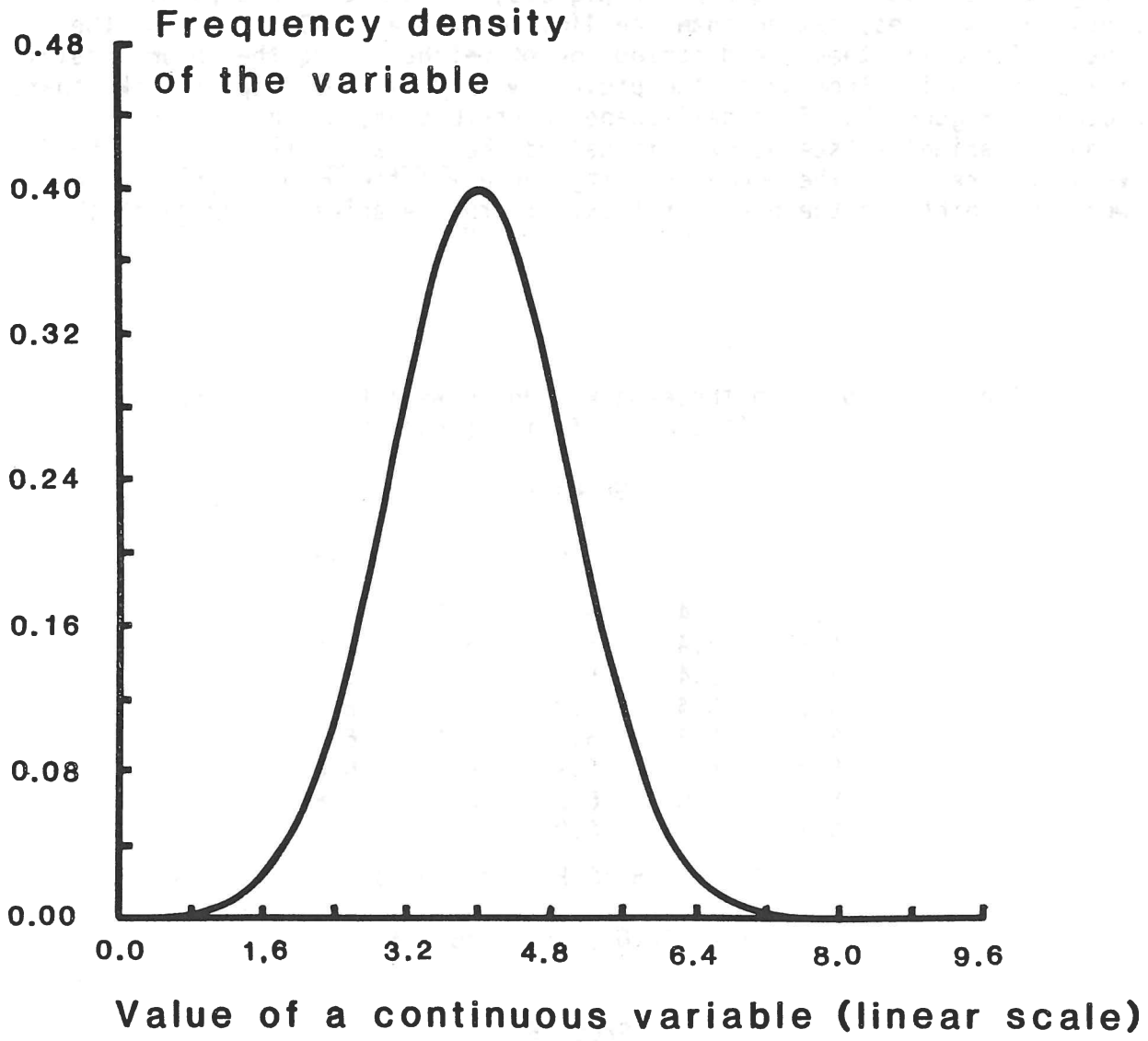

Fig. A1 Paradigm of the Normal distribution

continuous variable between these two points. Thus the height of the curve at a point is a measure of the frequency density of the variable at that point. The distribution is encountered commonly in biology. There are other distributions (including, notably, the binomial, Poisson and χ^2) which are described in standard statistical books.

Recorded characteristics (for example weight, girth, number of cases of disease, etc.) are variables. Variables may be either discrete or continuous. Continuous variables can take one of a theoretically infinite number of values. Weight is a continuous variable, (e.g. 3.6543....kg.). Discrete variables take only specified values, though note that the range may be countably infinite, e.g. 0, 1, 2, ... The number of cases of disease is a discrete variable; there may be 1, 2, or 3 cases of disease, but not 2.564 cases.

The study of statistics is concerned with the description, analysis and interpretation of measurements with random variation.

Descriptive statistics

A measure of position

A commonly adopted measure of position is the mean of a sample $(x_1, \ldots, x_n)$, which is conventionally denoted by $\bar{x}$ ('x-bar'). It is calculated using

$$\bar{x} = (x_1 + \ldots + x_n)/n = \Sigma x/n \ .$$

where Σ denotes summation.

In Table A1, $n = 49$ in each group and $\bar{x} = 5.76$ kg in Group A and 4.69 kg in Group B.

The sample has been assumed, implicitly, to be drawn from a much larger population and thus the mean of the sample is only an estimate of the true population mean μ (the Greek letter 'mu', pronounced 'mew'). Only if all the population is investigated will the true mean μ be known. The precision of $\bar{x}$ as an estimate of μ improves as the sample size increases.

Measures of spread

Measures of spread are a little more difficult to calculate than those of position. Two examples of simple measures of spread are the range and the mean of absolute deviations of the individual sample values from the sample mean. However, the range is sensitive to any excessively small or large values and the mean of the absolute deviations is difficult to deal with mathematically.

A more commonly adopted measure is the sample variance, s^2, which is calculated by

$$s^2 = \Sigma\{(x-\bar{x})^2\}/(n-1) \ .$$

An alternative formula that gives the same result, but is more easily calculated with small calculators, is:

$$s^2 = [\Sigma x^2 - \{(\Sigma x)^2/n\}]/(n-1) \ .$$

The square root of the sample variance is called the sample standard deviation.

The sample standard deviation, s, for the data from Group B in Table A1 may be calculated using the square root of the formula above. It is

$$s = \sqrt{([\Sigma x^2 - \{(\Sigma x)^2/n\}]/(n-1))}$$

$$= \sqrt{([1100.27 - \{229.9^2/49\}]/48)}$$

$$= \sqrt{(21.6167/48)}$$

$$= 0.67 \text{ kg.}$$

Just as the sample mean is an estimate of the population mean so the sample variance and the sample standard deviation are estimates of the population variance σ^2 (σ is the Greek letter 'sigma') and the population standard deviation σ.

When summary statistics are presented the sample standard deviation should be presented as well as the sample mean in order that the precision of the estimate of the population mean should be known.

Interval estimation

The sample mean gives a single estimate for the population mean μ and is therefore called a point estimate. Repeated sampling of the population will produce different sample means as estimates of the population mean. The square root of the variance of the sample means is termed the standard error of the mean (s.e.m.), to avoid confusion with the standard deviation of the individual values. It is given by:

$$\text{s.e.m.} = \sigma/\sqrt{n}.$$

This may be estimated by what is termed the estimated standard error of the mean (e.s.e.m.), obtained by replacing σ by s:

$$\text{e.s.e.m.} = s/\sqrt{n}.$$

It is sometimes more useful to quote a range within which one is reasonably confident that the true mean will lie. This range is known as a confidence interval. For example, on approximately 95% of occasions that samples are taken from a population of Normally distributed values, the confidence interval given by the sample mean ±2 s.e.m.s will contain the true population mean. Alternatively a single calculated confidence interval of the form (sample mean ±2 s.e.m.s) from a Normal population contains the true population mean with probability 0.95.

The upper and lower points of a confidence interval are termed confidence limits.

Using the values from Table A1, Group B,

$$\text{e.s.e.m.} = s/\sqrt{n}$$
$$= 0.67/\sqrt{49} \text{ kg.}$$
$$= 0.096 \text{ kg.}$$

and so the 95% confidence interval is estimated by

$$\bar{x} \pm 2 \text{ e.s.e.m.} = 4.69 \pm (2 \times 0.096) \text{kg.}$$
$$= 4.50 - 4.88 \text{ kg.}$$

assuming that the three week weaning weights are Normally distributed.

Statistical techniques for demonstrating association

A common application of statistics to epidemiology is the detection of association between variables. If a variable is the result of the action of another variable, then the former is termed the response (or dependent) variable, and the latter the explanatory (or independent) variable. Commonly, diseases are the response variables, and causes are the explanatory variables.

Demonstration of association can be approached in three ways.

1. The difference between the mean of the probability distribution of the values of a variable under two different circumstances can be measured. If there is a significant difference between the means in the two circumstances, then the different circumstances can be inferred to affect the values of the variable. For example, the weights of two groups of piglets, one group of which has developed neonatal diarrhoea and one group of which has not, can be measured. The effect of diarrhoea on weight can then be assessed by analysing the difference between the mean weights of the two groups.

2. Variables can be categorised, and a significant association sought between various categories. For example, cats can be categorised according to whether they have the feline urological syndrome and whether they eat dry cat food. Evidence of an association between the syndrome and the consumption of dry cat food can then be sought. The study could be further refined by estimating the degree of association between disease and the diet.

3. A correlation between variables can be sought. For example, the incidence of lameness in cattle and the amount of rainfall can be recorded, to investigate whether increased rainfall is significantly associated with an increased incidence of lameness.

The first two approaches are relevant to this paper, and are outlined below.

The principle of a significance test

The bell shape of the Normal distribution reveals that there is a probability, albeit a small one, of an observation occurring at the extreme ends of the distribution. This distribution may not only be used to describe the frequency distribution of the values of a continuous variable which has a

Normal distribution but also of the means of repeated samples taken from that population (here termed the 'reference population') though it has to be remembered that for the distribution of the means the variance is reduced by a factor of the square root of the sample size. There is, therefore, a high probability of the mean of a sample being under the hump, and a much lower probability of its being close to either of the two tails. If the mean is close to a tail, then this indicates either that the sample is one of those small number that are taken from the tails of the population's frequency distribution or that it has been drawn from a population with a different frequency distribution. This is the principle of a significance test.

The frequency distribution of the sample may be only slightly to the left or right of the reference population (its mean either just slightly to the left or the right of the peak of the hump). This is very probable if the sample is drawn from the reference population; there would be no justification in saying that the sample was drawn from a different population. It is therefore necessary to decide at what point a sample is considered not to have a frequency distribution similar to that of the reference population with which it is being compared. This decision is taken when the probability, P, of obtaining a value for the sample mean either as extreme as, or more extreme than, the one observed, assuming that the sample is drawn from the reference population, is less than a value known as the level of significance. This level is represented by α (the Greek letter 'alpha').

Conventionally, in biological sciences, α is taken to be 0.05 (expressed as $P < 0.05$, or the result is said to be 'significant at the 5% level' or 'P is significant *'). In practical terms, this indicates that the inference that the population from which the sample has been drawn is different from the reference population would be incorrect 5% of the time. The 5% level is purely conventional. If more caution in inferring, incorrectly, a difference were necessary, then the 1% level ($P < 0.01$ or 'P significant **') or the 0.1% level ($P < 0.001$, or 'P significant ***') could be chosen.

The null hypothesis

A significance test is conducted by first stating a null hypothesis. This is a hypothesis concerning the distribution of characteristics of the reference population. If the probability that the sample or anything more extreme has been drawn from the reference population is equal to or less than the chosen level of significance then the null hypothesis is rejected in favour of an alternative one which states that the sample has been drawn from a population which is different from the reference population. Demonstration of a significant difference implies rejection of the null hypothesis.

Notice that confidence intervals and the outcomes of significance test are closely related. For example, suppose that the null hypothesis states a particular value for the mean of a Normal distribution. A sample is taken and the significance test rejects the null hypothesis at the 5% level. Then the corresponding 95% confidence interval, sample mean ± 2 e.s.e.ms, will not contain the value of the mean specified by the null hypothesis. Conversely if the significance test does not reject the null hypothesis at the 5% level then the corresponding 95% confidence interval will contain the value of the mean specified by the null hypothesis.

Errors of inference

Five per cent of samples from a population lie within the region that would lead to rejection of the null hypothesis at the 5% level. If this happens then it constitutes a rejection of the null hypothesis when the hypothesis is true. This error is an example of a Type 1 error -false rejection of a true null hypothesis. The probability of a Type 1 error is just the level of significance discussed above.

A Type II error is a failure to reject the null hypothesis when it is untrue. The probability of committing this error is called β (the Greek letter 'beta'). Ideally, both α and β should be defined by the investigator before the study begins. In practice, this rarely occurs because β depends on α and the sample size, both of which are usually specified; β is therefore predetermined. The probabilities of Type I and II errors decrease as sample size increases. For a fixed sample size the larger the probability of a Type 1 error is chosen to be, the smaller the probability of a Type II error will be, and vice-versa.

Two remaining alternative decisions are possible. These represent correct inferences, rather than errors. The first is not rejecting the null hypothesis when it is true. The second is rejecting the null hypothesis when it is false (i.e. demonstrating a significant difference). The probability of the latter is called the power of a test; it is denoted by $1-\beta$.

The χ^2 test of association

Table 4 records the results of an investigation of the feline urological syndrome (FUS) in Denmark (Willeberg, 1975). The question being asked was "does an association exist between the development of the FUS and the eating of dry cat food?" The investigator categorised cats into those with the FUS whose partial diet was dry cat food, those with the FUS which did not eat dry cat food, those without the FUS whose partial diet was dry cat food, and those without the FUS which did not eat dry cat food. These four permutations allow the construction of a two-way table with four 'cells' in it, called a 2×2 contingency table.

The values in the table need to be assessed. A simple method of assessment would be to express the values in each row as percentages of the total of each row. If each row showed similar percentages, then this would imply that the row classification did not affect the column classification - that there was no association between the two classifications. This reasoning is sound, but requires large numbers, otherwise sampling variation could affect the result. Taking the data in Table 4, the percentage of animals with the FUS whose partial diet is dry cat food is 59% (44/75); the percentage with the FUS which do not eat dry cat food is 14% (9/64). This difference could be significant, but it could also merely result from the variation induced by selection of a relatively small sample of the total population at risk.

A common way of conducting a reliable test on these data is to calculate a test statistic called χ^2 (χ is the Greek letter 'chi', pronounced with a hard 'ch'). The distribution followed by this statistic is known as the χ^2 distribution. This statistic indicates the extent to which the observed values in the cells diverge from the values which would be expected if there were no association between row and column categories. A table of the values

of the χ^2 distribution is available, to decide whether the observed χ^2 value is larger than that which would be expected, based on a null hypothesis postulating no association.

This example involves only a 2 × 2 contingency table, and the χ^2 equation below is simplified to refer only to this type of table. The test can be performed on contingency tables with several rows and columns (i.e. with several categories). Details can be found in standard textbooks on statistics.

The χ^2 statistic is given by:

$$\chi^2 = \frac{n\{|ad - bc| - \frac{1}{2}n\}^2}{(a+b)\ (c+d)\ (a+c)\ (b+d)}$$

where $\frac{1}{2}n$ is a continuity correction, to improve the approximation, because the χ^2 distribution is a continuous one, yet the data (numbers of animals), and therefore the test statistic, are discrete. Notice that $|ab - bc|$ indicates the absolute value of ad-bc ; that is ad-bc if ad>bc and bc-ad if ad<bc.

Using the values in Table 4:

$$\chi^2 = \frac{139\{|2420-279| - (139/2)\}^2}{75 \times 64 \times 53 \times 86}$$

$$= 27.26 \ .$$

Percentage points of the χ^2 distribution are given in Table A2 at the end of this appendix for various significance levels and "degrees of freedom" As a general rule, the degrees of freedom (ν) to be selected are given by

$$\nu = (\text{number of rows} - 1) \times (\text{number of columns} - 1) \ ,$$

which, in this example, is

$$(2-1) \times (2-1) = 1 \ .$$

In a 2 × 2 table where the row and column totals are all known then knowledge of one of the values in the four cells in the body of the table immediately implies knowledge of the values in the other three cells. Similarly in a table with r rows and c columns, with the row and column totals all known, the knowledge of (r-1) × (c-1) of the values in the rc cells in the body of the table implies knowledge of the values in the other cells. This is the idea behind "degrees of freedom"; namely the freedom to choose the values in the body of a contingency table when the row and column totals are fixed.

Consulting row 1 (1 degree of freedom) of the table, the observed value, 27.26, is greater than the tabulated statistic at the 5% level of significance (3.841) and so an association can be inferred between the partial consumption of dry cat food and development of the FUS. Note that, in this example, the result is also significant at the 1% and 0.1% levels, too.

Table A2 Percentage points of the χ^2 distribution

Degrees of freedom	*Value of P*				
	0.99	0.95	0.05	0.01	0.001
1	0.000157	0.00393	3.841	6.635	10.83
2	0.0201	0.103	5.991	9.210	13.82
3	0.115	0.352	7.815	11.34	16.27
4	0.297	0.711	9.488	13.28	18.47
5	0.554	1.145	11.07	15.09	20.51
6	0.872	1.635	12.59	16.81	22.46
7	1.239	2.167	14.07	18.48	24.32
8	1.646	2.733	15.51	20.09	26.13
9	2.088	3.325	16.92	21.67	27.88
10	2.558	3.940	18.31	23.21	29.59
11	3.053	4.575	19.68	24.72	31.26
12	3.571	5.226	21.03	26.22	32.91
13	4.107	5.892	22.36	27.69	34.53
14	4.660	6.571	23.68	29.14	36.12
15	5.229	7.261	25.00	30.58	37.70
16	5.812	7.962	26.30	32.00	39.25
17	6.408	8.672	27.59	33.41	40.79
18	7.015	9.390	28.87	34.81	42.31
19	7.633	10.12	30.14	36.19	43.82
20	8.260	10.85	31.41	37.57	45.31
21	8.897	11.59	32.67	38.93	46.80
22	9.542	12.34	33.92	40.29	48.27
23	10.20	13.09	35.17	41.64	49.73
24	10.86	13.85	36.42	42.98	51.18
25	11.52	14.61	37.65	44.31	52.62
26	12.20	15.38	38.89	45.64	54.05
27	12.88	16.15	40.11	46.96	55.48
28	13.56	16.93	41.34	48.28	56.89
29	14.26	17.71	42.56	49.59	58.30
30	14.95	18.49	43.77	50.89	59.70

The table gives the percentage points most frequently required for significance tests based on χ^2. Thus the probability of observing a χ^2 with 4 degrees of freedom greater in value than 9.488 is 0.05 or 5 percent. Again the probability of observing a χ^2 with 4 degrees of freedom smaller in value than 0.297 is 1 - 0.99 = 0.01 or 1 percent.

[From Bailey (1983)]

REFERENCES

Aalund, O., Willeberg, P., Mandrup, M. and Riemann, H. (1976). Lung lesions at slaughter: association to factors in the pig herd. Nordisk Veterinaermedicin 28, 481-486.

Bailey, N.T.J. (1983). Statistical methods in biology. 2nd edition. Hodder and Stoughton (London).

Bender, A.P., Bender, G.P., Dorn, C.R. and Schneider, R. (1982). Associations between canine benign and maliganant neoplasms. Preventive Veterinary Medicine 1, 77-87.

Bender, A.P., Policello, G.E., Dorn, C.R. and Robinson, R.A. (1983). Cautions about the estimation of summary relative risk from veterinary epidemiologic studies. In: Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics. Arlington, Virginia, 6-10 September 1982 Pp. 60-67. Veterinary Medicine Publishing Company (Edwardsville).

Blot, W.J. and Day, N.E. (1979). Synergism and interaction: are they equivalent? American Journal of Epidemiology 110, 99-100.

Breslow, N.E. and Day, N.E. (1980). Statistical methods in cancer research Volume 1. The analysis of case-control studies. IRAC publication No. 32. International Agency for Research on Cancer (Lyon).

Cohen, D., Brodey, R.S. and Chen, S.M. (1964). Epidemiologic aspects of oral and pharyngeal neoplasms of the dog. American Journal of Veterinary Research 25, 1776-1779.

Copeland, K.T., Checkoway, H., McMichael, A,J. and Holbrook, R.H. (1977). Bias due to misclassification in the estimation of relative risk. American Journal of Epidemiology 105, 488-495.

Darke, P.G.G., Thrusfield, M.V. and Aitken, C.G.G. (1985). The association between tail injuries and docking in dogs. Veterinary Record. In press.

Doll, R. and Hill, A.B. (1964a). Mortality in relation to smoking. Ten years' observations of British doctors. British Medical Journal 1, 1399-1410.

Doll, R. and Hill, A.B. (1964b). Mortality in relation to smoking. Ten years' observations of British doctors. British Medical Journal 1, 1460-1467.

Donham, K.J., Berg, J.W. and Sawin, R.S. (1980). Epidemiologic relationships of the bovine population and human leukemia in Iowa. American Journal of Epidemiology 112, 80-92.

Ejigou, A. and McHugh, R. (1977). On the factorization of the crude relative risk. American Journal of Epidemiology 106, 188-191.

Evans, A.S. (1976). Causation and disease. The Henle-Koch postulates revisited. Yale Journal of Biology and Medicine 49, 175-195.

Feinstein, A. (1977). Clinical biostatistics. The C.V. Mosby Company (Saint Louis).

Fleiss, J.L. (1981). Statistical methods for rates and proportions. 2nd edition. Wiley (New York).

Goggin, J.E., Li, A. and Franti, C.E. (1970). Canine intervertebral disc disease: characterization by age, sex, breed and anatomic site of involvement. American Journal of Veterinary Research 31, 1687-1692.

Hayes, H.M. (1974a). Congenital umbilical and inguinal hernias in cattle, horses, swine, dogs and cats: risk by breed and sex among hospital patients. American Journal of Veterinary Research 35, 839-842.

Hayes, H.M. (1974b). Ectopic ureter in dogs: epidemiologic features. Teratology 10, 129-132.

Hayes, H.M. (1976). Canine bladder cancer: epidemiologic features. American Journal of Epidemiology 104, 673-677.

Hayes, H.M. and Fraumeni, J.F. (1975). Canine thyroid neoplasms: epidemiologic features. Journal of the National Cancer Institute 55, 931-934.

Hayes, H.M. and Fraumeni, J.F. (1977). Epidemiological features of canine renal neoplasms. Cancer Research 37, 2553-2556.

Hayes, H.M., Priester, W.A. and Pendergrass, T.W. (1975). Occurrence of nervous tissue tumours in cattle, horses, cats and dogs. International Journal of Cancer 15, 39-47.

Hayes, H.M., Selby, L.A., Wilson, G.P. and Hohn, R.B. (1979). Epidemiologic observations on canine elbow disease (emphasis on dysplasia). Journal of the American Animal Hospitals Association 15, 449-453.

Jarrett, W.F.H. (1980). Bracken fern and papilloma virus in bovine alimentary cancer. British Medical Bulletin 36, 79-81.

Jones, D.R. and Rushton, L. (1982). Simultaneous inference in epidemiological studies. International Journal of Epidemiology 11, 276-282.

Kellar, J., Marra, R. and Martin, W. (1976). Brucellosis in Ontario: a case-control study. Canadian Journal of Comparative Medicine 40, 119-128.

Keys, A. and Kihlberg, J.K. (1963). Effect of misclassification on estimated relative prevalence of a characteristic Part I: Two populations infallibly distinguished; Part II: Errors in two variables. American Journal of Public Health 53, 1656-1665.

Kleinbaum, D.G., Kupper, L.L. and Morganstern, H. (1982). Epidemiologic research. Principles and quantitative measures. Lifetime Learning Publications (Belmont).

Kupper, L.L. and Hogan, M.D. (1978). Interaction in epidemiologic studies. American Journal of Epidemiology 103, 447-453.

Last, J.M. (1983). A dictionary of epidemiology. Oxford University Press (New York).

MacMahon, B. (1972). Concepts of multiple factors. In: Lee, D.H.K. and Kotin, P. (Editors), Multiple factors in the causation of environmentally induced disease. Fogarty International Center Proceedings No. 12, Pp. 1-12. Academic Press (New York and London).

Mantel, H. and Haenzel, W. (1959). Statistical aspects of the analysis of data from retrospective studies of disease. Journal of the National Cancer Institute 22, 719-748.

Martin, S.W., Meek, A.H., Davis, D.G., Johnson, J.A. and Curtis, R.A. (1982). Factors associated with mortality and treatment costs in feedlot calves: the Bruce County beef project, years 1978, 1979, 1980. Canadian Journal of Comparative Medicine 46, 341-349.

Miettinen, O.S. (1972). Components of the crude risk ratio. American Journal of Epidemiology 96, 168-172.

Newell, K,W., Ross, A.D. and Renner, R.M. (1984). Phenoxy and picolinic acid herbicides and small intestinal adenocarcinoma in sheep. Lancet 2, (8415), 1301-1305.

Perry, B.D., Palmer, J.E., Birch, J.B., Magnusson, R.A., Morris, D. and Troutt, H.F. (1984). Epidemiological characterization of an acute equine diarrhoea syndrome: the case-control approach. In: Thrusfield, M.V. (Editor.) Society for Veterinary Epidemiology and Preventive Medicine. Proceedings of a meeting held at the University of Edinburgh on 10 and 11 July 1984. Pp. 148-153.

Plackett, R.L. (1981). The analysis of categorical data. 2nd edition. Charles Griffin & Co. Ltd. (London).

Priester, W.A. (1974a). Canine progressive retinal atrophy: occurrence by age, breed and sex. American Journal of Veterinary Research 35, 571-574.

Priester, W.A. (1974b). Data from eleven United States and Canadian colleges of Veterinary Medicine on pancreatic carcinoma in domestic animals. Cancer Research 34, 1372-1375.

Priester, W.A. and Mantel, N. (1971). Occurrence of tumours in domestic animals. Data from twelve United States and Canadian colleges of Veterinary Medicine. Journal of the National Cancer Institute 47, 1333-1344.

Priester, W.A. and McKay, F.W. (1980). The occurrence of tumours in domestic animals. National Cancer Institute Monograph Number 54. United States Department of Health and Human Services. National Cancer Institute (Bethesda).

Pritchard, D.G., Edwards, S., Morzaria, S.P., Andrews, A.H., Peters, A.R. and Gilmour, N.J.L. (1983). Case-control studies in the evaluation of serological data from respiratory disease outbreak in cattle. In: Thrusfield, M.V. (Editor) Society for Veterinary Epidemiology and Preventive Medicine. Proceedings of a meeting held at the University of Southampton on 12 and 13 April 1983. Pp. 131-138.

Rothman, K.J. (1975). A pictorial representation of confounding in epidemiologic studies. Journal of Chronic Diseases 28, 101-108.

Rothman, K,J, (1976). Causes. American Journal of Epidemiology 104, 587-592.

Sackett, D.1. (1979). Bias in analytic research. Journal of Chronic Diseases 32, 51-68.

Sartwell, P.E. (1950). The distribution of incubation periods of infectious disease. American Journal of Hygiene 51, 310-318.

Sartwell, P.E. (1966). The incubation period and the dynamics of infectious disease. American Journal of Epidemiology 83, 204-216.

Schlesselman, J.J. (1982). Case-control studies. Design, conduct, analysis. Oxford University Press (New York and London).

Snedocor, G.W. and Cochran, W.G. (1980). Statistical Methods. 7th edition. Iowa State University Press (Ames, Iowa).

Sponenberg, D.P. (1979). The inheritance of pathological conditions in dairy bulls. PhD thesis. Cornell University.

Trotter, W. (1930). Observation and experiment and their use in the medical sciences. British Medical Journal 2, 129-134.

Walter, S.D. (1977). Determination of significant relative risks and optimal sampling procedures in prospective and retrospective comparative studies of various sizes. American Journal of Epidemiology 105, 387-397.

Wang, C.Y., Chiu, C.W., Pamukcu, A.M. and Bryan, G.T. (1976). Identification of carcinogenic tannin isolated from bracken fern (Pteridium aquilinum). Journal of the National Cancer Institute 56, 33-36.

Willeberg, P. (1975a). A case-control study of some fundamental determinants in the epidemiology of the feline urological syndrome. Nordisk Veterinaermedicin 27, 1-14.

Willeberg, P. (1975b). Diets and the feline urological syndrome, a retrospective case-control study. Nordisk Veterinaermedicin 27, 15-19.

Willeberg, P. (1975c). Outdoor activity level as a factor in the feline urological syndrome. Nordisk Veterinaermedicin 27, 523-524.

Willeberg, P. (1976). Interaction effects of epidemiologic factors in the feline urological syndrome. Nordisk Veterinaermedicin, 28, 193-200.

Willeberg, P. (1977). Animal disease information processing: epidemiologic analyses of the feline urological syndrome. Acta Veterinaria Scandinavica 18, Supplement 64, 1-48.

Willeberg, P., Gerbola, M.A., Madsen, A., Mandrup, M., Nielsen, E.K., Riemann, H.P. and Aalund, O. (1978). A retrospective study of respiratory disease in a cohort of bacon pigs: I. Clinico-epidemiological analyses. Nordisk Veterinaermedicin 30, 513-525.

Willeberg, P. and Priester, W.A. (1976). Feline urological syndrome: interaction with same time, space, and individual patient factors. American Journal of Veterinary Research 37, 975-978.

SAMPLING IN ANIMAL HEALTH SURVEYS

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Veterinarians are frequently placed in the situation that they wish to acquire knowledge about the presence of disease or the levels of fertility or other important factors affecting production in a population of animals which, for some reason or other, cannot be studied by means of a complete census. Even where it is practicable to carry out a census it may not be necessary or advisable to do so. It has frequently been argued (e.g. Cochran (1977), Raj (1972), Yates (1981)) that a sample survey is frequently to be preferred to a full census on grounds of its smaller cost, the reduced time necessary for its completion, its greater flexibility - for example, measurements requiring specialised equipment or highly trained personnel are not usually possible in a census - and, perhaps surprisingly, its greater accuracy. This last advantage is likely to occur because the smaller scale of the exercise will make it easier to ensure that observations and measurements made on the selected units are accurate. However, all the value of these benefits may be reduced if the sample of units (farms, animals, etc) is unwisely chosen or if the analysis of the data is inappropriate to the sampling method adopted.

Although this paper will concentrate on the philosophy and methods of sampling in veterinary field studies it should be remembered that this is only one element in the execution of a survey. It is this author's experience that where the sampling procedure adopted in a study is sufficiently careless or ill-designed to make reliable conclusions impossible then usually there are other serious defects which would invalidate the investigation even if the sampling method had been appropriate. A survey is more likely to be irrevocably damaged by a badly designed questionnaire, ill-trained enumerators, inaccurate measurements or inadequate recording procedures than by an inappropriate sampling method. (See Zarkovic (1966) or Moser & Kalton (1971) for a discussion of the large variety of *non-sampling* errors which can arise in agricultural, demographic and social surveys.)

On the other hand, it is important to choose the sample of units to be surveyed in such a way that they will give a convincing and reasonably precise description of the most important features of the observation for as small a cost as possible. Information is a commodity with a high cost and its collection should be carried out to obtain a good return for the resources mentioned. There are many textbooks in print which cover, in detail, the many possible ways of obtaining efficient samples in many of the situations which arise commonly in practice; three have been mentioned above. By searching through these and similar publications it is possible to find a sample design for almost any survey together with the formulae required to calculate the various quantities which are commonly required as part of the report on the

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outcome. It is not the intention to go into that level of detail here. In the remainder of this paper the emphasis will be on introducing the concepts and terminology of sampling and discussing the motivation for different approaches to the sampling problem.

BASIC TERMINOLOGY

Statistical terminology suffers from the fact that many of the terms are words which, in ordinary language, have a meaning which may be similar to but rarely the same as the technical meaning with which it is employed by statisticians. A small number of such terms will be used repeatedly in the remainder of this discussion and it is important to understand their precise meaning.

Simple random sample

This is the form of sampling that is most commonly implied by the terms "sample" or "random sample". It is a sample selected by a procedure which gives all possible samples an equal chance of being chosen. It is the type of sample which will arise if, for example, each member of a population is represented by a plastic disc with a unique number, the plastic discs (identical except for the number) thoroughly mixed and then selected one at a time, without replacement, until the required size of sample has been drawn. Except in small populations or in populations with a very simple structure, simple random sampling will often be too costly or inefficient and more complex sampling procedures will be required.

Sampling error

This term has no pejorative connotation; no value judgement is implied by the word "error". When a sample is taken from a population and inferences are made about the population as a whole based on the characteristics of the sample, it should be clear that those inferences will rarely, if ever, be exactly correct. For example, if a dairy farm has a herd of 100 lactating cows, the average yield of ten cows, all sampled on the same day, will not be exactly equal to the average yield for the whole herd on that day. It could well be quite different if, by chance, the ten lowest- or ten highest-yielding animals were chosen. On the other hand, it is a common intuition, borne out by experience, that if the ten animals are chosen by simple random sampling then "most of the time" the mean of the sample will be "something like" the mean for the complete herd. Frequently the sample mean is used as as estimate of the population mean. The difference between the true value of the population mean and the value estimated by the sample is the sampling error. Of course, the magnitude of the sampling error will rarely be known since it can only be calculated if the true population value is known (in which case the survey would be unnecessary!) but sampling errors can be controlled by the use of adequate sampling procedures. Furthermore, the likely magnitude of the sampling error can usually be estimated from the data collected in the survey. Indeed, it is important to make some assessment of the level of sampling error for every quantity estimated from a sample.

Sampling unit

The sampling units are the items, people, animals, etc, which will be selected individually by a sampling procedure. There may be several distinct

types of unit defined at different levels of the sampling process. For example, if a simple random sample of counties is selected and then a sample of farms chosen within each county, the counties may be referred to as "primary" sampling units, the farms as "secondary" or "second-stage" sampling units, and so on.

Sampling fraction

This is the proportion of all the available units actually chosen for the sample. Again, there will frequently be several different sampling fractions in a single survey. A country may be divided into several regions and a different fraction of the available farms sampled in each region. Reasons for choice of sampling fractions are discussed in later sections. Beware of the "ten per cent" fallacy. It is not true that ten per cent is a good or necessary proportion to sample. In some circumstances ten per cent would be far too large a fraction; in others it may not be enough.

Sampling frame

This is a "list" of some kind which contains all the sampling units of a population. It may be an actual list such as a list of all the municipal districts in a region which we wish to survey and from which we can choose a sample of districts. It may be a map on which is marked all the farms from which a sample of farms is to be selected. It may be a queue of animals passing through a crush from which a sample of animals can be obtained. Notice that, even if farms, say, are the units of interest it is not necessary to have a list of all the farms in the region to be surveyed. The region can be broken down into smaller districts, perhaps local administrative districts of some kind, a few districts drawn by a suitable sampling method and then it is only necessary to find or draw up a list of farms in the chosen districts. Selecting the units in stages in this way is a common method of overcoming the deficiencies in sampling frames.

Representative sample

Raj (1972) points out that "this is an undefined term which appears to convey a great deal but which is unhelpful. If it means that the sample should be a miniature of the population in every respect, we do not know how to select such a sample. Actually, we can deliberately choose a distorted sample in order to make a better estimate." There is no known objective measure of representativeness; the term will not be employed in the remainder of this paper.

Standard error

This is the measure most commonly used to indicate the likely sampling error in an estimated value. If a survey were to be repeated a large number of times, selecting the same size of sample afresh at each repetition and always using the same sampling method, the estimated value of a quantity such as the total yield of milk or the prevalence of brucellosis in the population would be different for each sample although the population value remained the same. The standard error of an estimate is a measure of the variability that it would exhibit in repeated samples and it may usefully, if rather loosely, be thought of as the average value of the sampling error in the estimate. The value of the standard error will depend on the variability in the population, the sampling method, the size of sample and the rule (or formula)

used to calculate the estimate. The simplest way to interpret a standard error is by considering a *confidence interval*.

Suppose that a sample has been selected from a population to estimate the value of some quantity such as a prevalence, a mean or a total. Suppose that the true value of the quantity is T, the estimated value is E and the standard error is S. The values of E and S will be calculated from the sample but T will still be unknown. However, provided the sample is not very small, it can be said that "we are 95% confident that T is a value in the range E - 2S to E + 2S". Alternatively, "there is only a 5% chance that the true value of T lies outside that range". For example, if it is estimated from a survey that the prevalence of brucellosis in some population of cows is 12% with a standard error of 0.8%, then there is only one chance in twenty (5%) that the true prevalence lies outside the range 12 - 2 x 0.8 to 12 + 2 x 0.8, i.e. 10.4% to 13.6%. Clearly the quality and value of the information obtained via a survey depends onthe width of the confidence interval. To say that a prevalence lies in the range 10.4% to 13.6% is more informative than to say that it lies in the range 8.2% to 15.3%; the narrower the interval the better. A good sampling procedure will lead to standard errors which are as small as possible given the resources which are available to carry out the survey.

Bias

This is another term with a precise meaning which does not imply a judgement. It is not necessarily true in statistics that to be unbiased is good and to be biased is wrong. An estimate is said to be unbiased if its average value, in repeated samples, is equal to the true value of the quantity it is estimating. For example, suppose that, in a large herd, say 10,000 animals, there are 1,000 animals, i.e. 10%, with a particular disease. If a simple random sample of 100 animals is selected, it is rather unlikely that exactly 10 animals will be infected. The sample prevalence, p, calculated by dividing the number of infected animals in the sample by the total sample size might be any value, though it will probably be close to 10%. Suppose the first sample is returned to the herd and another sample is selected (which may or may not contain one or more animals from the first sample). Again, the sample prevalence is calculated. Lastly, suppose it were possible to repeat all this an indefinitely large number of times and that the true prevalence always remained at 10%. Each sample would provide an estimated prevalence. For some samples it would be rather less than 10%, for others it would be more. The occasional sample would show a very high prevalence. However, the long run average value of these estimated prevalences would be exactly 10% and for this reason we would say that the sample prevalence gives an unbiased estimate of the population prevalence. "Biased" and "unbiased", then, refer to long run properties of estimates not to the value of an estimate from a single sample. Small biases in estimates are perfectly acceptable if the estimate has a small standard error. Indeed, an estimate with small bias and small standard error would be preferable to an unbiased estimate with a large standard error. A useful analogy is to think in terms of a gun. A hunting rifle which fires on average at a point 5cms to the left of the intended target is biased. However, if the bullet usually lands within 1 cm of that point it will usually be only 4-6 cms away from the intended point and will therefore normally hit the animal at which it is fired. A different rifle which sprays its bullets in a circle of radius one metre around the intended point is unbiased (since it favours no particular direction off target) but will miss much more often than the biased rifle. Of course, a rifle with a large bias - say one which usually hits a point two metres above the target - will be effectively useless even if it hits the same point with every shot.

A NUMERICAL EXAMPLE

The most important concepts in sampling can be explained and exemplified using simple numerical examples. Consider a population which consists of just six farms of which only two are to be sampled. The quantity of interest is the mean number of animals per farm. The six farms have 24, 32, 122, 147, 173 and 186 animals respectively. The mean number of animals per farm (which we will denote by $\bar{X}$) is

$$\bar{X} = (24 + 32 + 122 + 147 + 173 + 186) \div 6$$

$$= (X_1 + X_2 + X_3 + X_4 + X_5 + X_6) \div N$$

(where X_1 = number of animals on first farm, etc, and N is the total number of sampling units (farms) in the population),

$$= \frac{\sum X_i}{N}$$

(where $\sum$ is the usual symbol to indicate that a set of numbers is to be added together),

$$= 684 \div 6$$

i.e. $$\bar{X} = 114.$$

The *variance*, S^2, of the number of animals per farm is defined in most texts on sampling by

$$S^2 = \frac{\sum (X_i - \bar{X})^2}{N-1} ,$$

the denominator N-1 being used, rather than N, because this simplifies many of the standard formulae. Note that $X_i - \bar{X}$ is the difference between the number of animals on the i-th farm and the mean number of animals per farm. In this case S^2 = 4,928.4 - the details of the calculations appear in Table 1.

Table 1. Hypothetical population of farms

Farm (sampling unit)	No. of animals (X_i)	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
1	24	-90	8100
2	32	-82	6724
3	122	8	64
4	147	33	1089
5	173	59	3481
6	186	72	5184
	684		24642

$\bar{X} = 684 \div 6 = 114$

$S^2 = 24642 \div 5 = 4928.4$

Now suppose a simple random sample of just two farms is chosen, x_1 and x_2 being the number of animals on the two farms chosen. The sample mean $\bar{x} = \frac{1}{2}(x_1 + x_2)$ can be used to estimate $\bar{X}$. Those readers who are unfamiliar with the processes of sampling and making inferences about a population from a sample may find it instructive to simulate this process by writing the six farm sizes on similar pieces of paper, drawing repeated samples of size two and considering the results of the sampling process. There are fifteen possible samples which can be drawn - see Table 2.

Table 2. Simple random sample

Sample	Farms in sample	Numbers of animals in chosen farms	Sample mean ($\bar{x}$)	Sample variance (s^2)
1	1, 2	24, 32	28.0	32.0
2	1, 3	24, 122	73.0	4802.0
3	1, 4	24, 147	85.5	7564.5
4	1, 5	24, 173	98.5	11100.5
5	1, 6	24, 186	105.0	13122.0
6	2, 3	32, 122	77.0	4050.0
7	2, 4	32, 147	89.5	6612.5
8	2, 5	32, 173	102.5	9940.5
9	2, 6	32, 186	109.0	11858.0
10	3, 4	122, 147	134.5	312.5
11	3, 5	122, 173	147.5	1300.5
12	3, 6	122, 186	154.0	2048.0
13	4, 5	147, 173	160.0	338.0
14	4, 6	147, 186	166.5	760.5
15	5, 6	173, 186	179.5	84.5
			1710.0	73926.0

Note: $1710 \div 15 = 114 = \bar{X}$

and $73926.0 \div 15 = 4928.4 = S^2$

The means of these samples vary from 28 to 179.5. All of these samples will appear equally often if the sampling is repeated indefinitely. The average value of the fifteen possible sample means is 114, exactly the value of $\bar{X}$, so that, in this case, the sample mean, $\bar{x}$ gives an unbiased estimate of the population mean, $\bar{X}$. Similarly, the sample variance, s^2, gives an unbiased estimate for the population variance, S^2.

The standard error of $\bar{x}$ is calculated from the formula

$$\text{standard error of } \bar{x} = \sqrt{(1-f)\frac{S^2}{n}}$$

where f is the sampling fraction, S^2 is the population variance and n is the sample size, so that here

$$\text{standard error of } \bar{x} = \sqrt{(1 - \tfrac{1}{3}) \times 4928.4 \div 2}$$

$$= 40.53$$

This example is used again below to develop and illustrate various sampling principles.

STRATIFIED SAMPLES

It is relatively rare to have no knowledge at all about the distribution of the values of an interesting variable in a population. For example, the prevalence or severity of infestation by a parasite will often depend on climate, humid areas probably being more hospitable to the parasite than dry regions. Management methods might be related to the size of an establishment implying that many health and production variables will also depend, to some extent, on the number of animals in a herd. Some information on the size of different herds may already be available in the results of a recent census, in tax records, etc. Constructive use of such prior knowledge can often increase, at no extra cost, the value of the information obtained by a sample. Let us return to the numerical example. Suppose it is known, a priori, that farms 1 and 2 are rather smaller than the others. It could then be argued that the population really consists of two subpopulations, or *strata*. One stratum consists of the two smaller farms, the other of the four larger farms. A simple random sample ignores this structure and from Table 1 it can be seen that samples 1 and 10-15 contain farms from only one stratum, with the obvious effect. Sample 1 seriously underestimates the mean size of herd while samples 10-15, and especially samples 13-15, give gross overestimates. It seems reasonable to suggest that samples which have observations from both strata should give better estimates, on average, than those where both units are sampled from the same stratum. This is borne out by Table 3.

Table 3. Stratified sample

Sample	Farms in sample	Ordinary sample mean ($\bar{x}$)	Stratified mean ($\bar{x}_{st}$)
1	1, 3	73.0	89.3
2	1, 4	85.5	106.0
3	1, 5	98.5	123.3
4	1, 6	105.0	132.0
5	2, 3	77.0	92.0
6	2, 4	89.5	108.7
7	2, 5	102.5	126.0
8	2, 6	109.0	134.7
		740.0	912.0
		740.0 ÷ 8 = 92.5	912 ÷ 8 = 114

A sample of size 2 is again selected from the population but with the restriction that one unit has to be chosen from each stratum. As a result only eight different samples are possible. The means of these samples appear

in column three and it can be seen that the ordinary sample mean now always underestimates the population mean. The complete population consists of the same six farms as before so that the overall mean herd size is still 114. However, the mean value of the eight sample means is 92.5, so that the sample mean is now seriously biased (because of the restriction imposed in the sampling method).

It should not be difficult to see why this has occurred. Farms 1 and 2 each appear in four possible samples while the others only appear in two samples each. In other words, because of the different numbers of units (farms) in the two strata, farms in the small farm stratum are more likely to appear inthe sample than those in the large farm stratum - twice as likely, in fact. Each small farm therefore has twice the influence or weight of each large farm in determining the value of the sample mean. This can be compensated by giving the large farm sampled more weight (twice as much) in the estimation of the population mean. In general, when the mean, $\bar{x}_{st}$, of a stratified sample is calculated each observation should be multiplied by a weight proportional to the size of the stratum from which it comes. In the example the small farms stratum comprises $\frac{1}{3}$ and the large farm stratum $\frac{2}{3}$ of the population and for the sample containing Farm 1 and Farm 3 the stratified mean is

$$\bar{x}_{st} = \frac{1}{3} \times 24 + \frac{2}{3} \times 122 = 89.3 \quad .$$

The values of $\bar{x}_{st}$ for all eight samples are given in the final column of Table 3. The mean value of these eight estimates is 114 so that $\bar{x}_{st}$ gives an unbiased estimate of the population mean.

We have seen that the ordinary sample mean, $\bar{x}$, from a simple random sample and the stratified mean, $\bar{x}_{st}$, both provide unbiased estimates of the mean size of herd in the population of farms. However, $\bar{x}$ takes values in the range 28.0 to 179.5 while $\bar{x}_{st}$ always lies between 89.3 and 134.7. It is clear that $\bar{x}_{st}$ is a more precise estimator than $\bar{x}$ and will usually give a more accurate estimate. The values of the corresponding standard errors confirm this. The standard error of $\bar{x}$ has been calculated above as 40.53 while the standard error of $\bar{x}_{st}$ is only 16.5. Exploiting the stratification has resulted in a tremendous gain in precision. There are various questions one might ask at this point. Will the stratified samples *always* lead to higher precision? Are there any other benefits to be gained? Does stratification have any disadvantages?

The answer to the first question is in the affirmative. Although technically it can be shown, especially if some of the population strata contain only a few units, that it is possible for a stratified sample to be less efficient than a simple random sample of the same total size, it is difficult to imagine circumstances under which it could happen in practice. However, the gain in precision may be substantial or trival depending on the characteristics of the population and on the relevance of the criterion used to stratify it. If the stratification criterion is only loosely related to the variable being measured, then it will cause little, or no, increase in precision. In the example of Table 3 the variable of interest

was "size of herd" and the farms were stratified into those with small herds (32 or less) and those with large herds (122 or greater). The average herd sizes of the two strata are 28 and 157, a difference of 129. This is much greater than the difference between any two herds *within* a stratum. The efficiency of a stratification will be higher the greater are the differences between the average values of different strata and the more similar are the values within the strata. A statistician would say that a successful stratification is one which achieves a between-stratum variance which is large relative to the within-stratum variance.

In the example, it was easy to construct efficient strata because the values were already known for the whole population! In practice, stratification will be carried out based on the value of some criterion other than the variable(s) being measured in the survey. In a survey intended to study management methods it might be sensible to stratify farms by size, but the current size of herds will not usually be known. However, a recent census would give a measure of size which might be quite accurate. At least, the very small herds will be likely to have remained small and the very large herds to be still large. If differences in management methods are related to differences in size and if the relative sizes of herds have remained fairly constant then such a stratification is likely to improve the efficiency of the survey. Similar considerations may arise in a health survey which, among other aims, is designed to study the prevalence of parasite borne diseases. The local prevalence of such diseases will be dependent on a variety of ecological and climatic factors. It may be important to know, for example, whether a farm is close to a small lake, what kind of vegetation grows on its land, and so on. The sampling frame from which the farms are chosen is unlikely to provide such information. Nevertheless, it may be the case that the survey area can be divided a priori into geographical regions which have general differences in climate or ecology likely to be associated with different prevalence levels of the parasites being studied. Provided most of the sampled farms have the characteristics expected from their geographical location and if it is true that the level of parasite activity is related to those conditions, then again the stratification will have had the desired effect.

When stratification is based on incomplete information anomalies can arise. Suppose farms are stratified into size groups (using a recent census) 1-20 animals, 21-50, 51-100, etc. A farm which had only 11 animals will be sampled in the 1-20 stratum but might now have 92 animals. Provided this kind of change does not happen too frequently it need not cause serious worry, although it will certainly reduce the efficiency of the stratification. However, it is important to handle such data carefully in the analysis. Although the farm above *now* has 92 animals it was selected as part of the stratum containing those farms with 1-20 animals at the time of the previous census and its historical status has not changed. When a population parameter such as a prevalence or the mean of some production variable is being estimated, together with its standard error, the data from each farm must be analysed in the stratum in which it was selected. There is a simple reason for this. In the example of Table 3 the ordinary sample mean was biased and $\bar{x}_{st}$, the stratified mean, was shown to give a more appropriate estimate. However, $\bar{x}_{st}$ is a weighted mean; each data value has to be multiplied by a factor equal to the proportion of units in the complete population which belong to the stratum in which the value was observed. If the weights used are not the correct ones, that is, if the relative sizes of the strata are

not known exactly then any estimates based on the stratified sample will be biased. In large samples this bias can mean that the stratified estimates are less accurate than those obtained from a simple random sample and all the benefits of the stratification will be lost. Furthermore, the standard errors which are calculated in the presence of such bias will understate the true error in the estimates.

The proportion of farms which fell into the 1-20 stratum at the time of the census will be known and the farm which now has 92 animals belonged to the 1-20 stratum *at that time*. The estimation of current population values should be carried out placing each farm in its correct historical stratum. The situation is different when the survey data are being analysed to look for relationships between variables. If size of farm is to be cross-tabulated or correlated with some other variable then the *actual* size observed in the survey should be used even when that is different from the historical size used for stratification. The same applies when a table is collated to show the distribution of farm size in the sample. If there have been more than a few instances of the value of the stratification variable being very different from that expected, the help of a professional statistician will be essential for the interpretation of the data.

Increased precision in estimates for the whole population is not the only motive for stratification; it may not even be the main one. Frequently the survey designer will have a special interest in specific subsections of the population. If the population can be stratified in such a way that each subpopulation (or *domain*) of interest coincides with a stratum this will be beneficial in at least two ways. First, the estimates for each subpopulation will be more efficient than if it crossed over several strata and second, it will be possible to judge, before carrying out the survey, whether enough data will be obtained in each subpopulation to enable reliable estimates to be obtained in it. This brings us to the general question of how many units to sample in each stratum.

Allocation of sample over strata

If the principle motivation for a stratification is to improve the precision of overall population estimates then it is known how the total sample should be distributed across the different strata. (Discussion of total sample size is left to the relevant section below.)

A common scheme is that of *proportional allocation*; if, say, one-fifth of the population units belong to a particular stratum then one-fifth of the sample is allocated to that stratum, and so on. Such a scheme is intuitively appealing and will often given good results. However, there are various features of the population and the strata which should be considered before opting for proportional allocation.

If a stratum contains only a small proportion of the population units then a simple proportional allocation might result in very few units being sampled in that stratum. Estimated values for the stratum will then have relatively large standard errors. It may be necessary to employ a greater sampling intensity in some of the smaller strata to obtain sufficient precision for the estimate in those strata. Furthermore, even if estimates for the whole population are the primary reason for the survey, proportional allocation will maximise precision only if the variability within all the strata is about the same. Often this will not be true. When strata are defined according to some

measure of size it is common to discover that the stratum containing the largest units exhibits more variability than the other strata. This is most likely to occur when there are a few very large units in the population. For example, if size of herd is used as the stratification criterion, the maximum difference in the stratum 101-200 animals is 99 while in the stratum containing farms with, say, more than 1000 animals, the difference between the smallest and largest herd will probably be several hundred and perhaps several thousand. To obtain maximum efficiency in population estimates the more variable strata should be more intensively sampled than the less variable strata. Of course, the variability that matters here is that of the quantities being measured in the survey: milk yields, herd size, disease status, etc. The stratum which is most variable with respect to one of these may be the least variable with respect to another so that, unless one of the quantities under study is clearly viewed as more important than the others, there may be unreasonable conflicts and something like proportional allocation will often provide a sensible compromise. In any case, there may not be much information about variability prior to the survey and it will certainly not be worth setting up a pilot study just to obtain the information required to make the optimum allocation.

Cost considerations also enter. Even if two strata are the same size and equally variable it may be more expensive to obtain data in one rather than the other. An efficient sampling scheme will take fewer observations in strata which are costly to sample (unless, of course, there are special reasons for wanting precise estimates *within* those strata) since to do otherwise will reduce the overall size of the sample that can be obtained with a fixed budget.

It can be shown that the allocation of a sample into the various strata which will give the greatest precision for estimates over the whole population (thought it may provide poor estimates in some of the individual strata) is to sample in each stratum a number of units proportional to the size of the stratum, proportional to the standard deviation in that stratum of the variable of primary interest and inversely proportional to the square root of the unit cost of sampling each unit in that stratum.

Number of strata

How many strata should be established? Again, the answer depends on whether the strata themselves are important domains or subpopulations of interest in which case the answer may be obvious. However, every increase in the number of strata will involve extra costs in planning and fieldwork which may restrict the options available.

If improvement in the precision of overall population estimates is the chief motivation for stratification there are good reasons to believe (Cochran, 1977, chapter 5A) that it is rarely worth having more than five or six strata and that frequently much of the benefit can be obtained using just two or three.

CLUSTER SAMPLES

It is quite common for survey practitioners to stratify the sampling units according to their geographical location; for example, a country may be divided into states and farms classified by the state into which they fall. If some farms are sampled in *every* state, then the outcome will be a stratified sample with as many strata as states. Frequently, however, the

motivation for geographical stratification is different from this. A simple random sample of all the farms in the country would result in a wide geographical scatter of the selected farms. This in turn would mean that interviewers or veterinary field teams would have to travel long distances to examine perhaps just a single farm. Each individual piece of information will then be expensive and the possible size of sample severely restricted on grounds of cost. It may therefore seem reasonable to restrict the study to just a few of the geographical strata leaving the others completely unsampled. Such a sampling strategy, where only a few of the strata are included, is usually called a cluster sample.

Suppose, in our example, that the six farms belong to three different regions,

Region 1	Farm 1 and Farm 2
Region 2	Farm 3 and Farm 4
Region 3	Farm 5 and Farm 6

Suppose that two regions are chosen at random and the two farms in that region then become the sample. (Conventionally, each of the three groups of farms would be referred to as a *cluster*.) Only three different samples are now possible - see Table 4.

Table 4. Cluster sample

(a) One cluster

Sample	Farms in sample	Sample mean ($\bar{x}$)
1	1, 2	28.0
2	3, 4	134.5
3	5, 6	179.5
		342.0

(b) Two clusters

Regions	Farms in sample	Sample mean ($\bar{x}$)
1, 2	1, 2, 3, 4	81.25
1, 3	1, 2, 5, 6	103.75
2, 3	3, 4, 5, 6	157.00
		342.00
	342 ÷ 3 = 114	

The average value of the sample means is 114 so that the ordinary sample mean is an unbiased estimate of the population mean in this case. (That would not be true if the clusters contained different numbers of farms. In that case a weighted mean would then be required.) However, the standard error of the mean is now 63.53, greater than the standard error of the mean from the simple random sample. Selecting a sample in clusters will usually give less precise estimates than would be obtained by a simple random sample of the

same size.* The extent of loss of precision will depend on how different are the characteristics of the individual clusters, but Leech and Sellers (1979) give examples showing that it is possible that estimates of prevalence from a cluster sample may have twice the standard error obtained by a simple random sample of the same size. In the present example, one of the clusters contains the two smallest farms and another contains the two largest. Thus, of the three possible cluster samples, each containing two farms, one of them greatly underestimates, and another seriously overestimates, the population mean.

If cluster samples are less precise than simple random samples, why should they ever be considered? Well, usually a cluster sample will be collected at much less cost than a simple random sample of the same size; reductions in the amount of travelling and the length of time spent in the field are often dramatic. As a result, the choice is not usually between a cluster sample and a simple random sample of the same size. For a given cost it will usually be possible to include substantially more units (farms in this example) than would be possible if they are selected completely at random. If, by restricting the choice of farms in clusters, we were able to choose two clusters comprising a total of four farms then the possible samples would be as in Table 4(b). The standard error of the estimate of the population mean would then be 31.76, smaller than that for a random sample of two farms although to collect the data from a simple random sample of two will usually mean travelling to two different regions anyway. Furthermore, simple random sampling requires more prior knowledge than cluster sampling, knowledge which may not be available. To choose a simple random sample it is necessary to have a sampling frame which is sufficiently accurate and detailed to enable individual sampling units to be chosen with equal probability. Most animal health measures are calculated on a per animal basis. A prevalence, for example, is the proportion of individual animals which suffer from a given disease. Unless there is available a register or a complete list of some kind of all those animals at risk then it will not be possible to select a simple random sample. Such a list will rarely be available. Usually the best that can be done is to select a sample of farms or villages and each of these primary units will contain a cluster of animals. However, even this would require that a list of farms be available. If there is no such list then it would still be possible to divide the region to be sampled into smallish subregions, select some of these and enumerate the farms only in the selected subregions. A sample of farms could then be chosen from all the farms in those subregions. Such *multistage samples* are common in animal health surveys and will be discussed further below.

Defining clusters

It is more difficult to give clear guidelines here than it was in the discussion of stratification criteria. The situation will vary depending on the amount of prior information available and on the number of sampling stages introduced. In general, the more similar the clusters are the better. (Contrast this with the case of stratification where most gains are made when the strata are quite different from one another.) In terms of a survey to estimate prevalence, say, in which farms are the primary units, this will mean that the survey will give estimates with optimum precision if all farms

* Raj (1972) gives an example where cluster sampling has actually improved precision. This can only happen when the values of the variable being studied are *negatively* correlated within a cluster, a circumstance which will very rarely occur in practice.

have about the same number of animals and the prevalence does not vary much from one farm to another. Neither is likely to be true. If the sampling had a prior stage of dividing the region of interest into subregions of which only a few are to be selected then the best division would be one which placed about the same number of farms in each subregion in such a way that the overall prevalence in each subregion was about the same. It will rarely be possible to achieve this. In general it will be necessary to accept clusters already defined: farms, administrative regions, etc. That these may not have "good" statistical properties just has to be lived with. Multistage sampling using different kinds of clusters will often be the only option available. Even if simple random sampling of the ultimate units (animals, say) is possible it is often or usually the case that the cheaper per unit cost of cluster sampling allows a total sample to be selected whose increased size more than compensates for the loss of precision induced by clustering.

Selecting clusters

Once the number of stages has been determined and the clusters defined at each stage there are two further problems to be addressed. How many clusters should be sampled at each stage and how should they be selected? The first of these questions will be dealt with in the two sections which follow. There are two common methods of selecting a sample of clusters at each stage.

The first is to select clusters *with replacement* such that each cluster has an equal chance of being selected. This sampling method could be carried out as follows. At each stage write the name (or other identifier) of each cluster on a plastic disc. Shake the discs in a box and choose one at random. Note which cluster has been chosen. *Return the disc to the box* and repeat the process until the required number of draws has been made. Of course, by this method, a sample of five draws might contain five different clusters or it might contain only four clusters, one of which has been drawn twice, and so on. This might seem to be a strange procedure. You may feel that it cannot be efficient to use the same cluster twice instead of using two different clusters. In a strict sense, you would be correct. The problem is that if the selection is made *without* replacement the estimates and their standard errors become much more difficult to calculate. Even with a computer available, the time required to program and carry out the calculations can be prohibitive unless the total number of clusters from which the sample is being drawn is small at every stage.

A second common way of selecting clusters is to choose each cluster with *probability proportional to its size*, again with replacement. This process is easily described. Suppose there were three herds containing 100, 50 and 20 animals precisely. We could think of this as a total of 170 animals of which numbers 1-100 belong to the first herd, 101-150 to the second and 151-170 to the third. Now choose a random number between 1 and 170. If it lies in the range 1-100 choose the first herd. If it is a number between 101 and 150 choose the second, etc. Then repeat the process as often as required. Again it is perfectly possible for the same herd to be chosen more than once but selection of herds without replacement has exactly the same disadvantage as in the previous case. If good information is available on the size of clusters and if the distribution in a cluster of the variable under study is associated with size of the cluster then sampling with probability proportional to size can improve the precision of estimates.

One consequence of the variety of designs that are possible in cluster sampling is that there are many different ways in which the data can be

combined to estimate population means and proportions. A discussion of this can be found in any of the texts mentioned in the introduction. It is good practice to seek the advice and help of an experienced survey statistician before carrying out any survey. This is even more true when some form of cluster sampling is to be used.

SAMPLE SIZE

The optimum sample size for a survey will depend on a number of factors, only some of which are under the control of the surveyor. A statistician who is asked, "How big should my sample be?", will respond with a series of questions.

What is the smallest population you wish to study?

The point here is that, although a survey may be intended to cover a whole country, the surveyor may want reliable estimates not just on a countrywide basis but also for specific subregions. In that case the subsamples within those subregions must be chosen large enough to give sufficiently precise local estimates. Once that has been taken care of it will usually be found that the total sample is then more than large enough to give acceptable estimates at the national level. Furthermore, a careful identification of subregions of interest will help to determine a sensible structure for the sampling plan.

What precision do you want for each estimate?

It is important to consider this carefully. A demand for unrealistically high precision will lead to the planned survey needing such large resources for its completion that it will probably never take place. It will be necessary here to distinguish between absolute and relative accuracy, especially where the quantity to be estimated is a proportion. Statisticians are accustomed to thinking in terms of absolute accuracy. A statement to the effect that you "would like to estimate a prevalence within 5%" may be interpreted to mean that if the true prevalence were 11% you would be content with an estimate in the range 6% (11% - 5%) to 16% (11% + 5%)! On the other hand, the same prevalence estimated to a *relative* accuracy of 5% would give an estimate in the range 10.88% to 11.12%, a very high degree of accuracy which would require a large sample. To estimate very small prevalences to high relative accuracy will need very large samples. Leech and Sellers (1979) give a useful rule of thumb when the estimation is to be *based on simple random samples* and an estimate, with high relative accuracy, is required of a low (i.e. less than 10%) prevalence. If the true prevalence is p% then a sample of 400 ÷ p will give a "reasonably accurate" and 900 ÷ p a "very accurate" estimate. For a prevalence of around 2% it would then require simple random samples of 200 and 450 respectively to reach these levels of accuracy. However, it was pointed out earlier that cluster sampling will cause estimates to be less precise for a given size of sample. Leech and Sellers suggest that the sample size estimated for a simple random sample should be multiplied by 4 if cluster sampling is used. The above samples would then need to be increased to 800 and 1800 respectively. Even this will be an underestimate if sampling is done by herd or farm, many herds are large and the disease being surveyed is highly contagious.

What answer do you expect?

This may seem a strange question. The survey has not been done yet so how can the answer be known? Yet, as we have just seen above, the sample size for

estimating a prevalence depends on the *true* prevalence. If a surveyor is unwilling to guess the range inside which this value is likely to be found it will not be possible to suggest a sample size which will provide the accuracy he requires. If the variable being studied is not a proportion but a quantity such as milk yield, live weight, etc, then a different kind of information will be required to enable the sample size to be determined. Now the statistician will ask about *variability* within and between herds, between different parts of the country, and so on. If a previous survey has been done in which the same variable was measured, the results of that could be used to extract the required information. Otherwise it will be impossible, with any degree of confidence, to suggest a sample size to achieve a required level of accuracy.

Are you interested in estimation or detection?

Following on a campaign to eradicate some disease a survey may be carried out to see if eradication has been achieved. For any given herd size it is possible to calculate how many animals need to be tested to, say, give 95% confidence that the disease will be discovered in at least one animal given a certain prevalence. However, such calculations need to be interpreted with care. Firstly, they do not usually take account of the sensitivity of the test method used to detect the disease in individual animals. If the test is not 100% effective then it is possible to miss the disease in a herd even if all the animals are checked. Secondly, if the survey is to be carried out on a regional basis then applying the formula at the herd level will lead to an unacceptibly high total sample size. Lastly, if the disease is present an estimate of the current prevalence will almost certainly be required and the sample should be chosen to give an efficient estimate.

What resources do you have?

Experience shows that this is usually the major determinant of sample size. Researchers planning a survey tend to be sanguine about the degree of precision which can be achieved. Frequently, when the sample size is determined approximately, based on the precision the researcher demands initially, it is discovered that he does not have sufficient resources available. The question then becomes one of designing the sample in such a way as to obtain the best results possible for a predetermined cost. It is important to enlist a statistician to help with this, if possible. In particular he should be able to make a rough calculation of the best precision you can hope for given the resources you have. It may then become apparent that there is no point in proceeding unless extra funds can be obtained.

The size of the sample

It can be seen, then, that a great deal of information is required before a sample size can be calculated for a given level of precision to be obtained. Even if all the information is available it is not possible to calculate a sample size which will *guarantee* a given precision (short of sampling virtually the whole population). A sample size would be offered which would have a such and such chance (often 95% chance) of giving sufficiently precise estimates. If the level of security chosen always were 95% then this would mean that, if all surveys were carried out with the "ideal" sample size the estimates would not have the required accuracy in one survey in every twenty (5%). Even this presupposes that there are no problems with data quality, that all tests are 100% sensitive and 100% specific, that all the farmers co-operate, that it is possible to reach all the farms chosen in the sample, that there are no errors

in the sampling frames, and so on. At best, questions about sample size can be answered only approximately. In the end, the usefulness of the results of a survey will depend on a good design of sample and on the care with which data is collected and analysed. Failure to get those right will have much more serious effects than having a sample size which is slightly smaller than one would like it to be. Besides, as already indicated, cost considerations are likely to restrict the total sample size below some ideal, theoretical optimum.

COMPLEX SURVEY DESIGNS

It is common for surveys to combine aspects of all the types of sampling discussed earlier. For example, a country might be stratified into three regions with differing climate and ecology. Within each stratum a sample of administrative districts (first stage clusters) might be chosen with probability proportional to the total number of farms in each district. Within each sampled district a sample of farms (stage 2 clusters) is chosen, perhaps by simple random sampling, and then a sample of animals (stage 3) is chosen in each farm. A particular animal health survey may be more or less complex than this but most will share some of its features.

Sampling intensities at different stages

If the major objective of a survey is to obtain estimates of prevalences or mean values of some other variables for the whole population there will be a continual conflict between the sampling intensities which give higher precision and those which give lower cost. The more units sampled in the earlier stages the more precise will be the population estimates. However, it is usually these earlier stage units that are most costly to sample. Increasing the number of administrative districts may mean setting up extra field team organisations in different parts of the country. Choosing extra farms within a given area will increase the time the field teams spend travelling. The cheapest way by far to increase the sample size is to select more animals from each farm already in the sample but this will have much less effect on the precision of population estimates than including more farms or more areas.

It will, therefore, rarely be efficient to choose large samples of animals at individual farms if the motive for that is simply to increase the sample size, unless this can be done without reducing the number of farms the field teams could visit. Once a whole day's sampling of animals has been carried out in a given farm then only for very special reasons should a second or third day be spent there. The extra days would be better spent visiting another farm. It is generally unsound practice to insist on sampling the same proportion of each herd if the herds are of very different sizes. A sampling plan which samples from each herd the number of animals which can be handled in one or two days will almost always be more efficient than one which spends several days in every large herd looking at more animals from each herd.

Systematic samples

Unless fairly complete records are kept by a farmer it will be difficult to select a simple random sample of animals. Even if such records are available it may be necessary to round up all the animals anyway to find those selected in the sample. Some form of systematic sampling is usually quicker and may even give better results than random sampling.

Suppose a survey of large beef producing farms is to be carried out and it is decided to select 80 animals from each farm. An owner states he has about 700 animals. If the herd is rounded up and passed through a crush and every ninth* animal selected the eventual sample will be about 80 animals. If the final number is not exactly 80 that will not matter. All the selected animals should be used even if there are a few more or less than expected (perhaps because the owner has mis-stated the size of his herd).

This kind of systematic sample will frequently have an age and sex structure which reflects that of the herd as a whole and will give quite precise estimates of herd characteristics. In the analysis of the survey the herd data should be treated as though it had been collected as a simple random sample.

Self weighting designs

When survey results had to be analysed by hand it was important to design complex surveys in a way which would reduce the amount and complexity of calculations required. This no longer has the same importance given the wider availability of electronic computing facilities and the major consideration should now be to give clear priority to the reduction of sampling costs. However, if the intention is to carry out a complex survey and analyse the results by computer it may be wise to consult a statistician familiar with statistical computing techniques *before* collecting the data.

DISCUSSION

This paper has set out to introduce the terminology, principles and methods of sampling for animal health surveys. It has been shown that stratified samples can give more efficient estimates than simple random samples of the same size and that, while the opposite is true for cluster samples, the improved use of resources can allow such an increase in the total sample size that cluster sampling will frequently be highly efficient. It has been argued that there is no "magic" sample size which will be so much better than any other and that the appropriate sample size will frequently be determined by economic rather than statistical considerations.

It has been pointed out repeatedly that a good management structure, well-trained and highly motivated personnel and the collection of good quality data matter more than the choice of an optimum sampling plan. Provided the individual data values are unambiguous and accurate a statistician ought to be able to salvage something worthwhile from any but the most inappropriate survey design though, justifiably, he may be annoyed at having to perform a rescue act when his advice could have prevented problems arising in the first place. If the data have been collected carelessly or recorded inaccurately then no amount of statistical analysis can save the situation.

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* A random number should be chosen between 1 and 9. Suppose it is 5. Then fifth, fourteenth, twenty-third, etc animal would be selected.

REFERENCES

Cochran, W.G. (1977). Sampling Techniques. John Wiley & Sons, London, 428 p.

Leech, F.B. and Sellers, K.C. (1979). Statistical Epidemiology in Veterinary Science. Charles Griffin, London, 158 p.

Moser, C. and Kalton, G. (1971). Survey Methods in Social Investigation. Heinemann, London, 555 p.

Raj, D. (1972). The Design of Sample Surveys. McGraw-Hill, New York, 390 p.

Yates, F. (1981). Sampling Methods for Censuses and Surveys. Charles Griffin, London, 458 p.

Zarkovic, S.S. (1966). Quality of Statistical Data. FAO, Rome, 213 p.

EQUINE EPIDEMIOLOGY AND PREVENTIVE MEDICINE

PREVENTIVE MEDICINE IN STUD AND STABLE

J. M. OWEN*

In 1975 the report on the Veterinary Profession by the Swan Committee made only one recommendation concerning veterinarians in equine practice. They were advised 'to make use of their qualifications by means of preventive medicine and the involvement of lay personnel'.

This puzzled veterinary surgeons in horse practice. The use of tetanus antiserum and vaccine have been widespread for many years. The avoidance of gastroenteric disorders by regular worming and the care of teeth have long been part of our work and more recently the vaccination of horses against flu and the swabbing of mares and stallions for venereal infections have become commonplace. All of these have required a commitment from owners and handlers.

The first reference to preventive medicine in the horse was made by the Greek, Xenophon in 390 B.C. (1962). He suggests how foot problems can be avoided by attention to the composition and drainage of the stable floor. Also, amongst many very useful comments, he states how horses should be fed to avoid laminitis.

Horsemen have always recognised that the maintenance of healthy feet is of paramount importance. By the time of Nero, pack mules in Rome were being shod with a canvas sock - a solea - which had an iron sole. Nero's wife Poppaea had her mules shod with gold. Permanent shoes as we know them are first mentioned in the literature as being used by the Romans in the 6th Century. Perhaps these became a necessity because Roman horses were being used on the excellent Roman roads.

In more recent times the Duke of Newcastle in his book 'A General System of Horsemanship' published in 1630 gives advice on the avoidance of skeletal problems by careful feeding. Also, he states the need for rotational grazing of pastures and recommends the types of management required to avoid low conception rates on studs.

We may conveniently consider preventive medicine today under seven headings - Nutrition, Parasites, Infectious Diseases, Reproduction, The Foal, Respiration and Locomotion.

NUTRITION

Preventive medicine in equine nutrition revolves almost entirely around the care of horse's teeth and the prevention of overfeeding. Horses need to grind their food well because, unlike ruminants, they don't get a second

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chance. Few horses have perfectly opposed teeth and the outer borders of the upper molars and the inner borders of the lower ones can become very sharp as a result. This can lead to poor grinding of food, inefficient digestion and colic. Also, sharp teeth can cut the inside of the horse's mouth when the horse is bridled and ridden. It is common, therefore, for teeth to be routinely examined and rasped if necessary.

Generally speaking, horses suffer from overfeeding rather than the opposite. As pets rather than economic units they reflect the over-indulgence of their owners.

In the foal, overfeeding and under-exercise may lead to so-called contracted tendons (Owen, 1975) as well as epiphisitis. In the adult animal laminitis, azoturea and 'filling' of the legs may all be the result of over-feeding. Fat, inactive mares often prove difficult to get in-foal.

Horses are more healthy and perform better when fit and lean.

Grass sickness occurs sporadically but in well recognised locations (Gilmour & Jolly, 1974). It is suggested that some farmers are able to avoid the disease in their horses by not allowing them to graze in certain fields.

PARASITES

The major parasite of horses and ponies is Strongylus vulgaris or 'red worm'. Generally speaking the horse owning public are well aware of the dangers of red worm infestation and the need to worm their charges. However, owners often fail to understand that only incomplete elimination of the larval forms of Strongylus species is obtained by the majority of anthelmintics. Also, the ease of re-infestation and, therefore, the need for frequent methodical treatment is not always understood.

Some owners still believe that anthelmintics are not really effective unless given through a stomach tube. Some veterinary surgeons help prolong that belief.

Most stud farms attempt to reduce the problem by frequent worming, by the picking up and removal of faeces from the paddocks and by the rotational grazing of pastures with sheep or cattle.

Our practice encourages owners to worm foals once a month for the first year or more and thereafter at least four times a year. Equine anthelmintics are available in saddlers shops, veterinary chemists and feed merchants as well as from veterinary surgeons.

Lungworm - Dietyocaulus arnfeldi - is more common in donkeys than horses (Round, 1976). Horses grazing with infected donkeys may have a high incidence of infection without signs of clinical disease. It is however generally considered sensible to avoid grazing horses with donkeys unless the donkeys are believed to be free of infection.

Worming programmes designed for the control of red worms are normally effective against the other two main worm problems in young horses; Strongiloides westeri - which causes diorrhoea and debilitation in foals - and Parascuris equorum - which may be found in animals up to 3 years of age.

INFECTIOUS DISEASES

Apart from diseases of the reproductive and respiratory system, there are few important infectious diseases of horses in this country. The need to give tetanus antiserum to unvaccinated horses with deep wounds is widely understood by owners. Many more horses are now vaccinated against tetanus because vaccines that combine 'flu and tetanus immunisation are available. This is an extra benefit derived from the vaccination programme against equine influenza.

Skin diseases are more of a problem. Mud fever, cracked heels and rain-scald, caused by Dermatophilus and staphylococcal species of bacteria can each be a debilitating condition. Prevention by good stable hygiene, cleanliness and barrier creams is recommended but is not always successful.

The prevention of infection by ringworm requires great care and good stable management.

REPRODUCTION

The increasing value of bloodstock makes preventive medicine in reproduction of the greatest importance.

Three hundred years ago the Duke of Newcastle recommended that a stallion's 'staff and stones' be cleansed with a good white wine and dried with a silk handerchief.

The sex life of a top class Thoroughbred mare makes 'Dallas' seem like a Sunday school outing; sharing in one year the male with forty or more females, she seldom returns to the same one two years running. Add to that the movement of stallions and mares from country to country and continent to continent and one can only be surprised that venereal infections are not more common.

The three major venereal problems are caused by:

1. Haemophilus equigenitalis - the C.E.M. organism
2. Klebsiella aerogenes types 1 and 5
3. Pseudamonas aeroginosa

The routine swabbing of mares and stallions to screen them for infection began in this country in the early 1960's. Most mares are now swabbed at least once a season if going to stud. The early type of metal speculum used for examinations and swabbing undoubtedly aided the spread of infection. They were dipped in diluted disinfectant for a few moments between mares but Klebsiella and Pseudamonas are both very difficult organisms to kill.

The development of the sterilisable re-usable plastic vaginascope was a great step forward allowing each mare to be examined with a sterile instrument.

Originally the cervix and uterus only were swabbed. Since the outbreak of C.E.M. in 1977, it has been recognised that pathogens may also be found at the urethral orifice as well as the clitoral sinuses and fossa of the mare. Another development of the C.E.M. outbreak is that many routine swabs are now put into a transport medium, thereby avoiding drying out of swabs on hot days.

Mini swabs are required for the clitoral sinuses because of their small openings. Normal sized swabs are still used in the cervix and uterus. In general, mares are only swabbed once a season for venereal infection unless an outbreak is suspected.

THE FOAL

The majority of foals have a veterinary examination within a few hours of birth, at which they are given tetanus antiserum and often antibiotic injections to cover them for their first days.

Retained meconium, particularly in the overdue colt foal, is a common problem. Prophylactic doses of oil by mouth are frequently given as routine to foals.

Haemolytic disease of the newborn, iso-immunisation, is found only in man and equidae (Rossdale & Ricketts, 1980). Many studs test the blood of firstborn foals against the colostrum or serum of the dam for signs of haemolysis. If the condition does occur, subsequent foals are muzzled for the first 36 hours and given colostrum from other mares.

Mares often 'run' their milk before foaling and thereby reduce the value of their colostrum. Kits are available to check the immunoglobulin levels of the foal's blood. Foals that are deficient in antibodies can be given antibiotic cover or serum from their dams.

Vaccinations against 'flu and tetanus are not given until the foal is 5 or 6 months of age, by which time maternal antibodies will have disappeared.

RESPIRATORY DISEASE

Compulsory vaccination of racehorses in the U.K. has led to the virtual elimination of equine influenza. No outbreaks have occurred since 1979 although the disease has been present in most European countries. The strict rules laid down by the Jockey Club for racehorses have been copied by horse societies, show organisers and auctioneers. Animals are given an initial course of two injections followed by a third six months later and then annually. Some horses are vaccinated more often than this.

The success of these measures is not fully appreciated by the laymen who think that the vaccination should eliminate all coughs. He also tends to blame any subsequent loss of performance, real or imaginary, on the vaccine.

Rhinopneumonitis, caused by Equine Herpes Virus Type 1, is very common and produces signs similar to those of 'flu. A good dead vaccine is not available in this country although a vaccine made to immunise mares against the strain of the virus that causes abortion has been used in vain attempts to avoid the respiratory disease.

Allergic conditions of the lung producing chronic obstructive pulmonary disease (C.O.P.D.) seem to be becoming more common in horses and ponies. The agents responsible are mainly Micropolyspora faeni and Aspergillus fumigatus (McPherson et al., 1979). These are widespread on hay and straw and, therefore, are found throughout the stable environment. Modern farm equipment allows hay to be made and cereals to be harvested in conditons that favour

the development of these moulds. The greatest care should be taken by horse owners to obtain only the best hay and straw.

LOCOMOTION

A recent wastage survey in Thoroughbreds in training shows that lameness is still the major reason for horses being 'out of work' (Rossdale et al., 1985).

Two recent innovations may help to avoid lameness in racehorses. A computerised gait analysis system is available with which it is possible to detect changes from the normal gait pattern of a horse. Such changes of limb action and hoof pressure are usually the prelude to a lameness. Another recent development is the use of thermography in which infra red cameras are used to pick up the earliest indications of inflammation.

The prevention of lameness requires good feet, good conformation and a fit animal. Feet will only remain healthy if the stable is well drained and the hooves are regularly attended to; we can do little about conformation after the horse has been purchased and, unfortunately, veterinary surgeons have little say in the training of horses. However unsatisfactory this situation may be I believe most of us will continue to obtain pleasure and satisfaction from horses and their owners.

REFERENCES

Gilmour, J. S. and Jolly, G. M. (1976). Some aspects of the epidemiology of equine grass sickness. Vet. Rec. 92, 565.

McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Breeze, R. G. and Pirie, H. M. (1979). Chronic Obstructive Pulmonary Disease (C.O.P.D.) in horses: Aetiological Studies: responses to intradermal and inhalation antigenic challenge. Equine Vet. J. 11, 159.

Owen, J. M. (1975). Abnormal flexion of the corono-pedal joint or 'contracted tendons' in unweaned foals. Equine Vet. J. 7, 40.

Rossdale, P. D., Hopes, R., Wingfield-Digby, N. J. and Offord, K. (1985). Epidemiological study of wastage among racehorses 1982 and 1983. Vet. Rec. 116, 66.

Rossdale, P. D. and Ricketts, S. W. (1980). Equine Stud Farm Medicine. Baillier Tindall (Cassell Ltd.) London 345p.

Round, M. C. (1976). Lungworm infection (D. arnfeldi) of horses and donkeys. Vet. Rec. 99, 393.

Xenophon (1962). The Art of Horsemanship. Translated by M. H. Morgan. Published by J. A. Allen & Co. Ltd., London.

RECENT STUDIES ON THE EPIDEMIOLOGY AND CONTROL OF EQUINE STRONGYLOSIS

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Herd et al. (1981) suggested that new approaches to equine strongyle control were urgently needed to overcome twin problems posed by the short egg reappearance period and the selection pressure for drug resistance. Conventional control programs are often ineffective and horses regularly treated with modern anthelmintics may be exposed to dangerous concentrations of infective larvae (L_3) on pastures. Repeated treatment at short intervals (e.g., 2 or 4 weeks) selects strongly for drug resistance, while treatment of longer intervals (e.g., 6, 8 or 12 weeks) generally fails to suppress faecal egg counts for the full interval between treatments. Veterinarians and horse owners are often unaware that a drug problem exists and continue to use drugs after they have lost their efficacy.

The formulation of rational control programmes for equine strongyles is largely dependent on a knowledge of the epidemiology of naturally occurring infection. In spite of this, relatively few epidemiologic studies have been done throughout the world and measurements of pasture infectivitiy with strongyle larvae are scanty. Epidemiologic studies have been much more common for cattle and sheep, and have led to major advances in ruminant parasite control (Anon, 1980). Unless one has a thorough understanding of the epidemiology, it is impossible to design effective parasite control programmes.

In this presentation, an attempt will be made to discuss: 1) Techniques for measuring pasture L_3 concentrations, 2) Epidemiology of equine strongylosis, and 3) Epidemiologic approaches to the control of parasites.

TECHNIQUES FOR MEASURING PASTURE L_3 CONCENTRATIONS

Most studies of concentrations of infective strongyle larvae on pastures (Duncan, 1974; Mirck, 1981; Craig et al., 1983; Herd et al., 1985) utilized the pasture sampling technique of Taylor (1931). This consists of collecting about 400 herbage samples on each of two W-shaped transects of the paddock, sometimes modified to N- or V-shaped transects. When this approach is adopted for horse pastures, it makes no allowance for the typical separation of paddocks into grazed areas of short grass (lawns) and ungrazed areas of high grass (roughs) around faeces. Although there is no regularity in the shape, position or size of the roughs and lawns, 50% or more of a field can be lost to grazing (Odberg and Francis-Smith, 1976; Archer, 1980).

Studies by Herd and Willardson (1985) indicated that the pasture sampling technique of Taylor (1931) was not appropriate for horse pastures, as it did not give an accurate measure of L_3 concentrations of herbage eaten by horses. Larval counts in the whole pasture or roughs were as much as 14-15 times higher than in the areas most grazed by horses (lawns), although all areas exhibited similar seasonal trends. Lower L_3 concentrations in lawns were attributed to the greater distance from larval reservoirs within faecal masses. The migration of L_3 from faecal mass to herbage can occur only under moist conditions and is likely to occur in waves coincident with falls

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of rain as low as 25 mm (Ogbourne, 1972, 1973; English, 1979; Craig et al., 1983). The distance of active migration may be only a few feet, but L_3 can be spread over a wider area by temporary localized flooding, hooves, farm equipment, etc.

Taylor (1954) suggested that horses have acquired an abhorrence of faeces as part of their adaptation to life in a wormy world. However, the protection afforded by an aversion to grazing near faeces may break down once conditions favour larval migration and dispersal. Although Herd and Willardson (1985) found L_3 concentrations in lawns were always less than in roughs, a 144-fold rise occurred in the lawns after late season rains. They suggested that problems might also occur earlier if horses were forced by overcrowding to graze the roughs, in spite of their natural repellance. Any horses with perverted appetites and a preference to graze the roughs would also be at greater risk.

There are a variety of techniques available for separating infective L_3 from pasture. The author has recently had considerable success with a modification of the Baermann technique (Herd, 1985a). A key feature of the modification is to conserve larval viability by processing the collected pasture within 3-4 days of collection and keeping all samples refrigerated at 4°C. Once the Baermann apparatus is set up, larvae are placed in water at 40°C instead of the usually recommended "lukewarm" temperature. The higher temperature appeared to trigger enhanced larval motility that resulted in 100% recovery.

EPIDEMIOLOGY OF EQUINE STRONGYLOSIS

A spring rise in worm egg output has been recorded in Kentucky, U.S.A. (Todd et al., 1949); Poland (Dobrowolska and Grabda, 1951); England (Poynter, 1954); Scotland (Duncan, 1974); Italy (Gencha et al., 1978); The Netherlands (Mirck, 1981) and Texas, U.S.A. (Craig et al., 1983). It has important epidemiologic implications because it occurs just prior to the period when seasonal conditions favour development of eggs to infective larvae, and it is likely to lead to massive increases in pasture infectivity in summer and/or autumn if not controlled. Summer and/or autumn peaks of infective L_3 on pasture have been observed in Scotland (Duncan, 1974); Italy (Gencha et al., 1978); Sweden (Nilsson and Andersson, 1979); The Netherlands (Mirck, 1981); Texas, U.S.A. (Craig et al., 1983) and Germany (Hasslinger and Bittner, 1984).

Recent studies in Ohio, U.S. (Herd et al., 1985) revealed two distinct spring and summer rises in strongyle egg counts with peaks in May and August/September respectively. This differed from previous studies of the spring rise where a single peak was observed, usually in July, August or September. In the Ohio study, pasture infectivity first reached high levels in June about a month after the spring rise in egg counts, then trebled in September, 2-4 weeks after the summer peak of egg output, coincident with above average rainfall in September in both 1981 and 1982. The interval between peak pasture contamination (with eggs) and peak pasture infectivity (with L_3) is likely to be short in wet conditions that favour larval migration, but delayed by dry conditions when there is inadequate moisture for migration of L_3 from faecal reservoirs to pasture.

Subsequent studies in one experiment showed that pasture L_3 counts did not fall to low levels until June of the following year when rising temperatures apparently caused enhanced larval activity, exhaustion of food reserves and death of larvae. It is obvious that the spring and summer rises in faecal egg counts can have far-reaching effects if not curbed, and horses may be exposed to potentially dangerous concentrations of pasture L_3 until the following spring. Once the rise in pasture infectivity has occurred, no amount of treatments will make it go away. Duncan (1974) and Herd et al. (1985) were in agreement that the rise in equine faecal egg

counts was seasonal in nature and unrelated to the date of foaling. There was no evidence to support the suggestion of Todd et al. (1949) that the spring rise in egg counts was linked to lowered resistance of mares following parturition and lactation.

Poynter (1954) was the first in England to record the spring rise in faecal egg counts when he observed minimal egg output in winter, a rise in spring and peak counts in August/September in ponies at the Animal Health Trust at Lanwades Park. No further studies were done in England for 30 years until Herd (1985b) measured both faecal egg counts and pasture L_3 counts at Lanwades Park in 1984. He observed a spring peak in faecal egg counts (578 epg) in late April followed by a larger summer peak (930 epg) in early September. The dry summer of 1984 delayed migration of infective L_3 from faecal masses to pasture, but concentrations of L_3 in the lawns escalated from zero in July to 18,486 L_3/kg of dry herbage on 19th September, following August and September rains. The results clearly emphasized the need to eliminate the seasonal rise in worm egg output in order to ensure safe grazing conditions.

During the course of the 1984 studies at Lanwades Park, the common cattle and sheep parasite, Trichostrongylus axei, was found to be highly prevalent (Herd, 1985b). T. axei and the cyathostomes (small strongyles) were the dominant species from August to October accounting for almost 100% of pasture infectivity in approximately equal proportions. It is possible that the T. axei infection originated from 8 head of cattle and/or some rabbits that had grazed the horse pastures in 1983, although this parasite can be transmitted by horses alone. The T. axei infection appeared to elicit a strong IgGT response and increased serum pepsinogen concentrations in susceptible ponies. The increased serum pepsinogen concentrations suggested that migrating T. axei larvae had interfered with the normal gastric physiology. It appears that T. axei may be more important in the horse than previously thought and the alternation of horse and ruminant grazing may not be as beneficial as generally believed.

EPIDEMIOLOGIC APPROACHES TO THE CONTROL OF PARASITES

Strategic treatments in spring/summer

Most equine parasite control programmes are based on regular year-round treatment with anthelmintics with little effort to tailor treatments to seasonal activities of the worm. Treatments are usually given at set intervals on the calendar, varying from 2-12 weeks. By contrast, epidemiologic control strategies involve only limited use of anthelmintics, rational timing, and reduced selection pressure for drug resistance.

Herd et. al (1981) suggested that if the spring rise in faecal egg counts was largely eliminated by a small number of prophylactic treatments early in the year, horses in northern latitudes could be protected from dangerous levels of pasture infectivity for the rest of the year. Studies by Herd et al. (1985) subsequently demonstrated the value of spring/summer treatments of adult horses for strongyle control in norther U.S.A. As few as two strategic treatments a year with oral ivermectin (Eqvalan) given in early May and early July resulted in a prolonged suppression of faecal egg counts and a six-fold reduction in pasture L_3 concentrations compared to control pastures. These two treatments also ensured a low level of residual pasture infectivity at the start of the next year's grazing season. The prolonged suppression of egg output by ivermectin (6-10 weeks) gives it an important epidemiologic edge over other anthelmintics that suppress faecal egg counts for only 4-5 weeks (Herd et al., 1981).

Cleaning horse pastures twice weekly

A non-chemical approach to equine parasite control based on removal of faeces twice weekly was evaluated at Lanwades Park in 1984 (Herd, 1985b). The rationale of this approach is that faeces are removed from paddocks before there is time for development of eggs to infective L_3 and migration of L_3 to pasture. Faeces can be collected with a mechanized paddock cleaner with a vacuum pump or some other collection mechanism, or manually by shovel.

This approach provided highly effective parasite control, superior to that achieved by anthelmintic treatments. Concentrations of infective L_3 on cleaned pasture reached a maximum of 1000 L_3/kg, compared to 18,486 L_3/kg for control pasture and 4850-10,210 L_3/kg for anthelmintic treatment groups. The low pasture L_3 counts on the cleaned pasture occurred even though the ponies in this group received no anthelmintic treatments and they had the highest mean faecal egg counts of all groups (peak 1722 epg).

The clean pasture approach has several important advantages that more than offset the cost of the labour involved: 1) It provided better parasite control than modern anthelmintic drugs, 2) It increased the grazing area by about 50% by eliminating the separation of paddocks into roughs and lawns. The entire paddock became one big lawn, 3) It eliminated the cost of repeated anthelmintic treatments and selection for drug resistance, and 4) It appears to offer an ideal control programme for both ascarid and strongyle control in susceptible weanlings and yearlings. Faeces removed from pasture are potentially dangerous if spread on other horse pastures without first composting them to allow the heat of fermentation to kill infective L_3. Pasture L_3 concentrations as high as 422,400 L_3/kg have been observed on pastures with freshly spread manure (Herd et al., 1985).

REFERENCES

Anon (1980). Grazing plans for the control of stomach and intestinal worms in sheep and in cattle. Ministry of Agriculture, Fisheries and Food, Booklet 2154, Publications, Tolcarne Drive, Pinner, Middlesex HAS 2DT.

Archer, K. (1980). Grassland management for horses. Vet. Rec. 107, 171-174.

Craig, T.M., Bowen, J.M. and Ludwig, K.G. (1983). Transmission of equine cyathostomes (Strongylidae) in central Texas. Am. J. Vet. Res. 44, 1867-1869.

Dobrowolska, D. and Grabda, E. (1951). Cykl roczny wydalania jaj nicieni u koni. Medycyna Weterynaryjna 7, 235-236.

Duncan, J.L. (1974). Field studies on the epidemiology of mixed strongyle infection in the horse. Vet. Rec. 94, 337-345.

English, A.W. (1979). The epidemiology of equine strongylosis in southern Queensland. 1. Bionomics of the free-living stages in faeces and on pasture. Aust. Vet. J. 55, 299-305.

Genchi, C., Malnati, G. and Carrara, L. (1978) Aspetti epidemiologici di nematodi gastrointestinali degli animali al pascolo. Clinica Veterinaria 101, 175-184.

Hasslinger, M.A. and Bittner, G. (1984). Zur saisondynamik der larven von pferdestrongyliden und deren beziehung zum infectionsrisiko auf der weide. Zbl. Vet. Med. B. 31, 25-31.

Herd, R.P. (1985a). Recovery of infective strongyle larvae from horse pastures. In preparation.

Herd, R.P. (1985b). Epidemiology, diagnosis and control of equine strongylosis at Newmarket. In preparation.

Herd, R.P. and Willardson, K.L. (1984). The seasonal distribution of infective strongyle larvae on horse pastures. Equine Vet. J. In press.

Herd, R.P., Miller, T.B. and Gabel, A.A. (1981). A field evaluation of probenzimidazole, benzimidazole and non-benzimidazole anthelmintics in horses. J. Am. Vet. Med. Assoc. 179, 686-691.

Herd, R.P., Willardson, K.L. and Gabel, A.A. (1984). Epidemiologic approach to the control of horse strongyles. Equine Vet. J. In press.

Mirck, M.M.H. (1981). An investigation into the epidemiology of Strongylidae infections in the horse in The Netherlands. Vet. Q. 3, 98-100.

Nilsson, O. and Andersson, T. (1979) Strongylus vulgaris hos hast - epizootologi och profylax. Svensk Veterinartidning 31, 148-156.

Odberg, F.O. and Francis-Smith, K. (1976). A study on eliminative and grazing behaviour - the use of the field by captive horses. Equine Vet. J. 8, 147-149.

Ogbourne, C.P. (1972). Observations on the free-living stages of strongylid nematodes of the horse. Parasitology 64, 461-477.

Ogbourne, C.P. (1973). Survival on herbage plots of infective larvae of strongylid nematodes of the horse. J. Helminth. 47, 9-16.

Poynter, D. (1954). Seasonal fluctuation in the number of strongyle eggs passed by horses. Vet. Rec. 66, 74-78.

Taylor, E.L. (1939). Technique for the estimation of pasture infestation by strongyloid larvae. Parasitology 31, 473-478.

Taylor, E.L. (1954). Grazing behaviour and helminthic disease. Brit. J. Anim. Behaviour 2, 61-62.

Todd, A.C., Hull, F.E., Kelly, G.W., Wyant, Z.N. and Hansen, M.F. (1949). Worm parasites in Thoroughbred mares. Bulletin 536, Kentucky Agricultural Experimental Station, University of Kentucky, Lexington, 16 pp.

RECENT STUDIES ON THE IMMUNOLOGICAL ASPECTS OF EQUINE STRONGYLOSIS

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There is a paucity of information on the immunological reactivity of horses to infection with the strongyle nematodes and most studies have examined infection with Strongylus vulgaris. Also, all studies usually necessarily have been performed with small numbers of animals. Nevertheless, some studies, but certainly not all, have demonstrated the development of protective immunity with a reduction in the clinical signs and the levels and prevalence of infection with age and following experimental immunization with normal or irradiated larvae of S. vulgaris (Amborski et al., 1974; Ogbourne, 1975; Duncan, 1975; Ogbourne & Duncan, 1977; Klei et al., 1982). Also, repeated infection of ponies with Trichonema spp. appeared to produce some protection as evidenced by a longer prepatent period and lower faecal egg counts although these experiments were not controlled (Smith, 1978).

GLOBULIN LEVELS

In S. vulgaris infection the gamma-globulin fraction and IgG immunoglobulin levels tend to be raised for 4 - 6 weeks from week two of a primary infection. This is followed by a rise in beta-globulins, contributed to mainly by IgG(T), with a return towards normality by about 30 weeks (1970; Patton et al., 1978; Kent, 1985). These parameters varied with level of infection. Thus, gamma-globulins and IgG increased in ponies infected with 700 larvae of S. vulgaris but little or no rise was apparent in ponies which received 200 larvae either as a single dose or repeatedly biweekly. Similarly, IgG(T) levels were increased earlier beginning at 7 - 10 weeks and were twice the magnitude in ponies infected with 700 larvae compared with a rise at 16 weeks in ponies infected with 200 larvae.

The pattern of these parameters following reinfection with S. vulgaris may vary. Increased levels of IgG were apparent early after infection in ponies reinfected with both 700 and 200 larvae (Kent, 1985). Round (1970) reported an earlier rise in beta-globulins after reinfection given 9 - 10 months after the primary infection although this rise failed to occur after a fourth infection. Also, no rise occurred in previously naturally infected ponies given an experimental infection even

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though some signs of colic and increased numbers of eosinophils were apparent after this infection (Amborski et al., 1974). On earlier reinfection, i.e. at 3 - 7.5 months, various authors have reported no rise in beta-globulins and a rise in IgG(T) was seen in only one of four ponies. This latter peaked 2 - 8 weeks after infection and the marked rise beginning at weeks 7 - 10 of a primary infection had not occurred by 17 weeks even though these ponies showed some clinical signs indicative of infection and arterial damage indicative of recent infection (Kent, 1985; Bailey et al., 1985).

Infection and reinfection with cyathostome species also increased beta-globulin levels (Schotman, 1963; Round, 1970) and elevated levels of beta-globulins and IgG(T) have been observed in other non-strongyle, helminth infections (Greer et al., 1974). Thus, levels of these proteins may be indicative of helminth infection but cannot demonstrate the presence of infection with a specific helminth and may not be elevated at times of disease i.e. acute, early colic due to infection with S. vulgaris larvae. For example, although a Chi-squared test revealed significant deviation from a random distribution when the values of total beta-globulins, IgG(T) and the ratio of the total beta-globulins to total protein were examined in 3 groups of 64 naturally infected horses in which anterior mesenteric artery larval numbers were known, marked variation within groups and between groups occurred. Thus, these parameters were not necessarily indicative of the levels of infection and some of the horses most heavily infected with S. vulgaris showed levels below the median value for all the horses examined and vice versa (Bailey et al., 1984a). Also, the relationship of elevated levels of beta-globulins and IgG(T) with other chronic infections (i.e. liver abscess, etc.) needs determination.

ANTIBODY RESPONSE

No direct relationship between these elevated immunoglobulin levels and either protection or the presence of specific antibody has been determined. Complement fixing (CF) antibody, which does not include IgG(T), is elevated during experimental infection with S. vulgaris (Enigk, 1950; Bailey et al., 1985). When detected by a complement consumption test, antibody titres were dependent on the levels of infection and were highest in ponies infected with 700 larvae rather than 200 larvae of S. vulgaris. The antibody response produced a biphasic curve between weeks 2 though 8 and 12 through 22 of a primary infection. The peaks in antibody titre correlated somewhat with the biphasic development of clinical signs and possibly correspond to the migration of larvae to and from the anterior mesenteric artery. However, antibody to S. vulgaris L4/L5 larval antigen was not detected when the larvae were resident but not migrating in the anterior mesenteric artery nor did titres increase on reinfection. A low CF antibody response was detected in only one of four ponies reinfected with larvae of S. vulgaris at 34 weeks (Bailey et al., 1985). Similarly, haemagglutinating (HA) antibody was detected in ponies examined during a primary infection. The response occurred within 2 - 9 weeks after infection with a

single dose of 5,000 larvae. Antibody titres then fell gradually from about 20 weeks in the surviving animals. In contrast, in ponies infected with 50 larvae/week for 20 weeks, only a gradual increase in titres, which did not appear significantly different from control values, occurred for the first 18 - 20 weeks but then a rapid rise in titre occurred at 20 weeks. In both these groups of animals reinfection at 32 weeks with 5,000 S. vulgaris larvae did not produce an anamnestic HA antibody response nor did challenge infection of conventionally reared, field infected ponies (Klei et al., 1983). Indirect fluorescent antibody titres increased within 1 - 2 weeks of the primary infection and again the response was more marked in the ponies infected with a single, large dose of larvae. In contrast to the CF and HA antibodies, however, examination of IFA antibody titres revealed an anamnestic response after reinfection (Klei et al., 1983).

Reasons for the variation in antibody responsiveness between the various tests remains speculative. Equine IgM, IgG_a and IgG_b fix complement and agglutinate antigen (McGuire et al., 1973). Of these, IgM undoubtedly would be the most efficient. Thus, the HA and CF antibody response to a primary but not a secondary infection with S. vulgaris might be accounted for by the detection of a predominantly IgM response in these tests. IFA examined with anti-horse-IgG could detect all the IgG isotypes, one or more of which would be the most likely to participate in an anamnestic response. The biphasic nature of the CF antibody response with the near absence of detectable titres for 3 - 4 weeks between weeks 8 - 12 of infection when larvae are resident in the anterior mesenteric artery is difficult to explain. However, based on the life cycle, where the moult from the fourth to the fifth stage can occur by 11 weeks (Klei et al., 1982), the two peaks in titre might be explained by the sequential exposure of the ponies to fourth and then fifth stage antigens, both of these antigens being present in the detecting somatic L4/L5 extract used in the test.

Intestinal stimulation by adult S. vulgaris appears not to induce a systemic antibody response. Antibody titres, particularly CF antibody titres, declined to negligable levels at about the time of patency of a S. vulgaris infection (Bailey et al., 1985). Also, no haemagglutinating or fluorescent antibody was seen following implantation of adult S. vulgaris into the caecum of ponies (Klei et al., 1983). Local intestinal stimulation, rather than systemic larval migration, also might account for the absence of CF antibody to larval cyathostome antigen in ponies naturally infected with and exposed to infection with Trichostrongylus axei and the cyathostominae (Herd et al., unpublished data).

The specificity of the antibody response remains in question. Of 11 samples examined which were positive for CF antibody from S. vulgaris experimentally infected ponies only about 50% produced a positive response to a cyathostome antigen (Bailey et al., 1985). The lack of an antibody response in ponies infected with adult S. vulgaris or larvae and adults of the cyathostome species (Klei et al., 1983; Herd et al.,

unpublished data) could suggest that cross-reactivity between these infections and the migrating larvae of S. vulgaris might be low. Also, horse serum which reacted positively on an IFA test with S. vulgaris L3 did not react with L3 of Strongylus edentatus and Strongylus equinus and only a slight reaction was apparent with mixed cyathostome larvae. Nevertheless, antibody produced during the migratory phase of a S. vulgaris infection cross-reacted with a somatic extract of adult S. vulgaris. Also, higher titres of haemagglutinating antibody were present in serum from strongyle-naive, two-year-old ponies previously infected with Parascaris equorum and Strongyloides westeri than were seen in serum from helminth-naive ponies (Klei et al., 1983).

Reactions indicative of an immediate type hypersensitivity response, perhaps an IgE response, and characterized by urticaria, gastric dilatation and torsion and infarction of the small and large intestine have been induced in sensitized horses by intravenous injection of an emulsion of S. vulgaris larvae or repeated infection (Ershov, 1970).

CELL RESPONSE

No marked differences usually are observed in the total WBC count or in the absolute numbers of circulating lymphocytes, neutrophils, monocytes or basophils during strongyle infections. However, eosinophil numbers show a biphasic response with an initial peak occurring weeks 4 through 7 and a second more sustained peak occurring from week 10 or later through weeks 20 - 25 of a primary infection with S. vulgaris (Amborski et al., 1974; Duncan and Pirie, 1975; Bailey et al., 1985). The latter peak followed the pattern of the IgG(T) response and began after week 12 in ponies infected with 700 larvae and after week 17 in ponies infected with 200 larvae (Bailey et al., 1985). In contrast, on reinfection of these ponies with the same numbers of larvae, while an enhanced initial peak in eosinophil numbers was observed the second sustained rise appeared almost absent. A gradual rise in eosinophil numbers was seen in only one (infected on both occasions with 200 larvae) of the four ponies by week 17, the end of the experiment.

A sustained eosinophilia was apparent in animals repeatedly infected with S. vulgaris (Patton & Drudge, 1977). Also, an eosinophilia, which was sustained for months, was seen in ponies experimentally reinfected with a large dose of Trichonema spp. (Smith, 1978).

As with beta-globulins and IgG(T), peripheral eosinophilia also is seen following infection with other non-strongyle helminths (Greer et al., 1974; Clayton and Duncan, 1977). The response therefore appears indicative of helminth infection. Further, eosinophilia was not indicative of the numbers of S. vulgaris found in the anterior mesenteric artery of 64 horses examined at post mortem. Indeed, five of the most heavily infected horses (>60 larvae) showed absolute numbers of eosinophils comparable to those seen in helminth-naive foals (Bailey

et al., 1984a).

A major role for eosinophils in antibody-dependent cell-mediated cytotoxicity (ADCC) and destruction of a variety of helminths in vitro now has been demonstrated. Their possible role in protection against infection with strongyle nematodes remains unknown. Histopathological examination at the site of infection and at the site of destruction of larvae in immune animals and in vitro ADCC studies may assist in this respect.

LYMPHOCYTE RESPONSIVENESS

Peripheral blood lymphocytes from experimentally infected ponies responded in vitro to the high molecular weight fraction of S. vulgaris L4/L5 antigen. The lymphocytes from ponies infected with 700 or 200 larvae of S. vulgaris produced positive responses between weeks 2 and 7 with a second more variable response around weeks 10 - 16. The lymphocyte responsiveness of these ponies mirrored most closely that of their CF antibody response. On reinfection with the same numbers of larvae little lymphocyte reactivity was seen in the more heavily infected ponies but a sustained response, from week 9 to the end of the experiment at week 17, was seen in the ponies infected with 200 larvae of S. vulgaris (Bailey et al., 1985).

Lymphocyte responsiveness has been examined also in 46 naturally infected horses in which the numbers of larvae of S. vulgaris in the anterior mesenteric artery were known. All the animals, whether or not they contained larvae, showed pathological evidence of previous infection. Higher responses to antigenic fractions and the major contribution to the significance of the results (Chi-squared test) came from horses in which no larvae were found compared with moderately or heavily infected animals. However, positive responses were seen in only 25 - 50% of the uninfected horses (Bailey et al., 1985).

S. VULGARIS LARVAL MITOGEN

Fraction III from Sephadex G-200 fractionation of the S. vulgaris L4/L5 extract has been shown to contain a mitogen which causes non-specific in vitro polyclonal activation of the lymphocytes from a number of animal species including horses. This mitogen induced proliferation of mouse T lymphocytes, perhaps T_H lymphocytes, with subsequent proliferation of B cells (Bailey et al., 1984b). Mitogens have been demonstrated in other parasites and have been postulated to be involved in suppressed immunological reactivity and perhaps survival of the parasite. A common feature of some of these conditions is a chronic hypergammaglobulinaemia postulated to be associated with mitogen-induced proliferation of B lymphocytes (Greenwood, 1974) or mitogen stimulation of T_H lymphocytes with subsequent T dependent, B cell proliferation (Selkirk et al., 1983). The hyperbetaglobulinaemia with elevated levels of IgG(T) in S. vulgaris infections might be related to the in vivo production of the S. vulgaris larval mitogen (Bailey et al., 1984b).

However, a relationship between production of this mitogen and poor protective immune reactivity remains purely speculative.

CONCLUSIONS

Studies on the immunological responsiveness of horses to infection with strongyles have lagged behind those on many other species of parasites in a variety of animals and man. No conclusive evidence for immunological protection against infection with either S. vulgaris or the cyathostominae yet is available. However, immunization of horses with normal or irradiated larvae of S. vulgaris has been shown to induce some protection against an experimental challenge infection although not against natural challenge infection. The migratory stages of S. vulgaris and the tissue stages of the cyathostominae should be susceptible to a protective immune response induced by vaccination. Studies are required to identify protective antigens, stage specific antigens, the sources of these antigens and their production as well as the life cycle stage of the parasites susceptible to immune destruction.

Enteritis and colic due to infection with S. vulgaris and the cyathostominae are clinical signs common to many other conditions. Although chemotherapy is available, specific diagnosis of arterial migration and the intensity of infection with S. vulgaris as well as the emergence and the intensity of infection with the cyathostominae is required. It is now known that antibody to S. vulgaris is present during the migratory phase of infection. Also, altered serum protein values occur during infection with S. vulgaris and the cyathostominae. These latter, however, may be only helminth specific such that extensive sudies are required on the development of serum protein changes. These should include the development of changes in a wide variety of experimental, monospecific helminth infections and in field infections in which the levels of infection can be determined as well as during other chronic bacterial or viral infections. Also, the specificity of the antibody response remains in question particularly if detected with modern, highly sensitive tests. Nevertheless, recent identification of specific antigens from some other helminths of man and animals bodes well for the probable identification of antigens specific for different species of strongyles. However, such an antigen must undergo intensive testing for the basic parameters of cross-reactivity, predictive value, sensitivity, specificity and source of potential errors using banks of sera from horses singly and multiply infected with a variety of helminths and from field infections of strongylosis and helminthiasis. Detection of specific circulating antigen might reveal the intensity of infection. Species specific immunodiagnostic tests would aid in epidemiological studies.

Little information is available on the role of immunopathology in the pathogenesis of strongylosis. Numerous parasites have been shown to affect the immunological responsiveness of the host, complement and coagulation cascades, the production and activity of the mediators of inflamation, etc. S. vulgaris

has been shown to affect at least some of these. However, the role of immunopathology in the pathogenesis of verminous colic and enteritis remain largely uninvestigated and information is required.

All these studies will be furthered only by a greater understanding of the biology of the parasite and the development of immunological responsiveness in the host.

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REFERENCES

Amborski, G.F., Bello, T.R. and Torbet, B.J. (1974). Host response to experimentally induced infections of Strongylus vulgaris in parasite free and naturally infected ponies. Am. J. vet. Res. 35, 1181-1188.

Bailey, M., Kent, J., Martin, S., Lloyd, S. and Soulsby, E.J.L. (1984a). Haematological and biochemical values in horses naturally infected with Strongylus vulgaris. Vet. Rec. 115, 144-147.

Bailey, M., Lloyd, S., Martin, S.C. and Soulsby, E.J.L. (1984b). In vitro induction of lymphocyte responsiveness by a Strongylus vulgaris-derived mitogen. Z. ParasitenKd. 70, 229-242.

Bailey, M., Martin, S.C., Lloyd, S. and Soulsby, E.J.L. (1985). The immunological and haematological response of ponies to infection with Strongylus vulgaris. (In preparation).

Clayton, H.M. and Duncan, C. (1977). Experimental Parascaris equorum infection of foals. Res. vet. Sci. 23, 109-114.

Duncan, J.L. (1975). Immunity to Strongylus vulgaris in the horse. Equine vet. J. 7, 192-197.

Duncan, J.L. and Pirie, H.M. (1975). The pathogenesis of single experimental infections with Strongylus vulgaris in foals. Res. vet. Sci. 18, 82-93.

Enigk, K. (1950). [On the development of Strongylus vulgaris (Nematoda) in the host animal.] Z. Tropenmed. Parasit. 2, 287-306.

Ershov, V.S. (1970) The biology of horse strongyles and the allergic reactions caused by them. In: Equine Infectious Diseases II. Eds. Bryans, J.T. and Gerber, H. Karger, Basel. pp 304-309.

Greenwood, B.M. (1974). Possible role of a B cell mitogen in hypergammaglobulinaemia in malaria and trypanosomiasis. Lancet i, 435-436.

Greer, G.J., Bello, T.R. and Amborski, G.F. (1974). Experimental infection of Strongyloides westeri in parasite free foals. J. Parasit. 60, 466-472.

Kent, J.C. (1985). Specific serum protein changes associated with primary and secondary Strongylus vulgaris infections in pony yearlings. (In preparation).

Klei, T.R., Torbet, B.J., Chapman, M.R. and Ochoa, R. (1982). Irradiated larval vaccination of ponies against Strongylus vulgaris. J. Parasit., 68, 561-569.

Klei, T.R., Chapman, M.R., Torbet, B.J. and McClure, J.R.(1983). Antibody responses of ponies to initial and challenge infections of Strongylus vulgaris. Vet. Parasit. 12, 187-198.

McGuire, T.C., Crawford, T.B. and Henson, J.B. (1973). The isolation, characterization and functional properties of equine immunoglobulin classes and subclasses. In: Equine Infectious Diseases III. Eds. Bryans, J.T. and Gerber, H. Karger, Basel. pp 364-381.

Ogbourne, C.P. (1975). Studies on the epidemiology of Strongylus infection in the horse. Int. J. Parasit., 5, 423-426.

Ogbourne, C.P. and Duncan, J.L. (1977). Strongylus vulgaris in the Horse: Its Biology and Veterinary Importance. Commonwealth Agricultural Bureaux, England.

Patton, S. and Drudge, J.H. (1977). Clinical response of pony foals experimentally infected with Strongylus vulgaris. Am. J. vet. Res. 38, 2059-2066.

Patton, S, Mock, R.E., Drudge, J.H. and Morgan, D. (1978). Increase if immunoglobulin T concentration in ponies as a response to experimental infection with the nematode S. vulgaris. Am. J. vet. Res., 39, 19-23.

Round, M.C. (1970). The development of strongyles in horses and the associated serum protein changes. In: Equine Infectious Diseases II. Eds. Bryans, J.T. and Gerber, H. Karger, Basel. pp 290-303.

Schotman, A.J.H. (1963). [The serum protein components of normal and diseased horses and ponies, determined by paper electrophoresis. Tijdschr. Diergeneesk. 88, 67-69.

Selkirk, M.E., Wilkins, S.R., Ogilvie, B.M. and Platts-Mills, T.A.E. (1983). In vitro induction of human helper T cell activity by Trypanosoma brucei. Clin. exp. Immun. 52, 512-518.

Smith, H.J. (1978). Experimental Trichonema infections in mature ponies. Vet. Parasit., 4, 265-273.

A TWO YEAR STUDY OF RESPIRATORY DISEASE IN A NEWMARKET STABLE : SOME PRELIMINARY OBSERVATIONS

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A recent survey of 5 flatracing training stables showed that respiratory disease was the second most likely cause of lost training days in young Thoroughbred racehorses (Rossdale et al, 1985). The flatracing horse has a limited period of its early life in which to prove its ability, and for potentially top class horses lost training time can result in considerable financial loss. While lost training days due to clinical respiratory disease are common another much publicised problem of the racing industry, which is linked to infectious respiratory disease, is the "loss of performance" syndrome (Mumford and Rossdale, 1980). This term is used to describe the occurrence of unexpectedly poor performance during training gallops, or in races, of many horses in one stable. In some cases mild and transient signs of respiratory disease may be present, but in others no signs are apparent.

During the 1970s an extensive survey was carried out to establish the prevalence of respiratory viruses in Britain (Powell et al, 1978). The viruses identified during this survey were equine influenza (influenza A/equi-1 [H7N7] and influenza A/equi-2 [H3N8]) equine herpesvirus-1 (EHV-1) (subtype 1 and 2), equine rhinovirus 1 (ERV-1) and 2 (ERV-2), an acid-stable picornavirus (ASPV) and equine adenovirus. Although up to 45 per cent of outbreaks of respiratory disease investigated were attributed to EHV-1 infection, in the absence of influenza epizootics, many infections were apparently not associated with clinical signs. Conversely, 25 per cent of the outbreaks investigated in that survey could not be associated with any of the known viral agents.

A recent study using endoscopy showed that visible intratracheal exudate was present in 50 per cent of examinations carried out after training exercise in one group of thoroughbreds without any obvious clinical signs (Burrell, 1985). A technique for endoscopic collection and assessment of tracheobronchial washes in the horse has recently been described and shown to be a practical means of improving diagnostic accuracy of pulmonary conditions (Whitwell and Greet, 1984).

The present paper presents some preliminary observations from a multidisciplinary survey of respiratory disease in a Newmarket flatracing training stable. Endoscopy and cytology were used to diagnose lower airway disease and virology and bacteriology were carried out in an attempt to identify causative agents. The work was carried out at the Animal Health Trust, Newmarket with the collaboration of Dr. P.D. Rossdale and his partners, Beaufort Cottage Stables, Newmarket. Financial support was

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MATERIALS AND METHODS

The stable used in this survey is an established commercial training yard with, in total, about 70 horses in training during the first year of study (1982/83) and about 100 during the second year (1983/84). At the beginning of the survey all horses were yearlings (becoming two year olds) or 2 years old (becoming 3 years old) in a ratio of approximately 2 to 1. There was an intake of about 40 yearlings (actually 18 to 22 months of age) in the autumn of 1982 and about 60 in 1983. Most of these yearlings were bought at public auction in England, USA and France. They were stabled and 'broken' away from the main yard, gradually being introduced from November onwards.

The study was carried out with two groups of horses. The first group consisted of 16 horses which were selected at the start for routine monitoring. By the end of the first season only 5 of these horses were available for continued monitoring and therefore another 10 were added to this group, making 15. Thus, a total of 26 horses were monitored routinely for varying lengths of time. The second group consisted of 56 horses with clinical respiratory disease. The success and continuity of the training yard always had priority over the needs of the research project and the use of horses and methods employed were governed by these restraints. As far as possible, the routinely monitored group were observed for clinical respiratory signs (temperatures were not recorded routinely), examined endoscopically each month with collection of tracheobronchial wash and a monthly serum sample was collected. Other horses outside the routinely monitored group were also regularly sampled for serology; up to 27 in the first year and 58 in the second.

Clinical cases of respiratory disease were investigated in a similar manner i.e. endoscopy with a tracheobronchial wash and acute and convalescent serum samples.

Endoscopy and the Collection of a Tracheobronchial Wash

In order to obtain a representative sample, endoscopy was carried out after exercise. Briefly, a 1.8m or 1.4m human colonoscope was introduced via the ventral nasal meatus into the nasopharynx and guided through the laryngeal additus and into the tracheal lumen. It was then passed down the length of the trachea until the carina could be seen. A plastic catheter with a sterile agar plug in the end was extruded from the distal end of the endoscope, via the biopsy channel. Thirty ml of sterile phosphate buffered saline was squirted from a 60ml syringe and directed into both mainstem bronchi. The fluid then drained cranially to the level of the thoracic inlet where it pooled. From there it was sucked back into the syringe via the catheter. The sample was divided into 3 aliquots for cytology, bacteriology and a total nucleated cell count. On passing down the trachea a visual assessment was made of the amount of exudate present and graded on

a scale 0 to 3, with zero representing no mucopus seen and 3 a profuse amount of mucopus along the length of the trachea.

Cytology

The cell counting and methods of cytological examination have been described previously by Whitwell and Greet (1984). These authors devised an airway inflammation score to summarise the endoscopic and cytological findings and this method of quantitating the degree of lower airway inflammation has been used in the present study. One point is given if grade 2 (moderate) or grade 3 (profuse) intratracheal mucopus is observed, one point is given for a nucleated cell count of >1000 cells/cm^3, and one point is given if there is a high or moderate proportion of neutrophils seen in the wash. This gives an inflammation score varying from 0 to 3. These authors considered an inflammation score of 2 or 3 to be 'significant' when evaluating lower airway disease. In the current study however, every positive inflammation score was considered.

Bacteriology

Tracheobronchial wash samples were plated onto blood agar and a chocolate blood agar and incubated for 24 hours in an 8.5% carbon dioxide enriched environment and onto a bordatella selective agar plate and incubated in an aerobic environment for 24 hours. They were also plated onto a blood agar and neomycin selective blood agar and incubated for 48 hours in an anaerobic environment. A direct smear was also made and an anaerobic enrichment culture in Robinsons cooked meat which was plated out after 48 hours incubation. The samples were also treated with a mucolytic agent and a series of dilutions made and plated onto blood agar plates which were incubated in enriched CO_2 to estimate the number of bacteria present.

All plates were examined and all potentially significant bacteria subcultured and purified for further identification. Standard bacteriological tests were used for specific identification.

Serology

Only antibody levels to EHV-1 (subtype 1, strain RACH, and subtype 2, strain MD) and ERV-1 using the Complement Fixation (CF) tests have been completed. A four-fold rise in CF titre is taken to indicate virus infection.

RESULTS AND DISCUSSION

Results from all the tracheobronchial washes have not been completed, nor have the serum samples been tested against all viruses. Therefore, this study represents a preliminary analysis of the results.

Incidence of Lower Airway Inflammation

A diagnosis of lower respiratory tract (LRT) inflammation was reached if the horse had a positive inflammation score at endoscopy with cytology. An episode of lower airway inflammation was defined as any period during which consecutive examinations which resulted in a positive inflammation score. The duration of each episode was taken as the time span covered by consecutive positive examinations plus two weeks at the beginning and the end of that period i.e. a single positive score equals 4 weeks duration. This method may over-estimate the duration of episodes slightly but it is thought unlikely that a disease episode will often take its course from onset to recovery in less than 4 weeks.

Of the 26 horses which were endoscoped approximately every month 24 horses had a total of 57 separate episodes of LRT inflammation. The mean duration of these episodes was 7 weeks, and ranged from 4 weeks to 22 weeks. On average, all horses spent 32.5 per cent of the period of surveillance with lower airway inflammation.

Coughing is the usual clinical sign associated with airway disease, particularly by lay persons. Analysis of the association between coughing and positive inflammation score gives an indication of how reliable coughing is as an indicator of LRT inflammation. The analysis was carried out on all endoscopic examinations undertaken which included the routine examinations and clinical cases.

A horse was considered to have had a coughing episode if it coughed at least 3 to 4 times a day, this being the usual level of coughing required before veterinary attention was sought in this stable. An association between positive inflammation score and coughing was only made if the horse was coughing on the day of examination or within the previous week. The results are shown in Table 1.

Table 1. Association between coughing and inflammation score from 338 tracheobronchial washes.

	Coughing	No Coughing
Positive inflammation score	57 (84%)	101 (37%)
Negative inflammation score	11 (16%)	169 (63%)
	68	270

The results demonstrate that 37 per cent of horses not coughing when examined had evidence of LRT inflammation and also that 16 per cent of horses which were coughing had no evidence of LRT inflammation by this method. Most of these latter cases had been coughing previously. The data suggest that coughing is an inaccurate and insensitive indicator of lower

airway inflammation in horses.

Serology

The number of seroconversions that occurred to EHV-1 and ERV-1 over each month of the two year period and the number of horses being sampled each month are shown in Fig. 1. Data from any particular horse are included only if there were at least three consecutive monthly samples. Although CF titres to both subtypes of EHV-1 were measured there is antigenic cross-reactivity between the two types and on serological grounds alone it is not possible to differentiate between the two. Therefore, in this analysis all seroconversions to EHV-1 are considered together. The data show that there were two peak periods of seroconversion to ERV-1, occurring from October to December of both years. This coincides with the arrival of yearlings from the sales. Virtually all infections were confined to these younger horses; for example 14 out of 20 yearlings tested seroconverted to ERV-1 in November 1982. Complement fixing titres to ERV-1 persist for many months or years at a level of $\frac{1}{10}$ to $\frac{1}{20}$ and there is apparently a corresponding period of immunity, as in only two horses in this study was a subsequent second seroconversion detected. Both occurred in two year old horses and were 5 months and 7 months apart respectively.

In contrast to ERV-1 the seroconversions to EHV-1 occurred sporadically throughout the survey period although there were two peaks of infection in each year. These occurred from October to December and again from March to May. The first peak again coincides with the arrival of yearlings and, although not recorded in these data, some of these arrived obviously having had recent infections, because they already had high or falling titres to EHV-1. A peak of infection rate at this time of year can be explained by the stresses of travel and the sales, and challenge by strains of virus not previously encountered. The second peak of infection rate occurring 3 to 4 months later may be due to a combination of factors. It occurs as titres wane from the previous infections and may simply be reinfection by the same virus strain circulating in the stable, or it may represent the introduction of strain variants by horses travelling to race meetings, which begins to happen in May.

EHV-1 is known to persist in a latent form in horses, and Burrows and Goodridge (1984) reported recrudescence and reinfection in a closed pony herd, possibly provoked by the stress of an outbreak of unrelated disease.

Association of Viral Infection with Disease

In attempting to link specific disease episodes with viral seroconversions only data taken from the 26 horses which were routinely monitored are considered. Clinical signs which may be attributed to the acute phase of a virus infection are pyrexia (>101.0^{o}F, 38.3^{o}C) anorexia or serous nasal discharge. The observation of these signs was by the stable staff and trainer. Temperatures were recorded daily for only part of the year following introduction of the yearlings, and it is likely that at other times of year cases were missed unless the horse appeared ill.

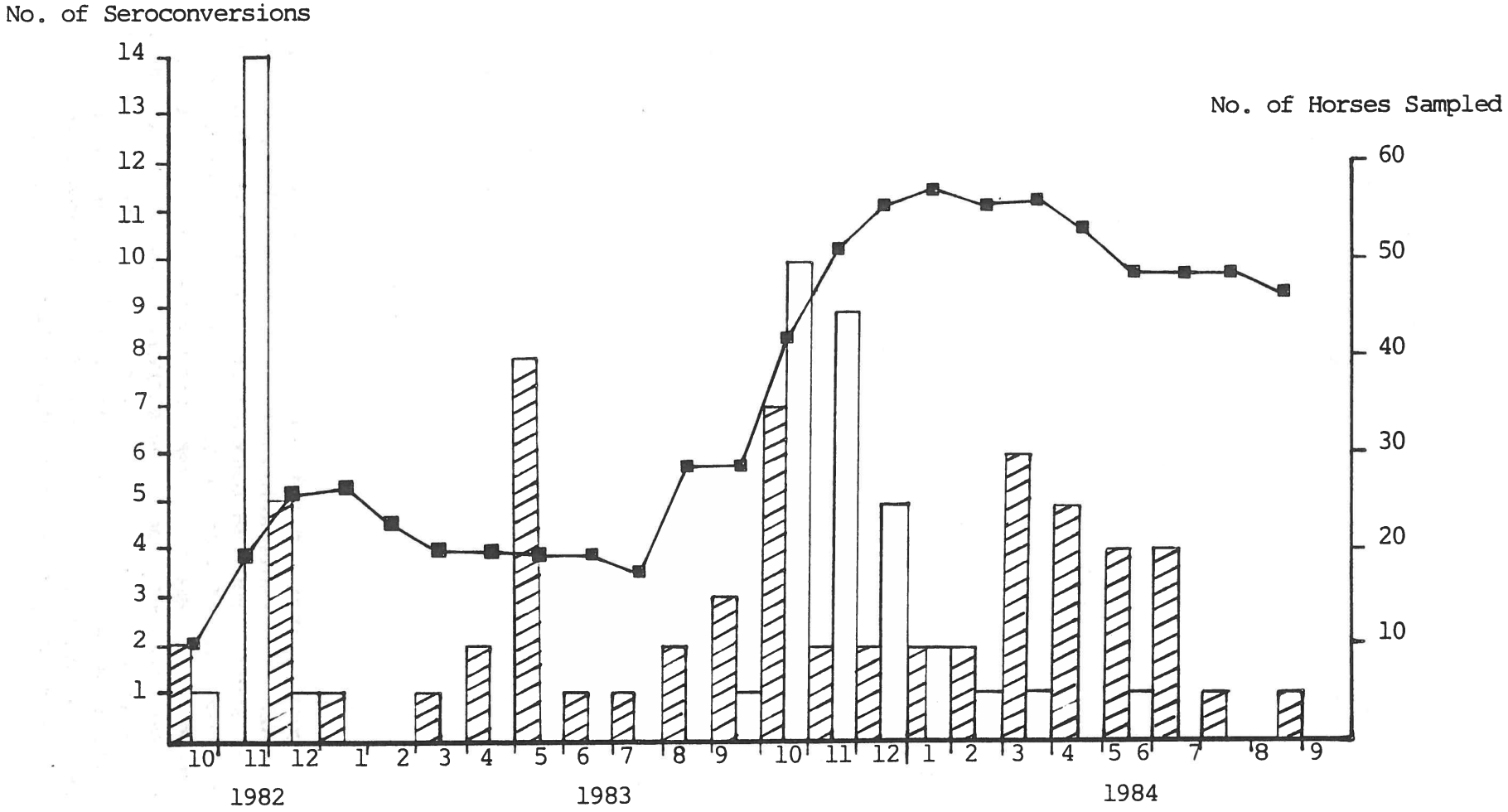

Fig. 1. Seroconversions to EHV-1 (▨) and ERV-1 (□) and the number of horses sampled each month (■——■)

Experimental infections with EHV-1 and ERV-1 have only been recorded in gnotobiotic or conventional ponies (Burrows and Goodridge, 1973; Thomson, 1978; Mumford and Thomson, 1978) so the sequelae to these infections in Thoroughbreds under the stress of training are unlikely to be directly comparable. Our understanding of the pathogenesis of these infections and the aetiology of lower airway inflammation is inadequate. In this analysis of the association of viral infection with lower airway inflammation only tentative conclusions can be drawn, particularly as the serology to other known viruses has yet to be completed.

The results in Table 2 show the number of seroconversions to EHV-1, ERV-1 or both concurrently, which were associated with acute signs of viral infection during the period of conversion, the number with subsequent onset of a disease episode of positive inflammation score up to 12 weeks later and those that were not followed by a positive inflammation score within 12 weeks.

Table 2. Seroconversions to EHV-1 and ERV-1 and their association with acute clinical signs and subsequent positive inflammation score (IS).

		Seroconversions		
		ERV-1	EHV-1	ERV-1 and EHV-1
No. with acute signs		4	1	0
No. with positive I.S.	During seroconversion	2	0	0
	Within 4 weeks	6	9	2
	Within 4-8 weeks	1	2	1
	Within 8-12 weeks	0	3	0
No. with no positive I.S. within 12 weeks		4	7	0
	TOTAL	13	21	3

These results confirm that, in the majority of cases of EHV-1 and ERV-1, initial infection and viral replication passed subclinically or at least unnoticed by stable staff. Routine temperature recording would probably increase the detection rate of infection. For each virus, about 30 per cent of infections did not result in lower airway inflammation within 12 weeks. If the associations made in this analysis are correct and not due to other undetected agents, then 69 per cent of ERV-1 infections and 66 per cent of EHV-1 infections resulted in a positive inflammation score. In some cases there is apparently a considerable time lag between EHV-1 infection and the onset of lower airway inflammation.

Disease Episodes of Unknown Origin

Forty-one out of 72 (57 per cent) recorded disease episodes (pyrexia or positive inflammation score) were not associated with seroconversions to EHV-1 or ERV-1 within the preceding 12 weeks. Attempting to explain the aetiology of these episodes is not possible without completing the serology to other known viruses. In the survey conducted by Powell et al (1978) 25 per cent of outbreaks of respiratory disease were not attributed to any of the known viruses. That survey showed that in the absence of influenza, EHV-1 and ERV-1 were the most common viral respiratory pathogens and that ERV-2, ASPV and equine adenovirus were associated with few of the incidents.

Bacteriology

The prevalence of the bacteria, cultured from the tracheobronchial washes is shown in Table 3. The results show that 18 per cent of all washes were sterile, of which only 9 per cent of these had an inflammation score 2 or 3. The majority of washes contained a mixed population of bacteria, from 2 to 9 different bacteria being identified in individual washes. In discussing significance, it has to be decided whether the bacteria, which are present in the normal flora of the nasopharynx, are contributing to the disease process in the lower respiratory tract, or if they are transients being inactivated and removed without provoking any inflammatory change. Using transtracheal aspiration Mansmann (1976) found that the distal trachea of normal horses was not sterile but contained a flora reflecting the horses' stable environment. Therefore the bacterial cultures must be quantitated and compared to the presence of disease before their significance can be determined. Unravelling the role played by the various bacteria isolated is a major aim of this project.

SUMMARY AND CONCLUSIONS

The arrival of many yearlings from sales and the stress of transportation and training provides a combination of factors conducive to the spread of infectious agents. This was shown by a high incidence of infection by ERV-1 and EHV-1 in the autumn months. Infections by EHV-1 continued sporadically with another peak in the spring/early summer. Most infections by these two viruses passed unnoticed in the acute phase but many were associated with subsequent LRT inflammation. Routine temperature recording is recommended for detection of viral infection. Coughing is an unreliable indicator of LRT inflammation compared to endoscopy and cytological evaluation of tracheobronchial washes. Therefore, endoscopic screening of young racehorses to identify LRT inflammation is advisable, particularly in the spring following the highest incidence of viral infection and before the onset of hard training work. Quantification of bacterial cultures from tracheobronchial washes and comparison with the presence of disease is necessary for assessment of their significance.

Table 3. The prevalence of bacteria cultured from all tracheobronchial washes (338) and from those with positive inflammation score (IS)

	No. of washes containing the organism (% of total washes)		No. of washes of positive IS containing the organism (% of washes that contain the organism)	
No Growth	62	(18%)	18	(29%)
AEROBES				
Strep. zooepidemicus	160	(47%)	97	(60%)
Strep. pneumoniae	72	(21%)	39	(54%)
*Gram negative rods	30	(8%)	14	(46%)
Bordatella bronchiseptica	11	(3.3%)	8	(72%)
E. coli and other coliforms	11	(3.3%)	7	(13%)
Staph sp.	145	(43%)	54	(39%)
Bacillus sp.	39	(11.5%)	21	(54%)
∝H Strep. sp.	33	(9.5%)	13	(33%)
Corynebacterium sp.	4	(1%)		
Enterobacter agglomerans	3	(<1%)		
Strep. equi	1	(<1%)		
Klebsiella pneumoniae	1	(<1%)		
Pseudomonas aeruginosa	1	(<1%)		
ANAEROBES				
Peptostrep sp.	32	(9.5%)	24	(75%)
Peptococcus sp.	39	(11.5%)	24	(61%)
Bacteroides sp.	46	(14%)	27	(58%)
Clostridium sp.	28	(8%)	11	(39%)
Fusobacterium sp.	24	(7%)	12	(50%)
Veillonella sp.	8	(2%)	1	(12%)
Propionobacter sp.	3	(<1%)		

*Gram negative rods consisted predominantly of Pasteurella sp. and Moxarella sp.

REFERENCES

Burrell, M.H. (1985) Endoscopic and virological observations on respiratory disease in a group of young Thoroughbred horses in training. Equine Vet. J. (In press).

Burrows, R. and Goodridge, D. (1973) In vivo and in vitro studies on Equine Rhinopneumonitis virus strains. Proc. 3rd int. Conf. Equine Infectious Diseases, Paris 1972, pp 306-321 (Karger, Basel 1973).

Burrows, R. and Goodridge, D. (1984) Studies of persistent and latent equid herpesvirus 1 and herpesvirus 3 infections in the Pirbright pony herd. Latent herpes virus infections in veterinary medicine. Eds. Wittman, G, Gaskell, R.M. and Rziha, H. Martines Nizhoff (CEC) p 307-319.

Mansmann, R.A. (1976) Evaluation of transtracheal aspiration in the horse. J. Am. Vet. med. Ass. 169, 631-633.

Mumford, J.A. and Rossdale, P.D. (1980) Virus and its relationship to the "poor performance" syndrome. Equine Vet. J. 12, 3-9.

Mumford, J.A. and Thomson, G.R. (1978) Studies on picornaviruses isolated from the respiratory tract of horses. 4th Int. Conf. Equine Infectious Diseases 419-429.

Powell, D.G., Burrows, R., Spooner, P., Goodridge, D., Thomson, G.R. and Mumford, J.A. (1978) A study of infectious respiratory diseases among horses in Great Britain 1971-1976. 4th Int. Conf. Equine Infectious Diseases, 451-460.

Thomson, G.R. (1978) PhD. Thesis, University of London.

Whitwell, K.E. and Greet, T.R.C. (1984) Collection and evaluation of tracheo-bronchial washes in the horse. Equine Vet. J. 16, 499-508.

EPIDEMIOLOGICAL FEATURES OF THE 1984 OUTBREAK OF EQUINE VIRAL ARTERITIS IN THE THOROUGHBRED POPULATION IN KENTUCKY, USA

P. J. TIMONEY*

Equine viral arteritis (EVA) was first defined as a separate and distinct disease of the horse in 1953, when the causative virus was isolated from aborted equine foetuses during an epizootic of abortion on a Standardbred farm in Bucyrus, Ohio (Doll et al., 1957). Seroepidemiological studies carried out in the USA in the early 1970's (McCollum & Bryans, 1972), revealed that whereas equine arteritis virus infection was endemic in the Standardbred breed, Thoroughbreds were largely susceptible, with evidence of infection in less than 5 percent of the population. There are relatively few reported occurrences of EVA in the scientific literature and the outbreak of the disease in Central Kentucky during the 1984 breeding season marked the first time EVA had been recorded in the Thoroughbred population in the USA.

A total of 38 farms were involved, with cases of the disease being reported over a 9 week period extending from the last week in April to the end of June. Maximal weekly totals of outbreaks of 11 and 10, respectively, were recorded during the last week in May and the first week in June. Clinical signs characteristic of acute EVA were reported in 159 horses comprising stallions, mares, foals and teaser stallions located on 37 farms. The preponderance of cases were in mares that had been bred to stallions on the farm on which the disease was first confirmed on May 25. The number of affected mares was approximately double that of foals and the foal total was in turn, nearly twice the number of affected stallions. No evidence of infection was detected in the yearlings on any of the affected premises. The severity of the disease observed varied considerably from severe cases to those of a very mild nature. Had there not been such an awareness of EVA and the symptomatology characteristically associated with this infection among horse owners and farm managers at that time, it is very possible that some of the horses mildly affected with the disease would have escaped detection.

CLINICAL CHARACTERISTICS OF THE DISEASE

The range of clinical signs observed in typical cases of EVA comprised: Fever up to 41° C that developed after an incubation period of 3 to 14 days (mean 7.3 days) and which persisted for 5 to 9 days;

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"stocking up" or limb oedema, especially of the hind limbs; nasal and ocular discharges, initially serous, in some instances becoming mucoid or mucopurulent later; inflammation of the conjunctival and nasal mucous membranes; periorbital oedema; a variable degree of depression and loss of appetite; maculo-papular skin rash most commonly on the sides of the neck, but sometimes generalised; oedema of the scrotum and prepuce in the stallion; infrequently, papular eruption involving the mucous membrane inside the upper lip; and rarely, oedema in the intermandibular space beneath the lower jaw or located beneath the sternum or in the shoulder region. Horses considered affected with EVA exhibited many or all of these clinical signs to a greater or lesser degree. Regardless of the severity of clinical signs, all affected horses made complete, uneventful recoveries. In no instance was there any associated mortality nor have there been any unequivocally confirmed cases of abortion. However, there was circumstantial evidence with respect to three farms, which suggested that equine arteritis virus infection may have resulted in a lower than average pregnancy rate in mares checked 18 to 30 days after their last covering date. On the basis of the interim results of a survey undertaken to investigate the possible effects of equine arteritis virus on the fertility of EVA affected mares, the percentage of these mares that were found barren was approximately 16 percent greater than that found in the normal mare population.

DIAGNOSIS

During the course of the outbreak of EVA, diagnosis of the disease was based on presence of the characteristic clinical signs together with corroborative virological data comprising virus isolation and/or detection of serological responses to equine arteritis virus. Numerous isolations of the virus were made from horses in the acute phase of the infection, with virus recovered 1 to 9 days (mean 2.3 days) after the onset of clinical illness, principally from swabs taken from the naso-pharynx. Three of the isolants were from mares/foals that had been vaccinated with the experimental modified live EVA vaccine 9 to 11 days previously. Horses affected with EVA developed good serological responses as monitored by the micro-neutralisation assay, with sero-conversions occurring approximately 7 to 14 days after clinical onset.

EPIDEMIOLOGY

Epidemiological investigation of the 1984 outbreak of EVA in Kentucky has revealed that primary cases of equine arteritis virus infection on 27 of the 38 farms involved, were in mares bred to stallions in one of the breeding sheds on the first farm known to have been affected. The original source of virus for that particular outbreak has not been established, though at one time it was speculated that infection was introduced with an imported mare. On the basis of the available data, the pattern of spread of EVA during this outbreak can be summarised as follows:

1. Equine arteritis virus infection occurred in stallions on 4 farms:

Farm A: 17 clinically affected stallions
Farm B: 7 clinically affected stallions
Farm C: 1 asymptomatically infected stallion
Farm D: 2 asymptomatically infected stallions

2. Primary cases of EVA on 27 of the 38 premises involved, were in mares bred to stallions on Farm A, the first farm known to have been affected.

3. Stallions at Farm B were exposed when a mare from one of the initially affected farms was bred to one of the stallions when incubating the disease.

4. Only one primary case of EVA was traced to a covering by one of the stallions on Farm B.

5. One of the stallions on Farm C transmitted EVA to mares on 2 farms additional to a mare on Farm C.

6. Two of the stallions on Farm D were implicated in transmitting equine arteritis virus infection to mares on 5 additional farms.

7. EVA occurred in mares/foals on 4 farms for which the respective sources of infection could not be determined.

Stallions on four farms were identified as having transmitted the disease, with clinical evidence of infection reported in 24 out of an infected stallion total of 27. The three asymptomatically infected stallions were located on two farms, neither of which had any apparent link with one another nor with the other two affected stallion premises. Cases of EVA in mares were reported on four additional farms for which the respective sources of infection could also not be determined.

In an attempt to obtain some estimation of the incidence of asymptomatic infection in mares bred to stallions shedding equine arteritis virus, a survey was undertaken to determine the serological status of 172 mares bled at least 14 days after their last breeding date. Out of this total, there were 39 mares with titres to equine arteritis virus: 23 of which had received the experimental modified live EVA vaccine at least 7 days previously; 12 had either been clinical cases of EVA or else were on affected premises and were regarded as having a high risk of exposure to the virus; and four were on individual unaffected farms. Epidemiological investigation of the latter four mares did not reveal evidence of recent equine arteritis virus infection nor any indication of lateral spread of infection to other horses with which they had been in contact. Whilst there was no evidence from this survey of widespread subclinical infection in mares, subsequent data have indicated that this may not necessarily have been the case in every instance. A high prevalence of serological positives to equine arteritis virus was found in mares on one farm, many of which had been bred to one of the asymptomatically infected stallions during the 1984 breeding season. The prevalence rate of 46.6 percent was greatly in excess of the 2.5 percent rate found in Thoroughbred mares randomly surveyed on farms in central Kentucky in the spring of 1984. As a corollary to this survey and in conjunction with the 1984 summer and autumn yearling sales in Kentucky, a total of 2,650 yearlings were tested of which 10 or 0.37 percent carried a serological titre to equine arteritis virus, confirming that there was little evidence of asymptomatic equine arteritis virus infection in the yearling population in Kentucky.

Lateral spread of infection between horses in adjacent stalls or having direct or across the fence contact at pasture, was not a notable feature of the 1984 outbreak of EVA. Detailed investigation of the number of cases of EVA per farm revealed that approximately 40 percent of the total number of affected farms had only one clinical case of infection, 58 percent had 1 to 2 cases and only 12 percent of the farms had more than five cases of the disease apiece. A clear-cut picture of the extent of asymptomatic infection on each affected premise, was difficult if not impossible to determine due to prophylactic vaccination of high risk horses on these farms with the experimental modified live EVA vaccine.

TRANSMISSION

Whilst transmission by the respiratory route has been traditionally regarded as the primary route of exposure in cases of naturally acquired equine arteritis virus infection, there was considerable evidence from the 1984 outbreak of EVA to indicate that the majority of the affected mares were exposed to infection in the breeding shed, most probably by the venereal route. Shedding of equine arteritis virus in the semen has been confirmed in 9 out of 25 stallions tested, either on the basis of virus isolation or the successful transmission of EVA to test mares. Secondary spread of infection on affected farms was thought to have taken place primarily as a result of aerosol transmission and in a few instances, through indirect contact with contaminated fomites and possibly infected teaser stallions or nurse mares.

The epidemiological significance of the teaser stallion in the spread of EVA in this outbreak was investigated. Clinical EVA was observed in only two out of 29 teasers on 25 of the affected farms, and although an additional three teasers were serologically positive for equine arteritis virus antibodies, significant rises in antibody titre could not be demonstrated. The two EVA affected teaser stallions which were located on separate farms, were not regarded as of major significance in the spread of the disease on those premises.

THE CARRIER STATE

The existence of the carrier state in the stallion has been confirmed in a relatively high percentage (36 percent) of the original group of infected stallions. The virus has been found to persist in the genito-urinary tract for at least 8 to 9 months after the stallions had clinically recovered from the disease. Many of the characteristics of the carrier state in the stallion have yet to be defined, especially the duration of persistence of equine arteritis virus in the carrier animal. Whilst none of the mares or foals involved in the 1984 outbreak of EVA have been discovered carriers of the virus, very little attempt has so far been made to monitor these animals for shedding virus.

CONTROL MEASURES

Measures to control the spread of EVA on Thoroughbred farms in Kentucky were instigated on May 25, the earliest date that virological

confirmation of the disease was available. Permission for the controlled use of an experimental modified live EVA vaccine developed many years earlier in the Department of Veterinary Science, University of Kentucky, was sanctioned on that date. A recommendation was made to vaccinate the following categories of horses: All mares to be bred to stallions clinically recovered from EVA, with vaccination carried out at least seven days before breeding; all horses in contact with known clinical cases of EVA; and horses on the farm on which the disease was initially confirmed and where it was feared the number of animals exposed to infection might be high. The occurrence of additional cases of EVA over the ensuing weeks on previously unaffected premises, led to an order issued by the Commissioner of Agriculture for Kentucky on June 25, banning the breeding of mares and the movement of horses for a period of two weeks. With no new outbreaks reported after June 22 and with the last clinical case of the disease confirmed on July 1, the ban restricting movement of horses from EVA unaffected farms was lifted on July 13. Restrictions remained in force, however, on horses from infected or suspect premises, until such time as detailed epidemiological investigation of these farms had been completed and it was considered safe to allow movement to recommence.

VACCINE

A total of 2,126 mares, stallions, yearlings, foals, teaser stallions and nurse mares on 87 different farms, were vaccinated with the experimental modified live EVA vaccine during the period May 25 to July 3. All of the vaccine distributed was prepared from the same batch of lyophilised vaccine containing a modified Bucyrus strain of equine arteritis virus of cell passage history HK131, RK111, EC1D25. The vaccination dose of 1 ml containing approximately 26,000 infective tissue culture doses of virus, was administered by the intramuscular route. Vaccination of all horses clinically affected with EVA and of foals less than 6 months of age was contraindicated. With the possible exception of a slight febrile response in a few foals, no undesirable clinical sequelae were observed following use of this vaccine. There were quite a number of reports, however, of mares, foals and stallions developing EVA at varying intervals after vaccination. These were individually investigated and it was found that the period between vaccination and the onset of clinical signs ranged from 0 to 10 days with a mean of 4.5 days. It was presumed that exposure to natural infection with equine arteritis virus had occurred some days before, at the time of, or a few days after vaccination and that the vaccinated horses had insufficient time to mount an adequate immune response before being challenged.

In conclusion, it is felt that control of the spread of EVA in the Thoroughbred population in central Kentucky during the 1984 breeding season was achieved following restriction of movement of breeding stock, closure of the breeding sheds, and the use of an experimental modified live EVA vaccine to curtail lateral spread of infection on affected premises.

REFERENCES

Doll, E.R., Bryans, J.T., McCollum, W.H. and Crowe, M.E.W. (1957) Isolation of a filterable agent causing arteritis of horses and abortion by mares. Its differentiation from the equine abortion (influenza) virus. Cornell Vet. 47, 3-41.

McCollum, W.H. and Bryans, J.T. (1972) Serological identification of infection by equine arteritis virus in horses of several countries. Proc. 3rd Int. Conf. Equine Infectious Diseases, Paris. 256-263. S. Karger, Basel, 1973.

INTERNATIONAL MOVEMENT OF HORSES AND ITS INFLUENCE ON THE SPREAD OF INFECTIOUS DISEASE

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Horses are moved from one country to another for one of three reasons: in connection with the sale of the horse, for breeding and for competition purposes. Competition horses account for the majority of movements especially within Europe but the sale of horses involves movement world wide and there is an increasing movement of horses between North America and Europe for breeding purposes. Within Europe the transportation of horses is most often undertaken by road and roll on/roll off sea ferry but for greater distances between the continents of Europe, North and South America, Asia and Australia, air transportation plays a prominent role. Formerly sea transportation of horses over long distances imposed a useful quarentine period but the increasing use of air transport has contributed to the spread of epidemic disease, the most obvious example being equine influenza. Table 1 lists a number of infectious and contagious equine diseases for which there is recent documented evidence of their spread following the international movement of horses. In the case of African Horse Sickness (AHS) and Venezuelan Equine Encephalomyelitis (VEE), the spread of the disease occurred primarily following the migration of the horse or the insects which acted as vectors of the disease. However, in the case of Contagious Equine Metritis (CEM), Equine Infectious Anaemia (EIA), influenza and piroplasmosis, the spread of disease was as a direct result of human intervention. In addition to the diseases listed in Table 1, a number of other pathogens, including Strep. equi (S. equi), equid herpes virus type 1 (EHV-1) and equine viral arteritis (EVA), have been disseminated following the movement of horses. The prevalence of S. equi and EHV-1 among the equine population is extensive and their incidence is not therefore regularly reported.

Primarily because of improved laboratory methods several previously unrecognised equine pathogens have been identified (Platt, Powell and Williams, 1985). These are listed in Table 2 but their distribution at the present time is obviously not understood.

CURRENT METHODS OF CONTROL

At present horses may be moved from one country to another under the authority of a licence issued by the state veterinary service of the exporting country. Within Europe and Scandinavia horses may be moved under a general licence which requires identification of the horse and also certifies freedom from certain named diseases. In addition it provides a declaration about the

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Table 1. Recent disease outbreaks following the movement of horses

Disease	Recipient country(ies)	Date and source
African horse sickness (AHS)	Middle East, S.W. Asia, India, Cyprus	1959 Extension of epidemic in Africa
	Spain	1966 Extension of epidemic in N. Africa
Contagious equine metritis (CEM)	U.K., Ireland	1977 Importation of carrier mare
	Australia	1977 Importation of carrier animal from Europe
	U.S.A.	1978 Importation of carrier stallions from Europe
	Japan	1980 Importation of carrier animal from Europe
Equine infectious anaemia (EIA)	U.K.	1974 Importation of carrier mare
Influenza	U.S.A.	1963 New subtype 2 imported from S. America
	Singapore and Malaysia	1977 Subtype 1 imported from Europe
	U.K.	1979 Subtype 2 imported during international horse show
Piroplasmosis	U.S.A.	1959 Importation of Cuban horses infected with B. caballi
Venezuelan equine encephalomyelitis (VEE)	U.S.A.	1971 Extension of epidemic from South and Central America

Table 2. Recently recognised infections of horses

Disease	Causal Agent	Reported
Encephalomyelitis	Encephalosis virus	S. Africa, 1970
Ehrlichiosis	unclassified Ehrlichia	California, U.S.A. 1969
Melioidosis	Pseudomonas pseudomallei	France 1978
Contagious Equine Metritis	Gram negative coccobacillus	U.K. 1977 Ireland 1977
Getah virus infection	Alphavirus	Japan 1978
Acute equine diarrhoea syndrome	unclassified Ehrlichia	U.S.A. 1984

disease freedom of the country of export, eg. the country was free of a particular disease during a previous stipulated period. To move horses over a wider distance it is the usual practice to issue a specific licence for an individual animal which covers one importation and is time limited. Between the United Kingdom, France and Ireland there has been in existence since 1974 the Tripartite Agreement which is an informal arrangement between the government veterinary services covering a common list of notifiable diseases and a common import policy. A recent example of this common policy was the temporary suspension of equine imports from the United States in the summer of 1984 following the outbreak of equine viral arteritis on Thoroughbred stud farms in Central Kentucky, The suspension was later lifted allowing horses to be imported under stricter veterinary conditions.

Information about the incidence of equine infectious disease in various countries may be obtained through the Office International des Epizootics (OIE), Paris, France, and via the many informal contacts that exist between individuals involved in equine disease investigations in various parts of the world. The OIE maintain two lists of notifiable diseases: list A comprising diseases to be notified monthly, and list B comprising diseases notified on an annual basis. The equine diseases included in lists A and B are shown in Table 3. Additionally, OIE requests that member countries should report within 24 hours by telegram or telex any disease in the lists which appear for the first time as an epizootic outbreak in a country.

IMPROVEMENTS OF PRESENT SYSTEM

There are reasonable controls at present to prevent the spread of serious notifiable and rapidly spreading equine diseases such as African Horse Sickness and Venezuelan Equine Encephalomyelitis. However, the precautions to prevent the spread of non-notifiable diseases including influenza, strangles, rhinopneumonitis, viral arteritis and certain venereally transmitted pathogens are presently vague and inadequate. These diseases in general do not give rise to mortality but nevertheless are a cause of economic loss and can seriously disrupt the international trade of horses.

Table 3. List of equine diseases to be reported to O.I.E.

List A, monthly	List B, annually
African horse sickness	Contagious equine metritis
Vesicular stomatitis	Dourine
	Epizootic lymphangitis
	Equine infectious anaemia
	Equine influenza
	Equine piroplasmosis
	Equine herpes virus type 1
	Equine viral arteritis
	Glanders
	Horse pox
	Japanese encephalitis
	Mange
	Salmonellosis (S. abortus equi)
	Surra
	Venezuelan equine encephalomyelitis
	of multiple species:
	Anthrax
	Hydatidosis
	Leptospirosis
	Rabies

There are several ways of improving the situation. When an outbreak of an infectious disease occurs among a group of horses it is imperative that a rapid but accurate diagnosis is made. An outbreak may occur in a country where the appropriate investigative and laboratory personnel and diagnostic facilities are not available. In such an outbreak resource to the extensive facilities available in other countries should be undertaken as quickly as possible. Once a diagnosis has been made and it is considered by those with experience in dealing with such problems that the disease could have a significant impact on international trade then it should be reported. At present there is no rapid mechanism for doing this except on an informal basis, although extensive collaboration is undertaken between equine epidemiologists of the United Kingdom, Ireland and France. Each year a report on the incidence of several non-notifiable equine diseases within the three countries is published. Such collaboration could be extended to include representation from other major equine importing and exporting countries including North America, Australia and Japan, as well as input from international equine organisations such as the Federation Equestrian International (FEI). By extending these contacts a system of emergency reporting could be developed as well as establishing a permanent system of disease communication. Such a system would require a collating centre to receive and transmit information as well as providing advice on resource facilities. The success would depend on the quality of information generated at the national level and to enhance this a better liaison needs to be established between the equine industries within each country and the state

or federal veterinary services responsible for the certification involved in the international movement of horses.

REFERENCES

Platt, H., Powell, D.G. and Williams, J.M. (1985) Constraints imposed by infectious diseases on the import and export of horses. Br. vet. J. (in press)

THE PRACTICAL APPLICATIONS OF COMPUTING

ARTIFICIAL INTELLIGENCE TECHNIQUES FOR BUILDING DIAGNOSTIC EXPERT SYSTEMS

J. BOWEN*

In the last decade, a range of techniques for incorporating human diagnostic expertise into computer programs have been developed by researchers in the field of Artificial Intelligence, a sub-field of Computer Science. These techniques have been applied in several areas of diagnostic expertise: e.g., medical diagnosis and crop pathology. This paper surveys these techniques and presents, by way of illustration, a simple system for diagnosis of equine colic.

1. Diagnosis as Search

In the field of Artificial Intelligence (AI), the general technique used to model problem-solving is to regard it as search through a space of possible solutions. In line with this general model of problem-solving, it is usual to regard diagnosis as search through a space of possible illnesses for one which matches the set of manifestations.

Although many different search methods have been described in the AI literature it is possible to classify each method into one of two groups: backward-chaining search methods and forward-chaining search methods. Every soluble problem has two essential elements: a body of data and a solution. A search method is regarded as backward- or forward-chaining depending on whether the search process is driven by observed data or by hypothesised solutions.

Forward-chaining search is so-called because it involves working forward from the data to the solution. In forward-chaining search new information is inferred from the data, further information is inferred from the newly derived information, and so on, until one of the items of information derived is the solution to the problem.

Backward-chaining search, on the other hand, involves choosing a hypothesis from a space of possible solutions and attempting to prove that this hypothesis is confirmed by the available data. If it is not possible to confirm the first hypothesis chosen, another candidate is considered, and so on, until one hypothesis is proven to be correct. Thus, backward-chaining search involves working backward from hypothesised solutions to supporting data. These two classes of search method are contrasted in Figure 1.

In diagnosis, the manifestations constitute the data and the set of possible illnesses constitutes the space of candidate solutions. In diagnosis of equine colic, for example, the data would include items of information such as those shown in Figure 2, while the space of candidate solutions would consist of various types of colic such as those shown in Figure 3.

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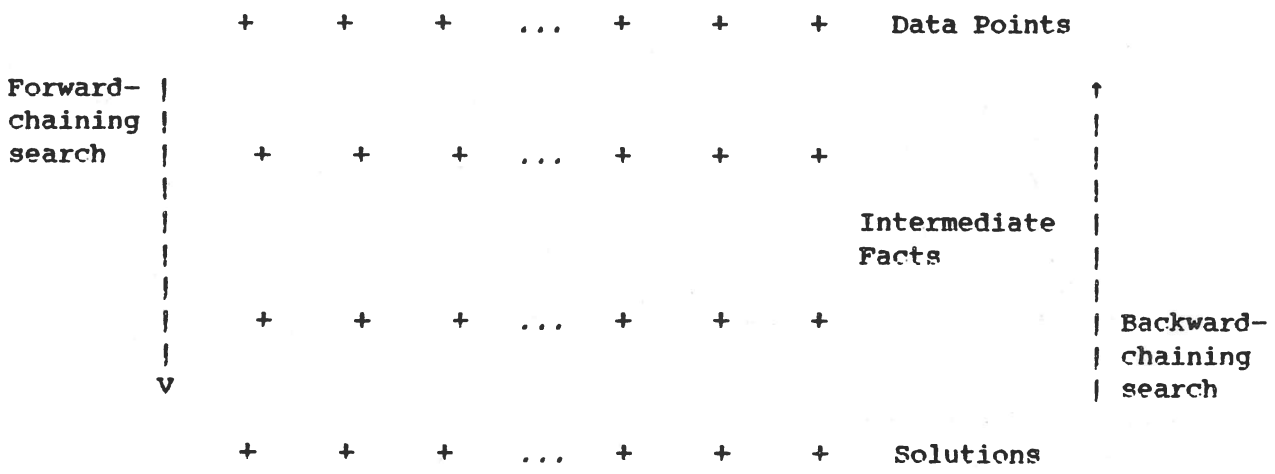

Figure 1: Forward- and Backward-chaining search.

* gut sounds are loud and gassy
* the abdomen is visibly distended
* the shape of the caecum or colon cannot be felt
* heart rate is increased up to 80 bpm
* feed includes finely chopped oat straw or similar food stuffs
* rectal examination reveals long columns of dry hard faecal material
* a mixture of faeces and water allowed to stand shows heavy sand sediment
* a stomach tube gives a gush of smelly watery fluid and relieves pain
* a rectal examination is impeded by large loops of the distended bowel

Figure 2: Extracts from the data space in diagnosis of equine colic

* impaction of the large bowel
* spasmodic colic
* sand colic
* oleocecal valve impaction
* acute gastric dilation
* subacute obstruction of the small intestine
* acute obstruction of the large intestine
* acute obstruction of the small intestine
* flatulent colic

Figure 3: Extracts from the solution space in diagnosis of equine colic.

In diagnosis, forward-chaining search involves acquiring information about the manifestations and case history, deducing further information from this, and so on, until the identity of the underlying illness is deduced. Backward-chaining search involves hypothesising the presence of a certain illness, making tests whose results confirm or reject the hypothesis, choosing another hypothesis, and so on until one hypothesis is confirmed.

If it is assumed that the patient is suffering from only one illness, then the search process, whether forward- or backward-chaining can terminate when the presence of one malady has been confirmed. This, of course, cannot be the case if the possibility of more than one illness being present is considered.

2. Criteria for Expert Systems

An expert system has been defined[Bramer81a] as "a computing system which embodies organised knowledge concerning some specific area of human expertise, sufficient to perform as a skillful and cost-effective consultant". This definition implies several criteria for expert systems.

An expert system must possess knowledge about its problem domain. It must be possible to easily update the domain knowledge possessed by the system: an expert, if he is to retain that status, must keep abreast of advances in knowledge of his problem domain. One distinguishing feature between experts and novices is that the former use short-cuts based on experience to solve problems with apparent ease while the latter adopt more exhaustive strategies based on first principles. This parsimony of effort should also be a feature of expert systems: an expert system for diagnosis, for example, should be selective in the questions it asks, particularly if these require expensive or time-consuming tests. A further characteristic of human experts, which must be emulated by expert systems, is that, if necessary , they are able to solve problems in the absence of complete data, or in the presence of incorrect or uncertain data.

The criteria just given must be satisfied if an expert system is to be a skillful and cost-effective problem solver. Two further criteria must be satisfied, however, if an expert system is to be acceptable to its users as a consultant. Firstly, an expert system must display a sensible line of reasoning to its users, if it is to acquire and retain their confidence. For example, the system should ask questions in a coherent way, not jumping about in a fashion which, although it might be computationally efficient, would seem odd to users who are in any way acquainted with the problem domain. At no stage in a consultation should the system ask questions which would not be asked by a human expert; for example the system should not ask questions whose answers are derivable from the answers to previously asked questions.

Secondly, an expert system must be able to explain its own behaviour to its users: a consultant diagnostician, for example, is able to explain why he needs whatever information he requests during a consultation and he is also able to justify whatever conclusions he reaches. In Figure 4, an extract from a consultation with a simple† system for diagnosis of equine colic is given. Whenever this system asks a question, the user can either reply to the question or he can himself ask why the question was posed.

† The system shown is in the early stages of its development. It is not yet able to cope with uncertain or incorrect data, and its performance level is still probably that of a novice rather than an expert. It is being developed as a project by Louise King, a third year computer science student.

In the latter case, the system is able to explain the line of reasoning which led to the question being asked. Finally, when the system reaches a conclusion and prints this out, the user is asked whether he desires justification of the finding; if so, this can be provided.

```
New Consultation
================
Is it true that the horse is old? no.
Is it true that the horse has poor teeth? why.

I am investigating whether the history suggests impaction.
To do this I need to know whether the horse has poor teeth
because I am using the following rule:
The history suggests impaction if the horse has poor teeth.

Would you like any further explanation? yes.

I am investigating whether the horse is suffering from impaction
of the large bowel. To do this I need to know whether the history
suggests impaction because I am using the following rule:
The horse is probably suffering from impaction of the large bowel if
the history suggests impaction and pain is moderate and no gut sounds
are present and rectal examination reveals long columns of dry hard
faecal material and the shape of the caecum or colon cannot be felt.

Now please answer the original question.
Is it true that the horse has poor teeth? no.
Is it true that grazing is on sandy country and feed is sparse? no.

      ( and so on ..... until the system reaches a conclusion)

Is is true that the horse has a history of recurrent moderate or
persistant mild colic? yes.
Is it true that there are distinct findings of thickened small intestinal
wall and distended loops in rectal examination? yes.
Is it true that gut sounds are normal to hypernormal? yes.

The horse is probably suffering from subacute obstruction of the small
intestine.
Do you want me to justify this? yes.

This diagnosis was made because the following was found to be true:
     the horse has a history of recurrent mild colic;
     there are distinct findings of thickened small intestinal wall
     and distended loops in rectal examination;
     gut sounds are normal to hypernormal.
```

Figure 4: Extract from a consultation with a simple system for diagnosis of equine colic, showing the ability to explain its question-asking behaviour and justify its findings.

3. Programming Tools for Expert Systems:

Although the main theme in the field of Artificial Intelligence is that problem-solving can be modelled as search, it is closely followed by a second theme which is that, in all but the most trivial of problem domains, knowledge must be used to constrain search. Consequently, much effort in AI research has been devoted to developing languages which facilitate the representation of real-world knowledge in a form close to the representations used by humans.

It would be possible to construct an expert system using one of the traditional programming languages such as Basic, Fortran or Pascal. However, it would be a very time-consuming task because, although these languages are often called high-level, the facilities they offer are too primitive to represent the kinds of information used by human experts, with any degree of ease.

Expert systems are usually implemented in one of the higher-level languages developed specifically for AI applications. Sometimes, one of the classic AI languages such as Prolog[Clocksin81a] or Lisp[Wilensky84a] is used. Most expert systems are not, however, implemented directly in these languages. Instead, Lisp and Prolog are used to implement even higher-level languages within which expert systems are then implemented. Such very high-level languages include OPS5[Forgy81a] which is implemented in Lisp and PROPS[Bowen85a] which is implemented in Prolog.

The expressive power of these higher-level languages can be gauged by the following extract from a toy expert system for configuring grocery orders. One rule of thumb associated with this task might be that when a grocery order contains potato_crisps but does not contain pepsi, then pepsi should be added to the order, on the basis that potato_crisps are salty and thirst-inducing. This rule can be stated in PROPS as follows:

```
when task_is_check_order
     and ordered(Purchases)
     and potato_crisps in Purchases
     and untrue(pepsi in Purchases)
then make Newpurchases = pepsi plus Purchases
     and replace ordered(Purchases)
                 by ordered(Newpurchases).
```

Although languages such as OPS5 and PROPS are designed to provide the kind of expressive power required for building expert systems, they are nevertheless general-purpose languages. The price of this generality is that constructing a particular expert system in one of these languages still requires a modicum of programming effort, albeit much less than would be required if the system were built in Lisp or Prolog, and certainly much less than would be the case if traditional programming languages were used. Recently, however, a third class of programming tool for expert system development has emerged which removes the need for programming: the expert system "shell".

An expert system requires both domain knowledge and some mechanism (often called an inference engine) for applying this knowledge to solving problems in the domain (Figure 5). An expert system shell provides a formalism for expressing domain knowledge and a ready-built inference engine for applying this knowledge to problem-solving. The inference engine includes facilities for asking questions, and providing explanations. The

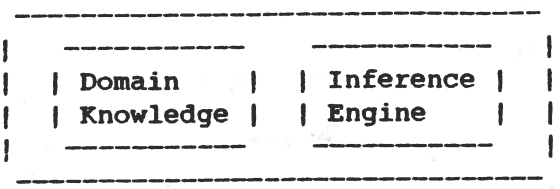

Figure 5: The two functional components of an expert system.

expert system developer merely has to identify the requisite domain knowledge (in most cases still a non-trivial task), express this knowledge in the formalism provided, and "plug" it into the shell.

The concept of the expert system shell evolved when the developers of MYCIN,[Shortliffe76a] the earliest diagnostic expert system, which was concerned with anti-microbial treatment, decided that the architecture of MYCIN could be applied to other, broadly similar, diagnostic problems. They constructed a shell called EMYCIN (or empty MYCIN)[Hayes-Roth83a] by stripping the MYCIN program of everything that was specific to the task of anti-microbial treatment. New diagnostic expert systems, in other domains, were then constructed by couching knowledge about these new domains in the formalism originally used to represent MYCIN's knowledge of anti-microbial treatment. Recently, however, a trend in purpose-built expert system shells has developed: these are constructed without going through the initial phase of building a specific expert system and stripping it of its domain-specific knowledge. While expert system shells can significantly reduce the effort involved in building an expert system, there is one major caveat. Expert system shells achieve their considerable power by narrowing their focus. A shell can only be used to construct an expert system if the target problem belongs to the class of problems for which the shell was designed.

The expert system whose use was illustrated in Figure 4 was built using a simple shell designed specifically for diagnostic tasks. The shell does not provide any facility for reasoning with incomplete or uncertain data. It uses a backward-chaining problem-solving strategy. That is, the inference engine chooses a hypothesis which it then tries to confirm through information acquired from the user. The shell assumes that only one illness is present and stops when a hypothesis has been confirmed.

All domain knowledge has to be expressed in one of the forms shown in Figure 6. Knowledge about possible solutions is expressed as "hypothesis" statements. Knowledge about the kinds of information necessary and sufficient to confirm these hypotheses is expressed as " .. if .. and .. " statements. A hypothesis may require multiple levels of evidence: that is, conditions in the RHS of some "if" statements may correspond to the conclusions in the LHS of other "if" statements. Knowledge that some possibilities are excluded by the presence of others is expressed in the form of "not .. if .. and .. " statements; conditions in the RHS side of a "not if" statement may correspond to answers to previously asked questions or they may correspond to conclusions in the LHS of "if" statements.

Although this shell offers only a very simple formalism for representing domain knowledge and restricts user replies to one of three words (yes, no, why), it nevertheless provides a quite effective user interface through its well-developed explanation facilities. It presents a sensible line of reasoning to the user because its backward-chaining search strategy means that at all times its questions are focussed on some specific hypothesis and because it avoids asking questions whose answers are derivable from the answers to previously asked questions.

More sophisticated shells offer other facilities including the handling of

uncertain data and acceptance of information volunteered by the user. Several techniques are used for handling uncertainty in expert systems. These include Bayesian probability theory,[Gaschnig80a] confidence factors,[Shortliffe76a] and fuzzy logic.[Zadeh83a] None of these methods is entirely satisfactory, however, and dealing with uncertainty is still the subject of research.

```
hypothesis : <text string>.

<text string> if <text string> and
                 <text string> and
                      ...
                 <text string>.

not <text string> if <text string> and
                     <text string> and
                          ...
                     <text string>.
```

Figure 6: The knowledge representation formalism provided by the shell used to construct the system shown in Figure 4.

The horse is probably suffering from impaction of the large bowel if the history suggests impaction and pain is moderate and no gut sounds are present and rectal examination reveals long columns of dry hard faecal material and the shape of the caecum or colon cannot be felt.

The horse is probably suffering from sand colic if some ingestion of sand or soil may have occurred and pain is severe and it is possible to palpate impacted loops containing sand and a mixture of faeces and water allowed to stand shows heavy sand sediment.

The horse is probably suffering from subacute obstruction of the small intestine if the horse has a history of recurrent moderate or persistent mild colic and there are distinct findings of thickened small intestinal wall and distended loops in rectal examination and gut sounds are normal to hypernormal.

The history suggests impaction if the horse is old.

The history suggests impaction if the horse has poor teeth.

Some ingestion of sand or soil may have occurred if grazing is on sandy country and feed is sparse.

Figure 7: Some extracts from the domain knowledge used by the simple system shown in Figure 4.

5. Knowledge Acquisition

Even in cases where a shell is used, construction of an expert system for any non-trivial domain is still a difficult task. This is because of the so-called "knowledge acquisition bottleneck": it is very difficult to formalise the knowledge used by experts to achieve their high levels of problem-solving performance.

In most cases, it seems,[Feigenbaum79a] the experts themselves find it difficult to describe the strategies and rules of thumb which they use with such evident success. Truly expert systems[McDermott81a] have required for their development many man-months of interaction between human experts and system builders. The system builders, the so-called knowledge engineers, interview the human experts and incorporate the knowledge thus acquired into a program which the experts are then invited to criticise. This process is performed repeatedly until the required level of performance is achieved; many iterations are usually required.

In order to expedite the knowledge acquisition process, research is currently being undertaken into automated knowledge acquisition. This research is based on the observation that human experts are usually better at expressing their knowledge through examples rather than as explicit principles. Research in automated knowledge acquisition is concerned with devising programs for inducing explicit rules from case examples provided by human experts. Some success has been achieved by systems which adopt a statistical approach to rule induction. Expert systems based on statistically induced rules have correctly solved problems in soybean pathology, for example.[Michalski80a] There is one major drawback, however, to such rules: they are often not very suitable for explaining expert system behaviour; the statistical basis of the rules means that, while they may produce effective problem-solving behaviour, the reasoning behind them is often opaque to humans. Consequently, efforts are being made to find alternative methods of rule induction.

References

Bowen85a. Bowen, J A, The PROPS User Manual, University of Reading, Dept. of Computer Science (1985). Forthcoming Technical Report

Bramer81a. Bramer, M. A., ``A survey and critical review of expert systems research,'' Proceedings BCS Conference, pp.486-515 (1981).

Clocksin81a. Clocksin, W. F. and Mellish, C. S., Programming in Prolog, Springer-Verlag, Berlin (1981).

Feigenbaum79a. Feigenbaum, E. A., ``Themes and case studies of knowledge engineering,'' pp. 3-25 in Expert systems in the microelectronic age, ed. Donald Michie, Edinburgh University Press (1979).

Forgy81a. Forgy, C. L., OPS5 User's Manual, Department of Computer Science, Carnegie-Mellon University (1981). Technical Report No. CMU-CS-81-135

Gaschnig80a. Gaschnig, John, ``An application of the PROSPECTOR system to DOE's National Uranium Resource Evaluation,'' Proceedings AAAI, pp.295-297 (1980).

Hayes-Roth83a. Hayes-Roth, R. D., Waterman, D., and Lenat, D., Building expert systems, Addison-Wesley (1983).

McDermott81a. McDermott, John, ``R1 The Formative Years,'' The AI Magazine, pp.21-29, American Association for Artificial Intelligence (Summer 1981).

Michalski80a. Michalski, R. S. and Chilausky, R. L., ``Knowledge Acquisition By Encoding Expert Rules Versus computer Induction From Examples: A Case Study Involving soybean Pathology,'' International Journal Of Man-Machine Studies 12, pp.63-87 (1980).

Shortliffe76a. Shortliffe, Edward Hance, Computer-based medical consultations: MYCIN, American Elsevier (1976).

Wilensky84a. Wilensky, R, LISPcraft, W W Norton & Co., London (1984).

Zadeh83a. Zadeh, Lofti A., ``Commonsense Knowledge Representation based on Fuzzy Logic,'' Computer, pp.61-65, IEEE, special issue on Knowledge Representation (October 1983).

THE USE OF SPREAD-SHEET MODELS IN ASSESSING ECONOMIC IMPLICATIONS OF DISEASE CONTROL POLICY

ALEXANDRA P. M. SHAW *

Spread-sheets are software packages which present the user with a large blank matrix, into which he can enter text headings, numbers and formulae linking those numbers, so as to write his own programmes without the need to learn a computer programming language. The best known spreadsheets are marketed under the names of Supercalc and Visicalc.

In order to understand how simple programming using a spread-sheet can be undertaken, a section of a farm budget is given in Table 1. So as to make it possible to follow how a spread-sheet is used, the letter headings for the columns and the number headings for the rows are left visible. Normally, for better presentation of tables, once the calculations have been accomplished, the column and row headings are suppressed.

The whole matrix available on Supercalc, which is the package used here, consists of 254 rows and 66 columns (lettered A to Z, AA to AZ, BA to BK). Each box in the matrix is called a cell and has a cell reference given by its column letter and row number. Pressing certain letter and control-key combinations enables you to move from cell to cell. Generally the programmes a user writes on Supercalc do not require the use of more than a fraction of the whole matrix. Depending on the capacity of the computer used and the complexity of the formulae entered, the amount of computer memory available may pose a constraint upon the size of the programme written.

Turning to Table 1, this shows that section of the Supercalc matrix starting at cell A1 and ending with cell D38. The widths of individual columns can be specified. Thus column A has been widened to incorporate the wide headings needed. Column B shows the normal print out. As stated at the beginning, three types of entry are possible into each cell:

a) Text
   This is given in cells B1, B7, D7 and throughout columns A and C (which contains the asterisks indicating which figures are to be entered when completing the farm budget).

b) Numbers
   These are the true 'variables' of the model written by the user. Once the basic model is written these can be changed again and again, in this case to show price variations. All the numbers required in column B are indicated by an asterisk given in column C.

c) Formulae
   These link the variables using simple algebraic notation, designating them by their cell references. To help understand how the spreadsheet works the formulae used in column B are listed as text in column B. The sign * (the asterisk) is used to indicate multiplication, / (a slash)to indicate division etc. Other formulae available but not

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used in this example are SUM (giving the sum of a specified row or column of figures), AVERAGE (giving the average of a row or column of figures), NPV (giving the net present value at a given discount rate of a given row or column of figures). Trigonomometric functions, logarithms, exponents and scientific notation can be used. More complex programming is possible using the OR, AND, NOT and IF functions related to the values or range of values calculated for certain items.

Table 1 Example of Part of a Farm Budget on Supercalc

	A	B	C	D
1	GROSS MARGIN PER COW FOR FRIESIAN DAIRY COWS		Filename: GM/DA	
2				
3	(Prices in UK pounds)	Figures		Formulae
4				
5	Yield (litres)	5250 *	or B9	B10*4.5461
6	Yield (gallons)	1155 *		B9/4.5461
7	Price per litre	.146 *		.146
8				
9	Milk Value	766.5		B9*B11
10				
11	- Concentrate Cost	210 *		210
12				
13	= Margin of Milk Value	556.5		B13-B15
14	over Concentrates			
15				
16	Data for Herd Depreciation:			
17	Cull cow Value	350 *		350
18	Replacement Heifer Cost	700 *		700
19	% of herd replaced annually	24 *		24
20				
21	- Herd Depreciation	84		(B23/100)*(B21-B22)
22				
23	+ Value of Calf	75 *		75
24				
25	- Miscellaneous Costs	60 *		60
26				
27	= GROSS MARGIN before deducting	487.5		B17-B25+B27-B29
28	forage variable costs			
29				
30	- Forage Variable Costs	75 *		75
31				
32	= GROSS MARGIN PER COW	412.5		B31-B34
33				
34				
35	INSTRUCTIONS: Complete all cells marked with an asterisk - *. Press			
36	! to initiate calculation			
37	NOTE: For the readers interest the formulae used have been			
38	entered as text in column D.			

Looking at Table 1, the calculation of the gross margin can be followed by moving down column B. The formulae first convert the litres milk yield to gallons or vice versa, then multiply these by the price of milk to derive the value of the milk produced. Herd depreciation and miscellaneous

costs are deducted from this and the value of the calf added, to obtain the Gross Margin before deducting forage variable costs. After deducting the forage variable costs from this the Gross Margin is obtained.

Thus the use of spreadsheets enables a beginner to start to write programmes in a very short period of time. These programmes are immediately presented in the tabular form given by the matrix indicates. They can be saved onto a floppy or hard disk and reloaded when needed. Some of the numbers or variables can then be altered, and then the new version of the calculations saved under a different file name, if the user wants to retain it. Thus the farm budget in Table 1 could be recalculated assuming a different yield, under a number of different price assumptions, or updated every year. These Supercalc files can be saved in such a way that they can later be incorporated into files of text written using a word-processing package - which is what has been done here. Thus once the results are obtained, they can be presented as tables in a text, without the need for retyping, and with the possibility of recalculating the figures and substituting more up to date or more accurate versions of the table as required.

A much more complex model model written using Supercalc is presented in Table 2. This is called 'NATDAIRY' and refers to the nation's population of dairy breed cattle - both males and females. This population is treated as a mixed herd, linked by the main production parameters which are entered by the user for each of ten years. These are:

- mortality for each age/sex group from cells A24 to G38,
- offtake for each age/sex group from cells A40 to G55,
- calving and milk production data from cells A56 to G71.

The initial inputs are the size of the herd, entered in cell C11 and the intial herd composition, entered in cells B18 to C21. Prices are also entered for each category of animal sold in cells D18 to E21 and for milk in cell F146.

The results calculated by the model begin at row 74. The herd composition in terms of males and females at the start of each year is given, together with the number of animals culled or slaughtered, and the number of live births. The live births are divided between the dairy breeds and the beef crosses. The latter are sold out of the herd aged a few weeks, after some mortality at the rate given in cell F71. The herd composition thus includes the dairy breed calves, both male and female, none of whom are actually slaughtered before they reach age one - thus there is no category for offtake in this age group. The calves aged 0 to 1 are fewer in number than the live births since some of the mortality (the proportion specified in cells F64 and F66) has already occurred.

The total value of output is calculated from cell A105 to G119. It is composed of milk, culled females, males sold for slaughter and beef cross calves sold out of the herd.

The last section of the model, from cell A121 to G135, gives the total herd size, growth in the total herd, the number of dairy cows and the final herd composition.

This basic format of model can be used to deal with individual farm herds, purely beef or dairy herds, mixed nomadic herds etc., according to

circumstances. The parts of the model that generally need adapting are the offtake rates, for example to reflect the sale of all male animals at a young age where a dairy herd is considered or conversely to reflect the retention of both male and female animals till an advanced age in a nomadic herd. Obviously far higher mortality rates would apply in this sort of herd, and it would not be practical to try and make distinction between loss of calves because of stillbirth and those due to neonatal mortality. The main individual feature of this model of a national herd of dairy animals is in providing the option of having pure dairy calves which provide the female replacements for the dairy herd or of having beef cross calves.

To demonstrate how such a model can be used for the economic evaluation of disease effects, the run of the model presented shows how two policies affecting milk production would be translated into output, into a different herd composition and into a change in dairy cow numbers. A three year national campaign to encourage the control of mastitis is reflected initially in higher culling rates for adult females (shown in cells B45 to B47) and, if accompanied by dry cow therapy, could lead to a once and for all improvement in milk yields of say, 10% in 10% of cows, or 1% overall. At the same time the milk yield of dairy cows is increasing by between 2% and 5% annually anyway. An average baseline increase of 3% per annum is taken, and to this is added on third of 1% for each of the first three years when the mastitis programme is underway. The effect of this (cells D62 to D71) is to increase yield by over 10% in three years, and from 5000 litres to 6600 in ten years.

However, at the same time as these effects are taking place, the European Economic Community is imposing quotas on milk production. In order to maintain milk production at the level it was in year one a change in the breeding policy, involving an increase in the proportion of cows producing beef calves is incorporated. This proportion is shown as increasing from 30% to 42% in four years. At this rate the decline in dairy cow numbers at 3.07% per year is much the same as the increase in milk yields. After some fluctuation, milk output settles at a similar level to what it was in year 1. If there had been no increase in the proportion of beef calves produced, with this remaining constant at 30% the number of dairy cows by year 10 would have been 3,361,000 as against 2,463,000 in this example (note that figues in the model are given in thousands - since the initial herd size was in thousands). Although the same amount and value of milk (since no price changes have been included at this stage), the actual value of output is lower, since more beef cross calves are being produced, and these obviously sell for less than the dairy males reared and considered as part of the herd do. However, this model does not include production costs. Although the cost per dairy cow may have increased somwhat, overall production costs will be miuch lower since fewer dairy cows and fewer dairy males are being maintained.

To complete the costs side of this economic evaluation the cost of dry cow therapy would have to be calculated. Here again the spead-sheet greatly facilitates matters, since the number of cows can be taken as a column from this model and cross-loaded into another model, where the numbers are multiplied by the unit costs. This is done by pressing a few command keys, and the retyping or reentering of columns of figures every time they are used in a different context is thus avoided. The effect of

culling is already reflected in the output calculation. To the benefits would be added an amount for veterinary and drug costs saved for each case of mastitis - the cost multiplied by the total number of cows and by the percentage affected.

As a final step the costs and benefits could be presented together and discounted so as to give a benefit cost ratio, net present value and internal rate of return - the standard measures of benfit-cost analysis. Here again the spread-sheet is ideally configured. The figures can be cross-loaded from the existing models and compared. Supercalc has an inbuilt discounting function.

Herd models of this type thus provide all the variables - mortality, fertility, culling rates and milk yields which are commonly affected by disease. The improvement in these parameters resulting from any disease control measure can thus be entered in the herd model, and the effect on output calculated. As well making as such changes in the production parameters or variables of the model, adjustments to the nature of the model can be made readily so as to reflect different production systems. To pursue the economic analysis further, the numbers in each age/sex category can then be multiplied by the cost of the measures and the production costs. Then the full costs and benefits of the disease control measure being evaluated can be calculated and compared over a period of years, using the techniques of cost-benefit analysis.

Table 2 An Output Model for the National Herd of Dairy Breed Cattle

	A	B	C	D	E	F	G
1	BASIC HERD MODEL ADAPTED TO THE NATIONAL DAIRY CATTLE HERD				NATDARY2		
2	==========				========		
3							
4	INSTRUCTIONS: Fill in C11, E11, G11, F17, E64, E66 and E71 and all						
5	labelled columns from B18 to G71 (except cells B22 and C22). Press /G Global), then R (for						
6	(for Global), then R (for rows), the press ! to calculate.						
7							
8	PARAMETER ENTRY						
9	----------						
10							
11	INITIAL SIZE OF HERD (number)?		7718	SPECIES ? Dairy Cattle		CURRENCY ?UK £	
12			(thousands)				
13							
14	INITIAL HERD COMPOSITION AND PRICES						
15							
16		% of Herd		Sale Prices		Price per	
17	Age	Females	Males	Females	Males	Litre of Milk	
18	0-1	13.01	12.73	65	55	.146	
19	1-2	11.67	11.15	250	500		
20	2-3	10.11	5.22	315	650		
21	3+	35.57	.54	400	700		
22	TOTAL	70.36	29.64				
23				Note: only beef cross calves are sold before age 1.			

	A	B	C	D	E	F	G
24	PRODUCTION PARAMETERS - MORTALITY						
25							
26		Adult	Young adult	Young animal	Calf	Females	Male
27	YEARS	Age 3+	Age 2 - 3	Age 1 - 2	Age 0 - 1	Stillborn	Stillborn
28		%	%	%	%	%	%
29	1	1.5	2	3	6	8	10
30	2	1.5	2	3	6	8	10
31	3	1.5	2	3	6	8	10
32	4	1.5	2	3	6	8	10
33	5	1.5	2	3	6	8	10
34	6	1.5	2	3	6	8	10
35	7	1.5	2	3	6	8	10
36	8	1.5	2	3	6	8	10
37	9	1.5	2	3	6	8	10
38	10	1.5	2	3	6	8	10
39							
40	PRODUCTION PARAMETERS - OFFTAKE (FEMALE CULLS AND MALE SLAUGHTER)						
41							
42		Female	Male	Female	Male	Female	Male
43		Age 3+	Age 3+	Age 2 - 3	Age 2 - 3	Age 1 - 2	Age 1 - 2
44	YEARS	%	%	%	%	%	%
45	1	26.5	75	12	90	10	50
46	2	26.5	75	12	90	10	50
47	3	26.5	75	12	90	10	50
48	4	22.5	75	12	90	10	50
49	5	22.5	75	12	90	10	50
50	6	22.5	75	12	90	10	50
51	7	22.5	75	12	90	10	50
52	8	22.5	75	12	90	10	50
53	9	22.5	75	12	90	10	50
54	10	22.5	75	12	90	10	50
55							
56	OTHER PARAMETERS - CALVING AND MILK PRODUCTION						
57							
58		Pregnancies	Pregnancies	Milk	Percentage	Proportion of	
59		to term per	to term per	produced per	of Calvings	Mortality 0-1	
60		Female aged 3+	Fem. aged 2-3	Live Birth	which are	occuring in	
61	YEARS	%	%	litres	beef crosses	1st six months	
62	1	92	95	5000	30	%	
63	2	92	95	5167	35	Females:	
64	3	92	95	5339	40	75	
65	4	92	95	5516	42	Males:	
66	5	92	95	5682	42	75	
67	6	92	95	5852	42	Percentage of	
68	7	92	95	6028	42	Beef calves	
69	8	92	95	6209	42	dying before	
70	9	92	95	6395	42	sale:	
71	10	92	95	6587	42	5	
72							
73							

	A	B	C	D	E	F	G	H
74	RESULTS							
75	-------							
76								
77	HERD COMPOSITION - FEMALES AT START OF YEAR					EVENTS OCCURING DURING YEAR:		
78						Culls of	Dairy	Beef crosses
79	YEARS	Age 3+	Age 2 - 3	Age 1 - 2	Age 0 -1	Dairy Females	Live Births	Live Births
80	1	2744.96	780.07	900.58	1004.46	911.08	1051.79	450.77
81	2	2647.24	783.51	904.54	907.97	885.99	950.76	511.95
82	3	2579.83	786.95	817.65	822.64	859.85	861.41	574.27
83	4	2534.25	711.36	740.81	766.24	729.65	802.35	581.01
84	5	2537.80	644.51	690.02	750.89	717.35	786.27	569.37
85	6	2483.00	600.32	676.20	727.35	698.33	761.62	551.52
86	7	2403.35	588.29	655.00	705.77	676.85	739.03	535.16
87	8	2332.48	569.85	635.56	684.69	656.75	716.95	519.17
88	9	2262.75	552.94	616.58	664.25	637.13	695.55	503.68
89	10	2195.22	536.43	598.18	644.43	618.11	674.79	488.64
90								
91	HERD COMPOSITION - MALES AT START OF YEAR					EVENTS OCCURING DURING YEAR:		
92						Sales of	Dairy	Beef Crosses
93	YEARS	Age 3+	Age 2 - 3	Age 1 - 2	Age 0 - 1	Dairy Males	Live Births	Live Births
94	1	41.87	402.67	860.51	982.63	824.86	1028.93	440.97
95	2	42.05	404.44	864.30	888.23	827.69	930.09	500.82
96	3	42.24	406.22	781.27	804.76	787.91	842.68	561.79
97	4	42.42	367.20	707.85	749.58	716.22	784.91	568.38
98	5	39.35	332.69	659.32	734.57	658.59	769.18	556.99
99	6	35.86	309.88	646.11	711.54	628.84	745.07	539.53
100	7	33.22	303.67	625.86	690.43	611.15	722.96	523.52
101	8	32.10	294.15	607.29	669.81	592.46	701.37	507.89
102	9	31.08	285.42	589.15	649.81	574.76	680.43	492.73
103	10	30.14	276.90	571.56	630.42	557.59	660.12	478.02

AMOUNT AND VALUE OF OUTPUT (thousands of UK £)

YEARS	Milk Total Litres	Milk Total Value	Value of Females Culled	Value of Males Sold	Value of Beef Cross Calves Sold	TOTAL OUTPUT VALUE
1	1633218200	238449857.15	34296738.75	47267213.37	50875.56	320064684.83
2	1642837517	239854277.51	33283717.20	47475093.99	57780.33	320670869.03
3	1666182565	243262654.42	32364949.26	45513233.63	64814.70	321205652.01
4	1658922462	242202679.46	27349222.19	41404674.12	65575.24	311022151.01
5	1674459423	244471075.75	27001458.71	38011052.35	64261.55	309547848.35
6	1670622704	243910914.80	26306694.54	36163546.18	62246.90	306443402.42
7	1669684968	243774005.40	25491404.93	35155192.87	60399.96	304481003.15
8	1668413532	243588375.69	24735226.70	34075315.52	58596.08	302457513.99
9	1667171677	243407064.86	23996323.48	33057536.45	56847.05	300517771.84
10	1665929601	243225721.73	23280094.16	32069931.46	55150.20	298630897.54

TOTAL HERD PRODUCTION PARAMETERS OVER THE YEARS

YEARS	Total Size of Herd (number)	Total Cows Aged 2.5 + (number)	Annual Growth Rate of Cow Numbers %
1	7718	3135	---
2	7442	3039	-3.86
3	7042	2973	-2.16
4	6628	2890	-2.80
5	6389	2860	-1.03
6	6190	2783	-2.69
7	6006	2697	-3.08
8	5826	2617	-2.97
9	5652	2539	-2.99
10	5483	2463	-2.98

FINAL HERD COMPOSITION

Age	% of Herd Females	% of Herd Males
0-1	11.75	11.50
1-2	10.91	10.42
2-3	9.78	5.05
3+	40.03	.55
TOTAL	72.48	27.52

THE COMPUTERISATION OF THE CENTRAL VETERINARY LABORATORY FACILITIES AND FUTURE PLANS FOR COMPUTING IN THE STATE VETERINARY SERVICE

K. W. ROBEY*

Computers have been used in the State Veterinary Service (SVS) for a number of years but are only now beginning to make a major impact on work of the Service. This paper will discuss the development and integration of computer applications within the SVS.

THE PAST

Up to 1983 MAFF used two ICL 1900 series mainframe computers installed at the Ministry's offices at Guildford. These were the only sources of significant computer power available. The SVS had several systems running on these computers. The main systems being:

- Brucellosis Eradication Scheme
- Veterinary Investigation Diagnosis and Analysis (VIDA)
- Zoonoses Order

In 1968 the Ministry were one of the first Civil Service departments to invest in this range of computers but in the intervening fifteen years newer and more powerful computers came on the market. By the late seventies the Ministry was finding that these computers could not meet the growing demands for facilities. The 1900 range of computers were no longer being manufactured and had by the standards of the day primitive software tools and only very limited capacity for on-line interactive computing.

The Central Veterinary Laboratory (CVL) partly overcame these limitations by using a commercial computer bureau to computerise its Notifiable Disease Systems referred to by Williams (1984).

The introduction of Prime computers

In 1980 the Ministry reviewed its computing requirements and from that review a decision was made to distribute computing facilities to a number of MAFF sites one of which was the CVL. There followed an open tender procurement exercise in which Prime Computers (UK) Ltd were awarded the contract. A Prime 550 model computer was installed at CVL in August 1983 and is supported by a small team of Data Processing staff on site. This computer is linked to other Prime computers in the Ministry and forms the MAFF Prime Network. This enables the Veterinary Service to access additional facilities available on other Prime computers within the Network.

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THE PRESENT

The Prime computers support powerful software tools and flexible interactive computing facilities. Software packages are available for:

- Test Handling
- Data Management
- Statistics
- Accounting
- Graphics

Computer systems

These software tools have been used to develop applications for the SVS. Each CVL Department is developing its own Departmental Bibliographic Index using STATUS, a text handling package, which runs on a Prime computer at Guildford. These Departmental indexes are created by amalgamating the data from officers personal index cards. The CVL Library's Current Awareness List which is also stored on computer and uses STATUS, provides additional information which can be inserted into the Departmental Index systems.

Financial accounting

The financial accounting applictions are being computerised. Purchase Ledger and Stores are two such systems which utilise finance packages purchased from Memory Computer Services Ltd. A computerised project costing system is also being developed, initially only accounting for manpower resources but eventually other costs will be transferred to this system from other computer applications. An example being the CVL Department of Animal Production system which will provide information on the costs associated with the housing of animals.

Statistical and numerical analysis

A major use of the CVL computing facilities is in the analysis of data arising from experiments and surveys carried out by the Veterinary Service. Various software packages and subroutines are used for this work such as GENSTAT and GLIM for statistical analysis and the NAG library for numerical analysis.

Notifiable disease systems

There are a set of computer systems on the CVL Prime which are associated with the control of notifiable disease. They have been transferred from the commercial bureau and enhanced using a data management package called INFORMATION. These systems contain information on strategic premises, outbreak records and the status of herds and flocks in relation to certain accredation schemes.

Existing ICL mainframe systems

There still exists a core of the systems that were implemented on the ICL computers at Guildford. The old 1900 series computers have been replaced by an ICL 2966 mainframe computer but by use of the Direct Machine Environment (DME) operating system the computer applications have been kept running with minimal modification.

Computer trials using microcomputers

Computer trials using microcomputers in Animal Health Offices (AHOs) and a Veterinary Investigation Centre (VIC) have recently been completed. These trails have demonstrated the advantages of computerisation to these offices. In Leeds AHO an epidemic management system was developed as an aid to tracing and spread of epidemic disease and has been described by Elliott (1984). Benefits such as the accessibility of information, speed of response and the increase in the amount of information available have been identified. The system is currently being expanded and implemented on the CVL Prime as a further trail exercise.

The VIC trial has shown that there are benefits to these offices as well. A sample handling system for both the control and recording of results has improvied the efficiency of the Centre.

THE FUTURE

The next few years will see the Prime computer systems continuing to be developed and becoming more integrated. The MAFF Prime network will expand to link up computer facilities in AHOs, VICs, CVL and Divisional Offices. Veterinary Service wide computer systems will typically have core information held centrally with more detailed local information residing in the AHOs and VICs. The introduction of these systems will allow the existing mainframe (ICL 2966) applications to be phased out. Investigations are underway to replace the Brucellosis system on these lines.

Preparation work has been done to provide a central Sample Handling System at CVL which will have the potential to link with the computer facilities in AHOs and VICs. Links will be made to the financial accounting systems for purpose of charging as well as providing better information to senior management.

The basic information in many of these systems will be the data on each herd enabling a fuller epidemic management system to evolve. With improvements in mapping procedures referred to by Williams (1984) will come the potential to view information in pictorial or graphical form which is likely to assist in identifying patterns of disease not easily recognised when presented as tabulations.

REFERENCES

Williams, P.A. (1984) The potential application of computerised mapping systems. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Edinburgh 10 & 11 July, 1984, pp.75-77.

Elliott, A.J. (1984) Preliminary investigation of computers as an aid to tracing the spread of epidemic disease. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Edinburgh 10 & 11 July, 1984, pp.71-74.

REMOTE USE OF COSREEL OVER THE BRITISH TELECOM PSS NETWORK

G.J. ROWLANDS* and A.M. RUSSELL*

Earlier publications (Lucey *et al.*, 1983; Rowlands *et al.*, 1983) have described the use of the COSREEL animal health recording system (Russell and Rowlands, 1983) by two agricultural colleges and their veterinary surgeons. Each college and veterinary practice was provided with a typewriter terminal connected by telephone over the British Telecom (BT) Public Switched Telephone Network (PSTN) to the COSREEL programs stored on an ICL System 4 computer at Rothamsted Experimental Station, Harpenden, Hertfordshire. The trial demonstrated the ease of use of the COSREEL coding system by veterinary surgeons in practice (Rowlands *et al.*, 1983), and identified the main problems as technical ones due to the use of an inefficient and outdated computer system.

In the summer of 1984 the Institute acquired its own Digital Equipment Corporation VAX 11/750 computer, one of a number of computers belonging to the Agricultural and Food Research Council's AGRENET computer network. The COSREEL programs were modified for the VAX computer and made simpler to use. At the same time a BBC microcomputer was installed at The Hale Veterinary Group, Chippenham so that it could be connected by telephone to the VAX computer at Compton. The purpose of this paper is to demonstrate how this link works and to discuss the potential value of this arrangement for the collection of data from a number of dairy herds for statistical analysis and epidemiological investigation.

MATERIALS AND METHODS

Figure 1 demonstrates how the BBC microcomputer in the veterinary practice is connected to the VAX computer at the Institute. In order to make the necessary connection, the telephone number of the nearest BT packet switching exchange (PSE), namely Bristol, is first dialled to gain access to the BT Packet Switch Stream (PSS). A 'packet assembler disassembler' (PAD) at the PSE controls the collection of data from the BBC, assembles groups of characters into 'packets', adds addressing information and transmits the packets into PSS. After the call is made, a series of codes are entered on the computer keyboard to identify the user and specify the address of the computer to which the packets of data are to be sent. The VAX computer at Compton is connected by a dedicated BT line to AGRENET's own PSE at the AFRC Computing Centre at Harpenden. The route from Chippenham to Compton is therefore via PSTN to Bristol, via PSS to Harpenden, into AGRENET and along a dedicated BT line to Compton.

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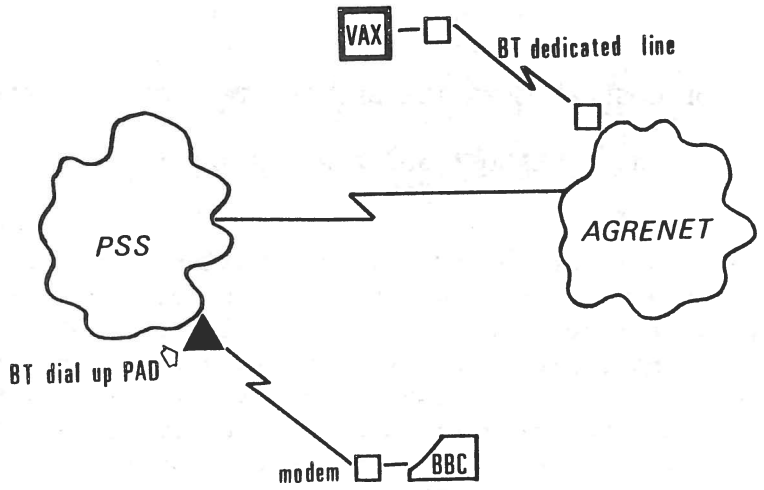

Fig. 1 A diagrammatical illustration of the telephone connection of a BBC microcomputer in a veterinary practice in Chippenham via a British Telecom dial up pad into PSS at Bristol to a VAX 11/750 computer at the AFRC Institute for Research on Animal Diseases, Compton. The VAX computer is part of the AFRC AGRENET computer network which has access to PSS at Harpenden.

The BBC microcomputer is a model B with a twin 200K single sided 40/80 track disk drive and an Epson FX80 parallel printer attached which prints at 160 characters per second. The microcomputer has software provided by the AFRC Computing Centre to enable it to both communicate as a terminal and transfer files to and from the VAX. It is connected to the BT network via a modem (Fig. 1). The cost of the hardware, including a modem capable of transmitting at 1200 bits (or about 150 characters) per second, is approximately £2,000.

Data are collected by the veterinary surgeon during each farm visit, and include management events such as calvings, services and oestruses, and veterinary events such as cases of disease diagnosed by the farmer, and treatments given. These data are entered weekly into the computer, as described by Lucey *et al.*, (1983), together with other clinical diagnoses and treatments and results of pregnancy diagnosis made by the veterinary surgeon (Rowlands *et al.*, 1983). Data may be entered directly into the VAX by using the microcomputer as a terminal. Alternatively data may first be entered into the BBC and stored on a floppy disk and when completed transmitted over the telephone line to the VAX. As soon as data have been transferred to the VAX, commands can be given to check the data for errors and update the databank. After updating, various action lists may be requested. Two of them are requested weekly, namely; (i) a fertility status of each cow listed in order of calving and (ii) a list of cows due in oestrus for each day in the coming week (Lucey *et al.*, 1983). A pregnancy diagnosis and infertility action list and second form of fertility status/oestrus detection list, as described by Lucey *et al.* (1983), are produced fortnightly. The reports may be printed at the Institute for posting to the farmer or transferred over the telephone line

for storage on the BBC microcomputer's disk and subsequent printing. Life histories of individual animals may also be retrieved and listed on the VDU screen.

Four dairy herds are currently being handled in this way.

RESULTS AND DISCUSSION

The new system has been in operation since September 1984 and many of the disadvantages of the previous system outlined by Lucey *et al.* (1983) have been overcome. Current problems are concerned mainly with the reliability of the telephone link.

Russell (1983) discussed alternative ways of storing data - in a microcomputer on the farm, in a larger computer at the veterinary practice or in an even larger computer at a central regional location. Most commercial livestock recording systems operate on microcomputers installed on farms but they offer extremely limited facilities for storing veterinary data so that the veterinary surgeon cannot be fully involved in herd analysis. Rather than place a computer in a veterinary practice, Russell (1983) argued that it would be better to keep both farm and veterinary records on a larger regional computer to which practices and farms could have direct access. This has the advantage that data collected from a number of farms and over several years can be stored in one database and be immediately accessible for research purposes (Rowlands, 1982). It is only when this is done that a proper statistical analysis can be undertaken which takes into account, for example, effects of herd, year, lactation number and previous milk production in assessing relationships between disease and milk production (Lucey, 1984; Lucey and Rowlands, 1984). Using data from 4 herds Lucey (1984) illustrated the differences in interpretation that can occur if such effects are not included. Only in Canada (Dohoo and Martin, 1984) have such research studies been done on a comparatively large number of dairy herds (32), and there is a similar need in this country to collect health and management data from this size of sample to ascertain the economic importance of disease. However, for this to be possible telephone access to the main computer must be reliable and, to be cost effective, calls must be kept short.

Two modems have been used for connecting the BBC microcomputer to PSS, one operating at 300 bits (about 40 characters) per second and one at 1200 bits per second, 4 times faster. Communication at the slower speed has been found generally reliable but the transfer rate is too slow. Communication at the higher speed is, on the contrary, much less reliable, and as yet it has not been possible to find a way of transferring a file of data from a floppy disk error free to the VAX. The transfer of action list files in the reverse direction, from VAX to BBC, is more reliable.

British Telecom software is unable to eliminate PSTN line interference at the faster speed, and data transmission can be corrupted, particularly over long distances. Such interference appears as spurious characters on the VDU screen. From Chippenham to Bristol these have occurred at a comparatively low frequency, one that can usually be tolerated. 'Noisy' lines can usually be avoided by redialling. PSS itself is designed specifically for the transfer of data, so that from the PSE exchange at Bristol to the VAX at Compton there is no further line interference. The main problem with PSS is that the telephone connection is occasionally lost. Fortunately it should soon be possible to guarantee error-free transfer of files, at least from VAX to BBC, when software for checking the accuracy of the transmitted data is

available from the AFRC Computing Centre.

It appears probable, therefore, that technical solutions can be found to solve current problems of telephone access. This being so, one needs to consider how best to utilise the computing capabilities of the BBC micro-computer to minimise telephone costs. Clearly the BBC can be used for entering and carrying out some of the validation of the data before they are sent to the main computer. As action lists require information mostly on events occurring during the current lactation it has also been thought (Lucey *et al.*, 1983) that a portion of the life histories could also be held on floppy disk on the BBC, thus enabling action lists to be produced locally. This facility would also enable the veterinary surgeon to look at life histories on the BBC screen without needing to dial the VAX computer. The main disadvantage of this approach is that it would require up to date versions of current life histories to be held on both computers, and this would be difficult to organise without writing further COSREEL programs for the BBC. A simpler approach would be to transmit from the VAX only the data values produced by the existing COSREEL action list programs, and to use simple programs on the BBC to reformat these values into the final reports. This method is being adopted.

Four veterinary surgeons serving a total of 9 farms are currently using the COSREEL system at Compton, and the East of Scotland College of Agriculture is also using COSREEL on its own VAX 11/750 computer in Edinburgh. A postal service is currently provided to the other 3 veterinary practices and farms, with one farm and veterinary practice entering their own data remotely and using VDU terminals to run the COSREEL programs. A useful databank of health records is thus being assembled so that some of Lucey's (1984) analyses can be repeated on a wider sample of herds.

As soon as current telecommunication problems are solved it is planned to evaluate the cost to the veterinary practice of running the BBC connection to the VAX with a view to assessing its viability for use by other veterinary practices.

ACKNOWLEDGEMENT

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REFERENCES

Dohoo, I.R. and Martin, S.W. (1984). Disease, production and culling in Holstein-Friesian cows. IV Effects of disease on production. Prev. Vet. Med. 2, 755-770.

Lucey, S. (1984). An investigation into the possibilities of modelling changes in milk yield, milk quality and fertility associated with disease. PhD Thesis, University of Reading.

Lucey, S., Rowlands, G.J., Russell, A.M., Foster, S.R., Wicks, B.T., Parsons, S.T.A. and Stimpson, P.M. (1983). Use of COSREEL, a computerised record system, for herd health management of two dairy herds. Vet. Rec. 113, 294-298.

Lucey, S. and Rowlands, G.J. (1984). The association between clinical mastitis and milk yield in dairy cows. Anim. Prod. 39, 165-175.

Rowlands, G.J. (1982). The use of herd health records in veterinary investigation. In 'Epidemiology in Animal Health', Proceedings of a symposium at the British Veterinary Association's Centenary Congress, Society for Veterinary Epidemiology and Preventive Veterinary Medicine, Reading, 1982, pp. 63-72.

Rowlands, G.J., Lucey, S., Russell, A.M., Wicks, B.T. and Stimpson, P.M. (1983). Use of COSREEL codes in large animal veterinary practice. Vet. Rec. 113, 336-339.

Russell, A.M. (1983). COSREEL - Computer system for recording events in economically-important livestock. Proceedings of Society for Veterinary Epidemiology and Preventive Veterinary Medicine, Southampton, 1983, pp. 29-36.

Russell, A.M. and Rowlands, G.J. (1983). COSREEL: Computerised recording system for herd health information management. Vet. Rec. 112, 189-193.

OPEN SESSION

AN ECONOMIST'S VIEW OF ANIMAL DISEASE

K.S. HOWE*

The importance of economic appraisal of the consequences of animal disease and its prevention or control has been recognised increasingly in recent years. Nevertheless, the approaches adopted have centred on quite narrowly defined cost/benefit studies and, to a lesser extent, estimates of resource wastage through livestock mortality. There has been no comprehensive attempt to interpret animal disease from the point of view of the part it plays within the economic process. Consequently, no well-defined methodology has yet been developed for analysing a wide range of important economic problems concerning, among others, the ways in which disease affects resource use in general for different types of livestock production, its relationship with choice of technology, how disease may influence the interpretation of the commercial efficiency of production in the long and short term, and what is the best way to deal with disease in farm livestock in an economic rather than a veterinary sense. This paper is intended as an introduction to some of the important conceptual issues which must be explored as the basis for subsequent applied work.

The role of economics

There is a tendency for non-economists to think of economics as concerned with problems of the nation's economy, and money. National problems, which include unemployment and inflation, belong to that part of the subject which is 'macro-economics'. Money 'per se' is, in fact, only a coincidental consideration to the main purpose of economics which is concerned with decisions affecting the availability and effective use of real resources in providing for real needs. In contrast to macro-economics, 'micro-economics' focusses attention on the choices made by the many producers and consumers who make up the national economy. Agricultural economists, for example, are applied micro-economists, and any study of the economics of animal disease draws on the concepts and techniques of micro-economic analysis. Fortunately micro-economics is free from the controversies which afflict macro-economics. A particular feature of the subject, seen exceptionally well in agriculture, is that the technical relationships which are the foundation of production are always prominent. In fact, micro-economic analysis would be largely nonsensical without them. The point is stressed, however, because sometimes economics is thought to be very different from other scientific disciplines. On first encounter some ideas can seem a little unusual, but economics is anything but a self-contained, exclusive preserve. The interpretation of animal disease from an economist's point of view should provide some justification for this argument.

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Economics and animal disease

Any production process involves the transformation of inputs into outputs. The production of animal products depends on biological processes whereby inputs of feed nutrients are changed into outputs such as meat, milk, eggs and wool. Such processes are studied widely by agricultural scientists, who describe the relationships between levels of inputs and the resulting outputs as 'response functions'. These relationships belong to a more general category known to economists as 'production functions'. Whereas agricultural scientists are interested exclusively with complex biochemical relationships between technical inputs and outputs, economists often include other inputs such as labour, the use of buildings and machinery, and management skill. For the most part these other inputs "facilitate the process" rather than undergo a direct physical transformation, but they are no less important to the economic activity of production. Without the use of labour, housing, and the rest, production of output for sale could not take place. That is one important example of where agricultural scientists and economists have different, but equally legitimate, frames of reference. Both interpretations are essential to the economic analysis of animal disease, as we shall see.

Suppose that we envisage a simple production process in which output is daily liveweight gain for a group of bullocks and the single variable input is the weight of concentrates fed per day. For obvious reasons it is easier to use a simple example, but it is intended that the interpretation should be perfectly general.

This implies a production function of the form

$$G = f(C|F, L, E, H, ...) \qquad (1)$$

where

G	=	liveweight gain per day	variable
C	=	weight of concentrates fed per day	variable
F	=	intake of forage per day	fixed
L	=	labour use	fixed
E	=	managerial expertise of the farmer	fixed
H	=	housing system	fixed

Essentially it is the response of liveweight gain to concentrates which is under examination. For animals which are deemed 'healthy' by some criteria, the response function may be described in general terms by curve 'H' in Fig.1.

Curve H indicates that higher rates of concentrate feeding give higher growth rates, but that there are diminishing returns to successive increases in concentrate use. Leaving aside for the moment the question of economic efficiency, consider the effect of disease on the response function. Insofar as disease affects the animals' biological performance, it will tend to shift the response function downwards, say to curve 'U'. Rather than contribute to production, disease actively works against it, i.e. disease is a 'negative input'. For some arbitrary level of concentrates input C_1, growth is now G_2 rather than G_1. The mathematical expression for the function should be supplemented by another variable, say 'D' for disease.

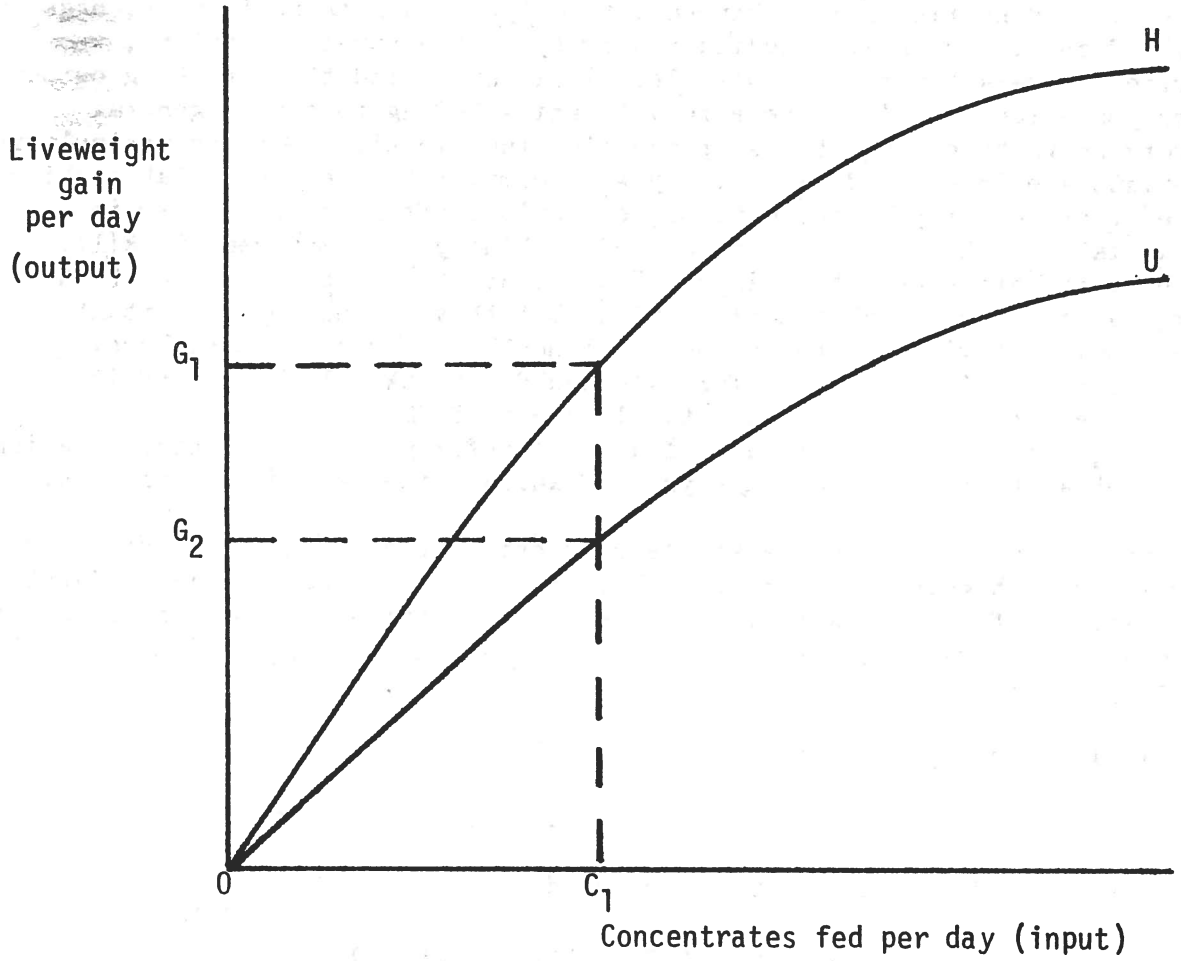

Fig. 1 . The impact of disease on output

The crucial question is how to recover growth rate G_1 having been forced back to G_2 by disease. The obvious answer is to obtain the kind of veterinary inputs which are capable of restoring G_1. An additional 'positive input' in the form of veterinary attention is necessary to offset the 'negative input' of disease. Often this will be the only course of action, and whether or not it is worthwhile from an economic point of view depends on the financial value of $(G_1 - G_2)$. If G_1 and G_2 represented the finished weight of cattle for a given quantity of feed, then the difference in market value, effectively $P(G_1 - G_2)$, where P is price, would be the maximum expenditure which could be justified on veterinary attention to recover the higher weight. In the model in Fig.1 the example is rather different, since it concerns loss of the rate of liveweight gain. The economic interpretation is made more complicated, but essentially the conclusion is the same for the reasons as follows. The loss in the rate of gain may imply the reduction in finished weight by the time it is planned to market the cattle in the sense described above. On the other hand, it can mean that the cattle will have to be kept longer to reach the same sale weight because their progress has been checked. In that case even if the

growth rate recovers to something like its initial level, the average rate of gain over time must have been reduced. The implications may be very significant for any farmer with limited housing facilities, for example, who is conscious of the economic importance of animal turnover in generating income from cattle. The retention of a batch of cattle for longer than is usually necessary often will mean a delay in bringing in the next and subsequent batches, thereby reducing annual throughput. The value of $P(G_1 - G_2)$ then becomes the daily revenue foregone over a period of time as a direct result of the fall in turnover.

Until now the example has been explained as if the only reaction to disease is to make use of veterinary inputs. In many instances this will be so, but not always. There are circumstances when it is feasible to substitute other inputs for veterinary attention. In justification of this statement we first reconstruct Fig.1 in the shape of Fig. 2 which, though strikingly similar, has a different interpretation.

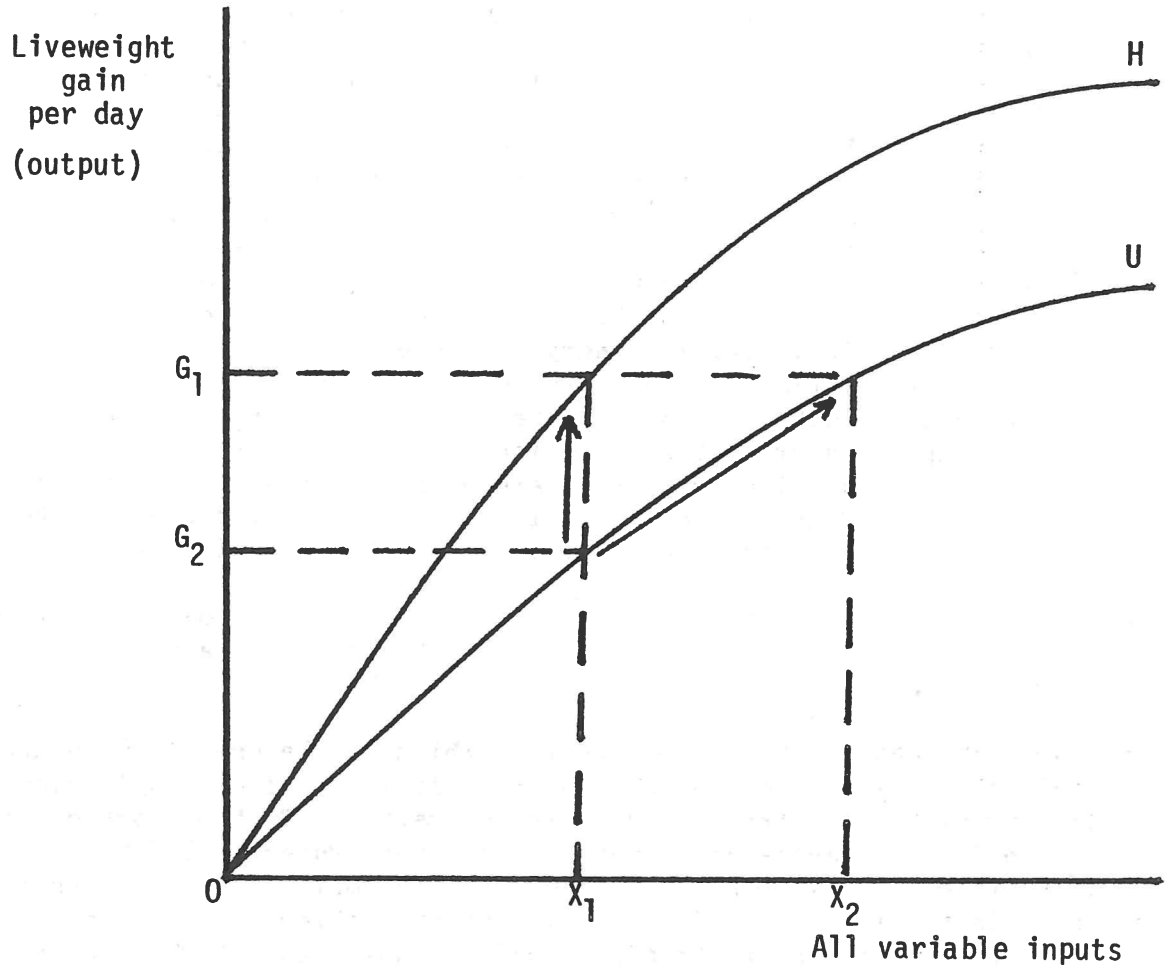

Fig. 2 The recovery of planned output : either veterinary inputs or non-veterinary inputs

Instead of confining attention to the response function for liveweight gain in relation to concentrates fed, now we interpret the horizontal axis to mean a measure of some "composite" input comprising a package of all relevant variable inputs, i.e.

$$G = f(C|F, L, E, H, ...) \quad (1) \text{ above}$$

becomes $$G = g(X) \quad (2)$$

where $$X = \{C, F, L, E, H, ...\} \quad (3)$$

This is an example of an 'economic production function', which expresses the relationship between output and increasing quantities of all feed, labour use, managerial skill and other environmental variables such as lairage space. In contrast with point C_1 in Fig. 1, X_1 now represents a collection of quantities of all of the inputs which happens to yield growth rate G_1 as before. For reasons which will become clear, veterinary inputs are presently excluded.

Again we suppose that disease causes a reduction in technical efficiency $(G_1 - G_2)$, and again a question to be answered is how to recover G_1. The solution may be as before, by incurring veterinary expenditures up to a maximum outlay of the value of $P(G_1 - G_2)$. But there is an alternative (in fact more than one, as we shall see) which is to increase the use of other inputs from X_1 to X_2. The arrows on Fig. 2 describe these two paths back to G_1. However, it will clarify the explanation to state the problem in rather a different way. If a farmer wants his cattle to achieve a growth rate G_1, he has to choose between the alternative production techniques which he expects will give the same result. At the two extremes, either he may devote relatively few resources to adequate nutrition, housing and supervision of his stock, while employing veterinary inputs to forestall disease, or he may take considerable care with his animal husbandry reflected in the use of more non-veterinary instead of veterinary inputs, to reach the same end. From that perspective curves H ('healthy') and U ('unhealthy') are inaptly named. Really they are simply the production functions for two different, but equally valid, techniques of production, but which are differentiated in practical terms by health status of the animals. Which is chosen will depend on the relative costs of the necessary veterinary and non-veterinary inputs.

As intimated above, it is too restrictive to conceive only of two extreme alternatives. Figure 3 illustrates another possibility, with a more appropriate lebelling of the curves and horizontal axis.

Technique 3 introduces another possibility which may be one of any number which is available between Techniques 1 and 2. It is a 'middle way' which involves lifting production from G_2 to G_1 in two parts. That proportion of $(G_1 - G_2)$ given by RS is achieved by veterinary expenditures while the remainder, equivalent to climbing from S to T, is accomplished by increasing non-veterinary inputs from X_1 to X_3. An important problem to an economist is the identification of the specific path corresponding to RST which enables G_1 rather than G_2 to be achieved most cheaply, i.e. discovering the combination of veterinary and non-veterinary inputs which meets the objective but a minimum total cost. By extension, frequently it is necessary to evaluate if the additional total outlays are at least covered by the consequent additional receipts.

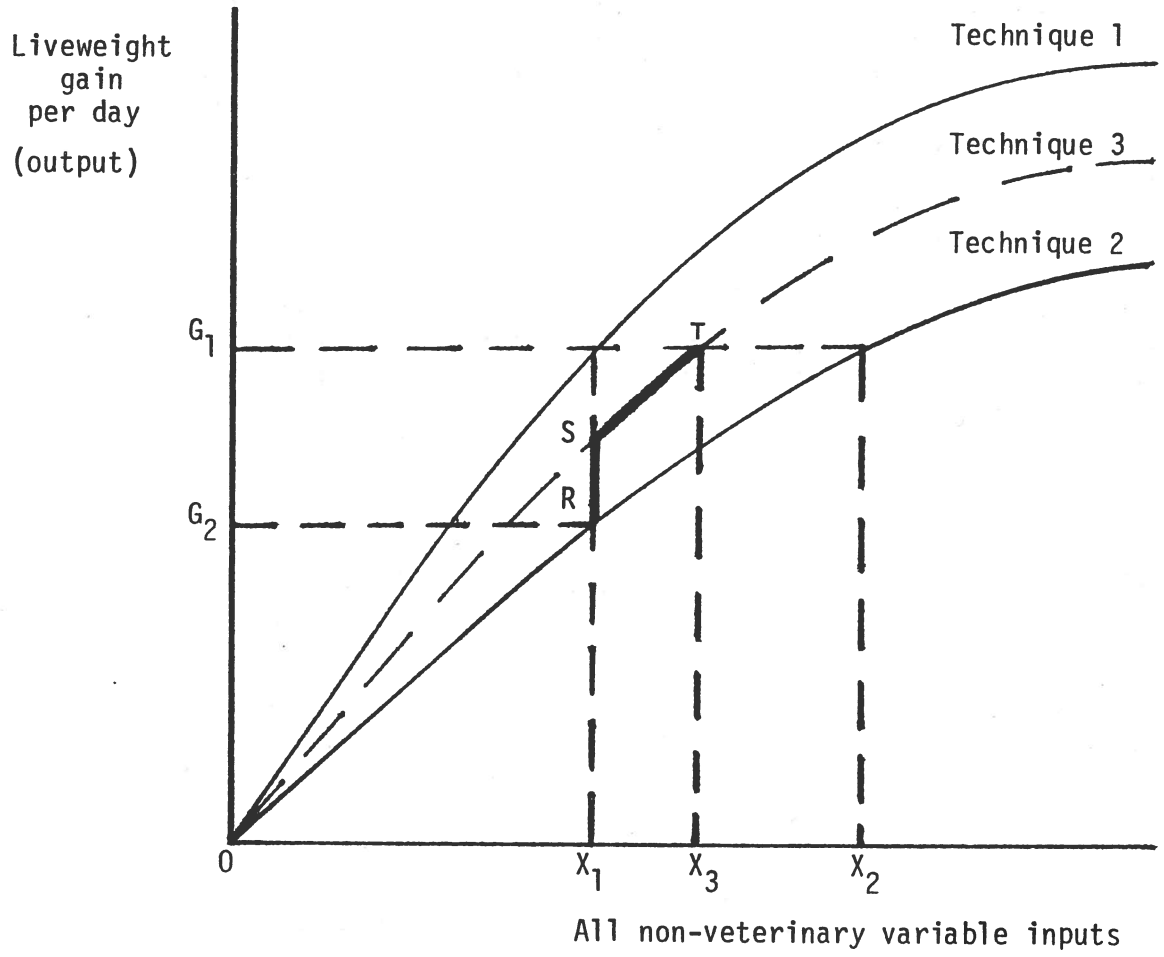

Fig. 3 The recovery of planned output : both veterinary inputs and non-veterinary inputs

Data requirements

It is self-evident that data are essential to give substance to the concepts outlined above. Clearly, the precise requirements will vary with the specific disease condition or conditions to be studied. In keeping with the general perspective which has been taken here, the following is offered as a broad framework which can guide the initial steps in the economic analysis of animal disease.

1. Identification of the 'best performance' conditions

In all circumstances it is necessary to ascertain what conditions are necessary if animals are to produce in the neighbourhood of the current technical optimum e.g. daily liveweight gain, food conversion ratio, lactation yield, lambing percentage, etc. Such data may be provided from experimental results, but with the disadvantage that they will seldom be representative of the potential under the less controlled environment of

commercial farming. Reference to the performance of 'élite' herds and flocks, often identifiable from the enterprise costings conducted, for example, by universities and the Meat and Livestock Commission, is likely to be the more useful. The idea of the current technical optimum is stressed, since it is to be expected that the implementation of new scientific knowledge and husbandry practices will tend to increase potential efficiency over time. There may be occasions when it is useful to seek out expert opinion on the likely direction of future developments in the control of animal disease.

2. Quantification of 'best performance' relationships

In essence, this activity involves using the empirical data to describe the relationships which underpin Technique 1 of Fig. 3. Detailed case studies may suffice in some instances, while recourse to the use of more advanced statistical techniques on sample data will be helpful in others. The direct estimation of a production function using multiple regression analysis has the advantage that the relative importance of various determinants of efficiency can be measured.

3. Study of livestock enterprises subject to health problems

This amounts to repeating the type of analysis outlined in points 1 and 2 above, but for livestock subjected to health problems i.e. defining Technique 2 of Fig. 3, and also investigating the pathways between Techniques 1 and 2 as described and summarised with reference to Technique 3. Obviously it is seldom possible to draw a clear distinction between 'healthy' and 'diseased' livestock, and an important part of the exercise is to delimit the threshold points which are considered to be significant.

4. Economic interpretation of the technical relationships

Using the procedures which have been outlined above, it is possible to make important insights into the economic implications of animal disease. The analysis has been couched in terms of the type of problems which afflict the individual livestock enterprise. Technical and economic efficiency are seldom synonymous, and the characteristics of their interrelationship is susceptible to analysis. Moreover, farmers usually are concerned more with the profitability of their farming systems overall than with particular enterprises, and it is not difficult to evaluate the economic consequences of animal health problems causing time and physical resources to be diverted from alternative uses on the farm. As an extension, knowledge of individual farm circumstances and additional epidemiological data can enable inferences to be made about the economic impact of animal disease on the national farm. By implication, the scope and direction of veterinary research and practices could be guided by reference to the kind of results forthcoming from the approach to economic appraisal which has been discussed, as well as policy with regard to disease control programmes. A prerequisite for the formulation of appropriate economic models is the classification of disease types according to their particular characteristics.

Conclusion

It has been argued that hitherto the application of economics to the problems of animal disease has tended to abstract from the central role of the discipline as a science choice. Economics helps with decisions about how scarce resources are to be allocated and used. Veterinary skills and medicines

are no different from any other scarce resource, and it is both necessary and desirable to interpret the economic efficiency with which they are deployed in the cause of improving animal health. As part of the appraisal the relationship between veterinary inputs and its substitutes must be studied. The paper attempts to explain some of the basic conceptual and empirical issues which should be addressed. These ideas are being pursued in more detail, with attempts to relate them more specifically to actual disease conditions and veterinary and farming practice, in a major research study proposed by Professor John McInerney and the author at Exeter University.

TIME SERIES ANALYSIS OF OVINE PNEUMONIA USING SCOTTISH SLAUGHTERHOUSE DATA

A. SIMMONS* and J.C. CUTHBERTSON**

The post-mortem inspection of animals slaughtered for human consumption is, in many countries, a well established routine designed to protect the consumer from exposure to potential pathogens. Accurate records of the numbers of condemnations of carcases and offal and the reasons for them are compiled for both public health and accounting purposes. Consequently there is a large amount of data available to workers with an interest in animal health.

The analysis of slaughterhouse data is an inexpensive method of assessing the prevalence of certain animal diseases because the data are collected routinely and are readily available. When data, collected over several years, have been analysed the results of the analyses have been used to monitor changes in condemnation rates (Biering-Sorensen, 1965; Blamire *et al.*, 1970; Blamire *et al.*, 1980; Cuthbertson, 1983).

Time series analysis may be used to detect seasonal, secular and cyclical patterns in data which may otherwise be obscured by random variation. The aim of this study was to determine the value of time series analysis of slaughterhouse data using ovine pneumonia as an example. It is unlikely that the condemnation rates reflect the true prevalence rate of pneumonia because of the bias inherent in the data. One source of bias lies in the fact that the great majority of sheep (greater than 90%) in this survey were classified as lambs or hoggs and therefore they did not reflect the age distribution of the populations from which they were selected. Similarly animals selected for slaughter have to be clinically healthy and therefore they do not necessarily reflect the health status of the populations from which they were selected. However it is probable that trends exhibited by condemnation data will parallel those in the sheep population as a whole.

At meat inspection no differentiation is made between the various types of ovine pneumonia. However because of the classes of sheep (lambs and hoggs) which predominated in this survey lesions of pneumonia and/or pleurisy were most likely to belong to the atypical pneumonia category.

MATERIALS AND METHODS

The number of sheep lungs condemned per month because of pneumonia and/or pleurisy and the number of sheep slaughtered per month were extracted from the meat inspection returns of a Scottish slaughterhouse (Table 1). These data covered the five years from 1979 to 1983 inclusive with the exception of the period February to March 1982 when the slaughterhouse was closed. The monthly percentage condemnation rates (Table 2) were calculated and were analysed by a time series method to determine the underlying pattern of condemnations attributable to pneumonia and/or pleurisy. The method was used to identify secular and seasonal changes in the data.

The analysis was carried out in 4 parts.

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Table 1. Monthly totals of sheep slaughtered and of lungs condemned because of pneumonia and/or pleurisy (1979–83)

		JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
1979	Sheep slaughtered	11405	12394	16216	10616	7701	9089	8997	11751	18157	16107	15882	17463
	Lungs condemned	38	30	75	61	50	21	24	44	26	49	38	25
1980	Sheep slaughtered	11795	11605	13180	11126	14116	10761	10769	18750	15321	13559	21422	12642
	Lungs condemned	47	44	51	72	82	53	53	36	41	46	65	55
1981	Sheep slaughtered	12453	11280	9863	8505	10442	8418	9488	16138	13277	13965	11351	10433
	Lungs condemned	60	65	61	64	53	37	20	28	24	29	40	28
1982	Sheep slaughtered	8249	–	–	2561	4140	5601	6813	8798	8063	9990	6946	10121
	Lungs condemned	60	–	–	22	20	20	19	40	77	60	98	66
1983	Sheep slaughtered	7910	9721	10534	7132	7833	6417	9850	16680	14382	12959	11288	10326
	Lungs condemned	56	62	51	60	30	31	68	135	157	98	141	100

Table 2. Percentages of sheep lungs condemned monthly because of pneumonia and/or pleurisy and average monthly and yearly percentage condemnation rates (1979–83)

	JAN. %	FEB. %	MAR. %	APR. %	MAY %	JUNE %	JULY %	AUG. %	SEPT. %	OCT. %	NOV. %	DEC. %	YEARLY PERCENTAGE CONDEMNATION RATE
1979	0.33	0.24	0.46	0.57	0.65	0.23	0.27	0.37	0.14	0.30	0.24	0.14	0.33
1980	0.40	0.38	0.39	0.65	0.58	0.49	0.49	0.19	0.27	0.34	0.30	0.44	0.41
1981	0.48	0.58	0.62	0.75	0.51	0.44	0.21	0.17	0.18	0.21	0.35	0.27	0.40
1982	0.72	0.71[a]	0.75[a]	0.85	0.45	0.34	0.26	0.43	0.95	0.60	1.41	0.63	0.68
1983	0.71	0.64	0.48	0.84	0.38	0.48	0.69	0.80	1.09	0.76	1.25	0.97	0.76
Average monthly percentage condemnation rate	0.53	0.51	0.54	0.73	0.51	0.40	0.38	0.39	0.53	0.44	0.71	0.49	

[a]Estimated

Table 3. Three month rolling average percentage condemnation rates because of pneumonia and/or pleurisy (1979–83)

	January %	February %	March %	April %	May %	June %	July %	August %	September %	October %	November %	December %
1979		0.34	0.42	0.56	0.48	0.38	0.29	0.26	0.27	0.23	0.23	0.26
1980	0.31	0.39	0.47	0.54	0.57	0.52	0.39	0.32	0.27	0.30	0.36	0.41
1981	0.50	0.56	0.65	0.63	0.57	0.39	0.27	0.19	0.19	0.25	0.28	0.45
1982	0.56	0.72	0.77	0.68	0.55	0.35	0.34	0.55	0.66	0.99	0.88	0.92
1983	0.66	0.61	0.65	0.57	0.57	0.52	0.66	0.86	0.88	1.03	0.99	

1. Removal of random variation

It is desirable to remove random variation from a time series because this frequently obscures any non-random variation. The calculation of a rolling average for each month has the effect of "smoothing" the data and allows detection of patterns that would otherwise be concealed (Sard, 1979). In this case a three month rolling average was calculated.

2. Regression analysis

To determine whether a secular trend existed in the data, a regression analysis was carried out using the Minitab computer statistics programmes (Ryan *et al.*, 1976).

The equation analysed was:

$$Y = a + bX + e \qquad (1)$$

where Y = % lungs condemned per month X100 (pneumonia/pleurisy),
a = point of interception by regression line on Y axis,
X = months 1 – 60 (1 = January 1979 ..... 60 = December 1983),
b = regression coefficient,
e = random error around the secular trend.

Regression analysis was also carried out using the values of X squared. Further analyses included calculation of correction factors for each month to determine the presence of any significant seasonality. December was used as the indicator month and dummy variables were used for the other eleven months of the year. This arbitrarily chosen indicator month was used as a basis to calculate the seasonal effects of the other months. The equation for this was:-

$$Y = a + b_1X + b_{2.1}D_1 + b_{2.2}D_2 + b_{2.3}D_3 \ldots\ldots\ldots\ldots + b_{2.11}D_{11} + ut, \qquad (2)$$

where $b_{2.1}$ ..... $b_{2.11}$ = the seasonality factor for each month relative to the indicator month (December),
D_1 .....D_{11} = dummy variable for each month of the year,
ut = a random disturbance term reflecting the residual errors (average value = 0), whose variance was assumed to be constant for all values of X.

3. Removal of the secular trend

Regression analysis and the detection of a secular trend, if any, allows the effects of the secular trend to be removed from the data. The effect, therefore, is one of removing the influence of the passage of time from the readings and leaving the effect of other influences considered in the regression as random error. Removal of the secular trend was carried out by adding to each monthly condemnation rate the quantity $b(x-\bar{x})$ where $(x-\bar{x})$ = deviation from the mean time (Sard, 1979).

4. Removal of seasonal trend

Seasonal variation is an important factor in time series analysis and it is often useful to examine the data with its effects removed. In this case removal of the seasonal trend was carried out as follows:-

a) An average value for each month over the five years was calculated

$$\text{e.g. } \frac{\%\text{ Jan 1979} + \%\text{ Jan 1980} + \%\text{ Jan 1981} + \%\text{ Jan 1982} + \%\text{ Jan 1983}}{5}$$

b) An average of the twelve derived values was calculated.

c) The seasonal index, one for each of the twelve months was expressed as a proportion:-

$$\frac{\text{average calculated in (a)}}{\text{average calculated in (b)}}$$

Removal of the seasonal fluctations was carried out by dividing the appropriate seasonal factor into each monthly percentage.

The two months for which no data were available (February and March 1982) were dealt with as follows. Average monthly indices were calculated without using any data for 1982. The values for February and March 1982 were then estimated by expressing the average monthly indices for both months as proportions of the January 1982 percentage condemnation rate. This was based on the assumption that the pattern of seasonality prevailing in the other years also had prevailed in 1982.

RESULTS

There was considerable variation in the monthly percentage condemnation rates (Fig. 1). The highest rates occurred in the spring (April 1979, 0.57%; April 1980, 0.65%; April 1981, 0.75%; April 1982, 0.85%) and to a lesser extent in the autumn of each year. In each of the last two years (1982 and 1983) there was a sustained rise in the percentage condemnation rate with peaks in November 1982 (1.4%) and November 1983 (1.25%).

It is evident from an examination of the condemnation rates displayed in Fig. 1 that there was a degree of seasonal variation and an increasing secular trend. The results of the time series analysis carried out to detect the trends are given below.

1. Removal of random variation

Three month rolling averages calculated to remove random variation are presented in Table 3. There was a series of peaks and troughs in the monthly condemnation rates over the five year period. In addition there was an overall increasing trend in condemnation rates which became more noticeable from August 1982 onwards (Fig. 2).

2. Regression analysis

The result of regressing Y (percentage condemnation rate x 100) on X (time) was as follows:

$$Y = 24.4 + 0.918\,X \qquad R^2 = 0.30 \qquad (3)$$

where R^2 = coefficient of determination of the regression.

The resultant regression curve is shown in Fig. 1.

When the values of X were squared the following regression resulted

$$Y = 33.4 + 0.0155X^2, \qquad R^2 = 0.35 \qquad (4)$$

The higher value obtained for R^2 when the values of X were squared indicates that more of the variation in the data is explained by the quadratic equation than the linear equation. This shows that the trend in Y increased over time, i.e. in the year 1983 the trend was faster than in 1979.

Regression analysis using 11 dummy variables gave the following results:-

$$Y = 37.0 + 0.980X + 14.80\,D_1 + 16.20\,D_2 + 21.60\,D_3 + 32.00\,D_4 + 9.30\,D_5 - 3.52\,D_6 - 5.70\,D_7 - 5.88\,D_8 + 6.54\,D_9 - 2.84\,D_{10} + 22.98\,D_{11} \qquad R^2 = 0.51 \qquad (5)$$

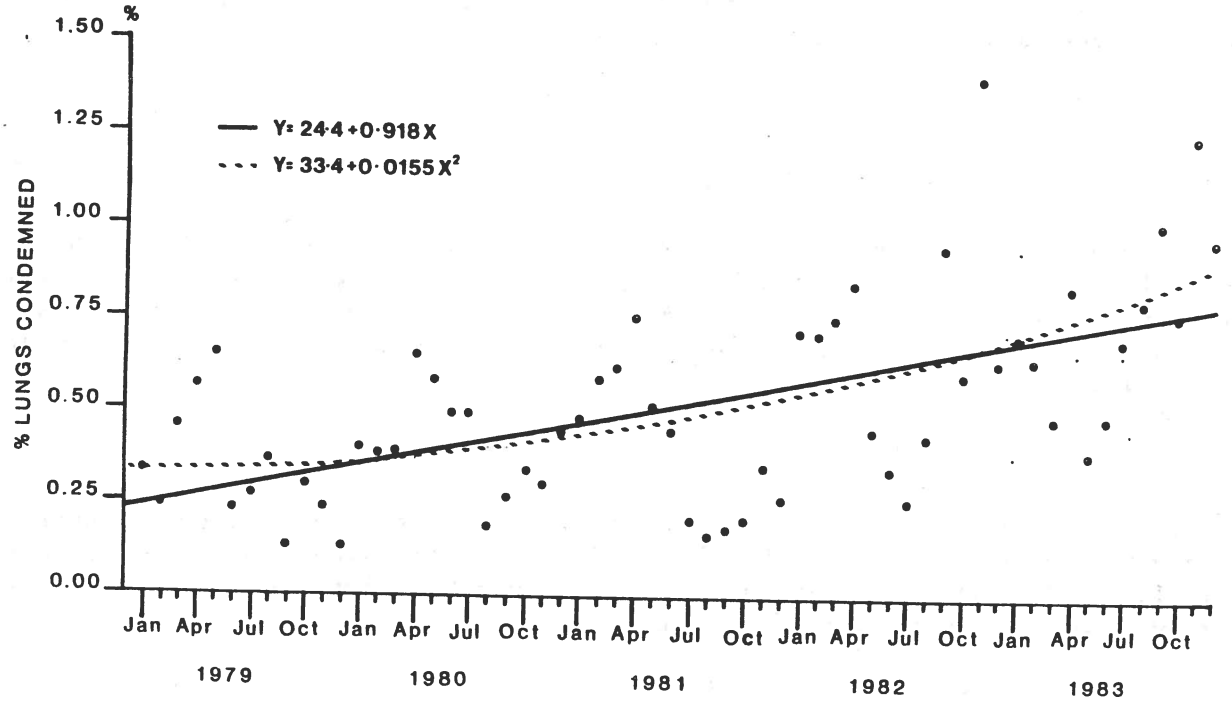

Fig. 1 Percentages of lungs condemned monthly because of pneumonia and/or pleurisy and regression curves ($y = 24.4 + 0.918x$; $y = 33.4 + 0.0155x^2$.)

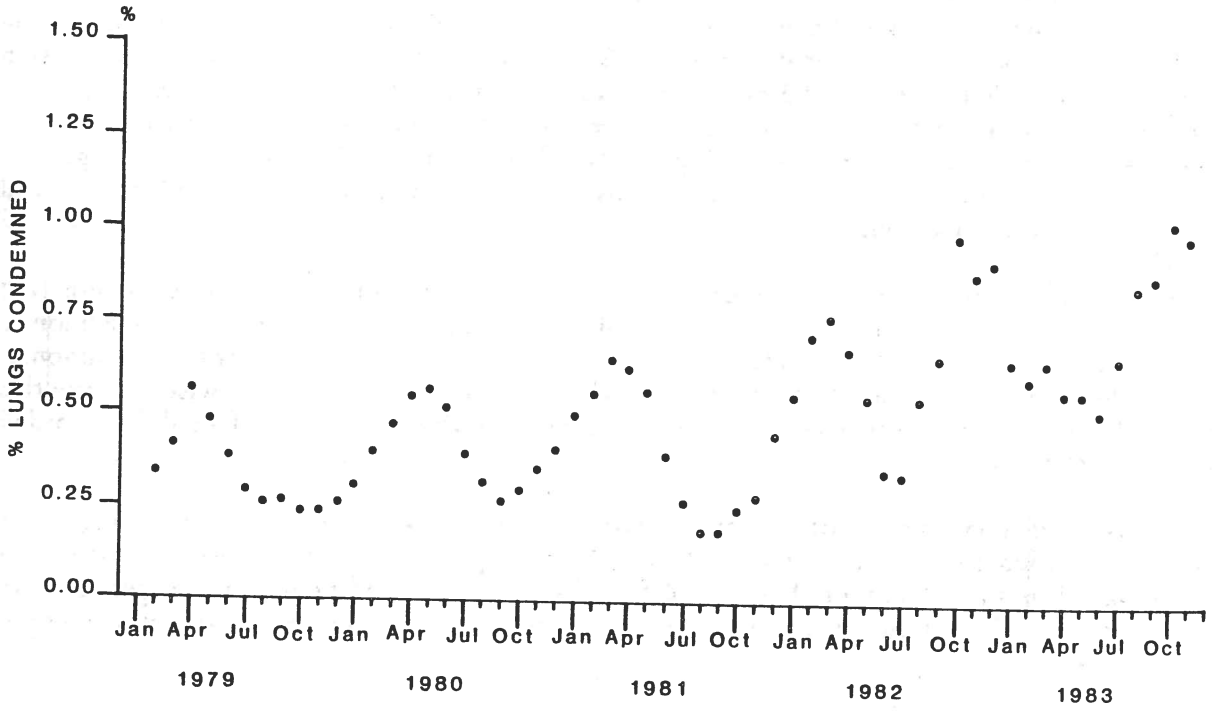

Fig. 2 Three month rolling average percentage lung condemnation rate (pneumonia and/or pleurisy).

There was not a sufficient number of values for each month to detect small differences from trend values because data were only available for five years and especially because the data were quite variable about the trend. However, the further increase in the value of R^2 shows that this multiple regression equation accounts for more variation than the two previous regressions i.e. the equation represents a curve which better fits the data.

3. Removal of the secular trend

A secular trend was described by Eq. (3).

The value of b = 0.918 was used to remove the secular trend from the data. The results are shown in Table 4 and are shown graphically in Fig. 3. The variation evident in Fig. 3 was a result of random variation and seasonal influences. A pattern of increases and decreases in percentage condemnation rates was evident, particularly between July 1980 and July 1982 and between April 1983 and October 1983.

4. Removal of the seasonal trend

The data, processed using the seasonal indices to remove seasonal trend are presented in Table 5 and shown graphically in Fig. 4. A degree of clustering of the values was evident (February to June 1981, July to December 1981 and July to December 1983) but there was a considerable amount of variation despite the removal of seasonal trends.

DISCUSSION

Examination of the raw data provided evidence of an increasing secular trend and a pattern of seasonality (Fig. 1).

Regression analysis confirmed the increasing secular trend i.e. the percentage lung condemnation rate increases with time and it is shown by the curve described by Eq. (3) and drawn on Fig. 1. Further regression analysis as described by Eq. (4) and illustrated by the second regression curve in Fig. 1 revealed that in 1983 the trend was much faster than in 1979. These regression analyses have R^2 values of 0.30 and 0.35 respectively indicating the proportion of variation in the data that is explained by these regression equations. These values of R^2 show that a large proportion of the variation is not accounted for and this may be assumed to be attributable to seasonal influences and random variation.

There are a number of possible explanations for a long term increase in the condemnation rates. The most obvious is that the overall increase in the condemnation rates for pneumonia/pleurisy in slaughtered sheep was merely reflecting a similar trend in the occurrence of pneumonia/pleurisy in the population of sheep from which they were drawn. However it is unwise to make this assumption because other factors have been exerting influences which may have affected the recorded condemnation rate.

The change in consumers' tastes over the last few years towards a leaner carcase may have affected the recorded condemnation rates. The peak of susceptibility to atypical pneumonia is 3–4 months of age (Jones and Gilmour, 1983) and if lambs are slaughtered now at a younger age than previously because of consumer tastes then the recorded condemnation rate may show a corresponding increase.

If the efficiency of inspection is increased while the true prevalence rate of pneumonia/pleurisy remains steady then a corresponding increase in recorded condemnation rates would occur. If this was because of a change either in technique or of operators then there would be a sudden increase and not a gradual change as demonstrated in this survey.

Examination of the raw data (Fig. 1) provides evidence of a pattern of seasonality. Successive

Table 4. Percentages of sheep lungs condemned monthly (corrected to remove secular trend) because of pneumonia and/or pleurisy (1979–1983)

	January %	February %	March %	April %	May %	June %	July %	August %	September %	October %	November %	December %
1979	0.60	0.50	0.71	0.81	0.88	0.45	0.49	0.58	0.34	0.49	0.42	0.31
1980	0.56	0.53	0.53	0.78	0.70	0.60	0.60	0.29	0.36	0.42	0.37	0.50
1981	0.53	0.62	0.65	0.77	0.52	0.44	0.21	0.16	0.16	0.18	0.31	0.22
1982	0.66	0.64	0.67	0.76	0.35	0.23	0.15	0.31	0.82	0.46	1.26	0.47
1983	0.54	0.46	0.29	0.64	0.17	0.26	0.47	0.57	0.85	0.51	0.99	0.70

Table 5. Percentages of sheep lungs condemned monthly (corrected to remove seasonal trend) because of pneumonia and/or pleurisy (1979–1983)

	January %	February %	March %	April %	May %	June %	July %	August %	September %	October %	November %	December %
1979	0.32	0.24	0.43	0.40	0.65	0.29	0.36	0.49	0.13	0.35	0.17	0.15
1980	0.38	0.38	0.37	0.45	0.58	0.62	0.65	0.25	0.26	0.40	0.22	0.46
1981	0.46	0.58	0.58	0.52	0.51	0.56	0.28	0.22	0.17	0.24	0.25	0.28
1982	0.69	0.71	0.71	0.59	0.45	0.44	0.35	0.57	0.91	0.70	1.01	0.66
1983	0.68	0.64	0.45	0.59	0.38	0.62	0.92	1.05	1.05	0.88	0.90	1.01
Seasonal index	1.04	1.00	1.06	1.43	1.00	0.78	0.75	0.76	1.04	0.86	1.39	0.96

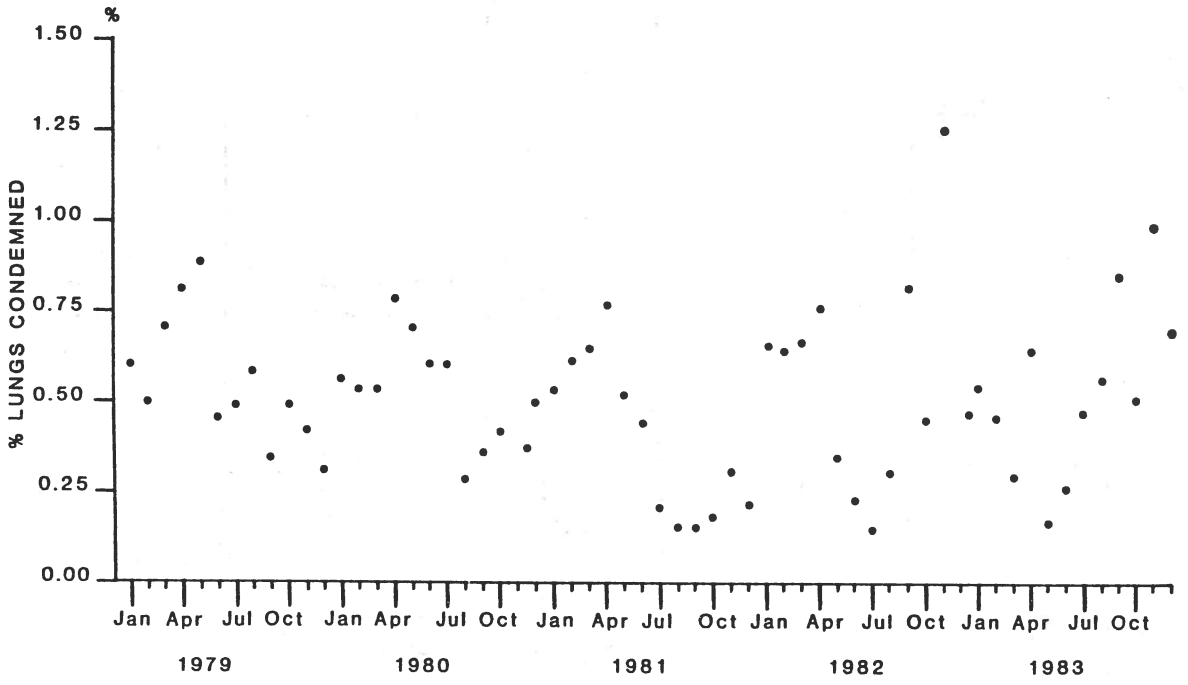

Fig. 3 Percentages of lungs condemned monthly (corrected to remove secular trend) because of pneumonia and/or pleurisy.

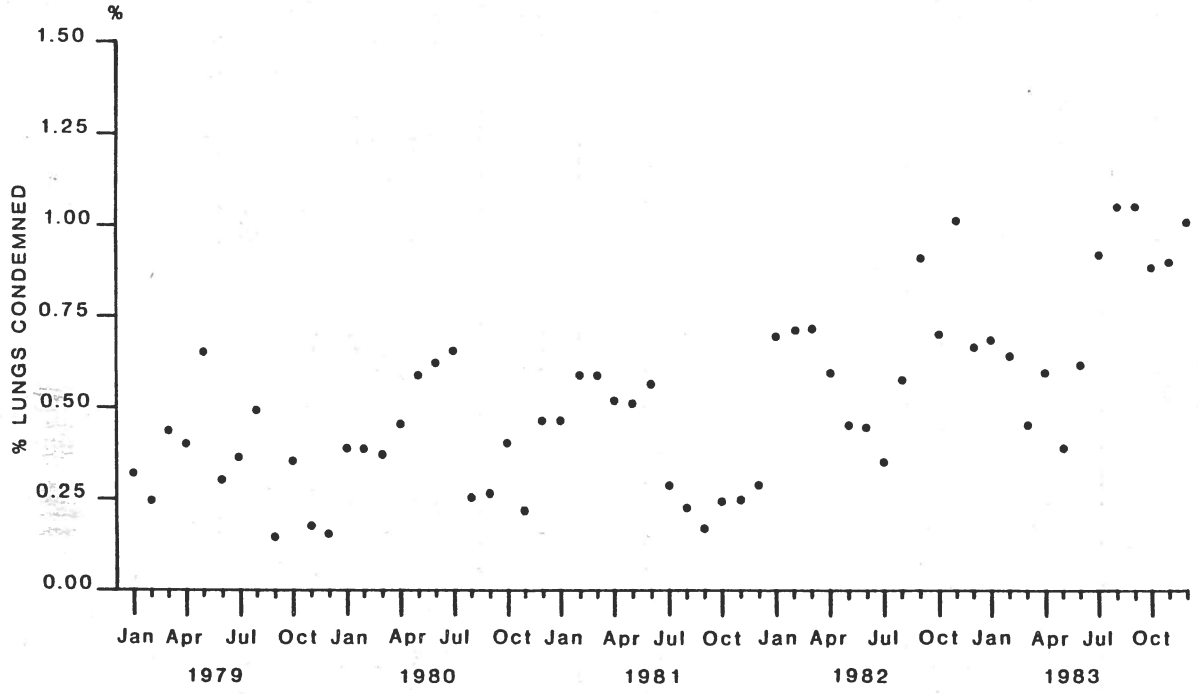

Fig. 4 Percentages of lung condemned monthly (corrected to remove seasonal trend) because of pneumonia and/or pleurisy.

peaks and troughs in the condemnation rate reflect seasonal fluctuations. With the data "smoothed" using a three month rolling average the pattern becomes more clear (Fig. 2). An increasing secular trend is evident but in addition there is an obvious peak in the condemnation rate each spring. This is followed by a trough in the summer and a slow rise during the autumn and winter to reach a new peak in the next spring. The significance of the seasonal trend was demonstrated by multiple regression. The R^2 value of 0.51 represents an increase over that obtained in the simple regression i.e. the multiple regression accounts for more of the variation in the data.

There are no clear reasons for the seasonality in the recorded condemnation rates. However it is reasonable to assume that in Scotland animal husbandry and management exert a strong influence on the prevalence of pneumonia in sheep. The prevalence of atypical pneumonia increases as the stocking density increases and as the altitude at which the sheep are reared decreases (Jones and Gilmour, 1983). It is reasonable to assume that lowland intensively reared early lambs have a higher prevalence rate of atypical pneumonia than hill sheep but reach slaughter weight more quickly because of superior nutrition. The lambs going to slaughter in the spring will be those reared in lowland areas and they will exhibit a higher prevalence of pneumonia than lambs slaughtered in the summer months and this will be reflected in the recorded condemnation rate. It is thought that there are two reasons why the condemnation rate was lower during the summer. First, the fastest growing lambs fattened on grass in the upland areas would have been slaughtered in the early summer and they would have been relatively free of pneumonia. Second, lambs born on the hill but fattened on the lower areas on a more extensive basis than early lambs would have been slaughtered in the late summer and early autumn. These again would have been relatively free of pneumonia. The rise in condemnation rates during the late winter may also be attributable to management practices. Lambs and hoggs housed over the winter have an increased risk of atypical pneumonia (Jones and Gilmour, 1983) and this may be reflected in the condemnation rate recorded at slaughter.

There is a considerable amount of unexplained variation in the data. Multiple regression indicated that only 51.0% of the variation was accounted for by seasonal and secular influences. If data from several slaughterhouses were available or a longer time series had been used then the effects of random variation would have been minimised and a significant trend would have been more clear. The reasons for such a high degree of random variation are not clear. However the slaughterhouse received large batches of sheep from all parts of Scotland. If the prevalence rate of pneumonia in a particularly large batch was significantly lower or higher than the average expected for that month because of different types of management then this would have been reflected in the returns.

The calculation of a rolling average to "smooth" the data provides evidence of the seasonal and secular trends (Fig. 2) because much of the random variation evident in the raw data (Fig. 1) has been removed. Rolling averages provide a quick method of removing random variation from a time series but suffer the disadvantages of excluding the first and last items of data and may be unduly affected by extreme values (Sard, 1979).

Despite the removal of the secular trend from the data (Fig. 3) the effects of seasonality are not obvious because of the random variation in the data. Similarly random variation obscures an obvious secular trend in the data when the effects of seasonality are removed (Fig. 4).

Thus it can be seen that time series analysis provides statistical evidence of seasonal and secular trends in the recorded condemnation rate. However, this has to be related to the prevalence rate of pneumonia in the population as a whole and it is recognised that the variation in condemantion rates did not reflect accurately the changes in the true prevalence rate. Two factors are thought to be responsible for this unavoidable discrepancy.

1. Not all the instances of the occurrence of pneumonia are recorded despite being recognised and dealt with by the inspector. Pneumonia is only recorded if the organ is affected severely enough to warrant condemnation. This is usually if the lesion occupies one third or more of the organ. Localised lesions confined to a single lobe only require trimming and are not recorded.

2. Sheep selected for slaughter are not a true random sample of the population. Most are free from clinical disease and are unrepresentative of the age structure and the sex ratio of the population

from which they are derived (Cannon and Roe, 1982).

However, despite these drawbacks, some valid conclusions may be drawn from studies of this type, particularly when data that cover a period of several years are analysed. For example, the efficacy of the bovine tuberculosis eradication scheme in Denmark was monitored by Biering-Sorensen (1965). Initially an increase in the proportion of cattle affected with tuberculosis was recorded in the returns because of the slaughter of numerous positive reactors but as the eradication programme progressed the returns showed a fall in the proportion of cases to a very low level.

Similarly Blamire *et al.* (1980) recorded a considerable reduction in the number of sheep and cattle livers condemned as a result of fascioliasis compared to that recorded in the previous 10 years (Blamire *et al.*, 1970). They attributed this reduction to various factors including gradual climatic changes, improved management and the increased availability of effective fasciolicides.

It is likely that time series analysis of slaughterhouse data as carried out in this survey would be of value in disease surveillance and in the monitoring of the efficacy of disease control programmes. However because of the bias in the available data it may be desirable to improve data quality. It must be borne in mind that the time available for inspection of each carcase and offal is usually short. Therefore it is unreasonable to expect a meat inspector to record extra details. For this reason a specific survey would be required to broaden the data base.

A specific survey would not be constrained by the limitations imposed by routine inspection procedures. By using trained personnel more information on the severity and pathology of a particular condition could be obtained. If information on the age, breed, sex and physical condition of the animals that are slaughtered is available a comprehensive bank of data could be amassed enabling detailed investigations into particular conditions to be carried out. The greatest benefits would be realised if traceback were to be made possible. Therefore every effort should be made to obtain information on the origin of the animals destined for slaughter.

Obviously this type of survey would be considerably more expensive than the present survey. The cost of trained personnel and laboratory facilities to process samples could be prohibitive. However, costs may be reduced if, rather than subjecting every carcase and offal to a detailed examination, only a representative sample is examined. Cannon and Roe (1982) outline methods which define sampling frames with confidence limits for a given expected prevalence rate.

With the available data a discrepancy between the recorded condemnation rates and the true prevalence rates was unavoidable. However, the secular and seasonal trends detected by the time series analysis indicate that studies of this type may be useful in the monitoring of fluctuations in disease prevalence rates. However, the large amount of random variation could have been reduced by increasing the number of sheep in the sample. To further improve the quality of the data, i.e. to more closely equate the condemnation rate with the true prevalence rate, it would probably be necessary to organise a specific survey.

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REFERENCES

Biering-Sorensen, U. (1965). The value of recording disease conditions observed at slaughterhouses and disposal plants. The Veterinarian 3, 87–97.

Blamire, R.V., Crowley, A.J. and Goodhand, R.H. (1970). A review of some animal diseases encountered at meat inspection 1960–1968. Vet. Rec. 87, 234–238.

Blamire, R.V., Goodhand, R.H. and Taylor, K.C. (1980). A review of some animal diseases encountered at meat inspection in England and Wales, 1969 to 1978. Vet. Rec. 106, 195–199.

Cannon, R.M. and Roe, R.T. (1982). Livestock Disease Surveys: A field manual for veterinarians. Australian Bureau of Animal Health, Canberra. 35pp.

Cuthbertson, J.C. (1983). Sheep disease surveillance based on condemnations at three Scottish abattoirs. Vet. Rec. 112, 219–221.

Jones, G.E. and Gilmour, J.S. (1983). Atypical Pneumonia. In: Diseases of Sheep (Ed. W.B. Martin), pp. 17–23. Blackwell, Oxford. 282 pp.

Ryan, T.A., Joiner, B.L. and Ryan, B.F. (1976). Minitab Student Handbook. Duxbury Press, Boston, Ma.

Sard, D.M. (1979). Dealing with data: the practical use of numerical information: (13) Predictable differences. Vet. Rec. 105, 196–199.

DATA RECORDING AND ANALYSIS IN DAIRY HERDS

COMPUTERISED ANALYSIS OF THE INCIDENCE OF BOVINE REPRODUCTIVE DISORDERS : A TOOL FOR MANAGING HERD FERTILITY PROBLEMS

G. FRANCOS* AND E. MAYER*

Fertility control, based on a routine of regular weekly visits to dairy herds, has been a cornerstone of our clinical veterinary service† for over 4 decades. This fertility control includes routine post-partum rectal examinations and pregnancy diagnosis, treatment of fertility disturbances and the supervision of dairy herd fertility problems. The data on the monthly conception rate calculated by the veterinarian during his visits to the farm from the records of inseminations and from the results of his pregnancy examinations, added to the data supplied by the artificial insemination (A.I.) centres on yearly conception rates and other general indicators of fertility (days to conception, insemination index etc.), served for many years for the evaluation of herd fertility status. The computerisation of all A.I. records in the early seventies supplied a wider spectrum of general fertility data: days to first insemination, days from the first to the last insemination, inter-insemination intervals and their subdivision according to their duration and the percentage of cows pregnant. All these data were very valuable for classifying herds according to their fertility status, but gave only limited indications of the reasons for the big differences in fertility between herds.

In order to try to diagnose the possible causes of herd fertility problems, the above commonly applied general indicators are insufficient. The experience we gathered brought us to the conclusion that in order to investigate herd fertility problems efficiently additional data such as those relating to herd management, nutrition, (including the amounts actually fed to the various production groups), as well as more specific information about the character of fertility disorders, is necessary.

For this purpose a method of analysis of the incidence of reproductive disorders (R.D.) was elaborated. This method has been applied for the last two decades in many dairy herds, particularly in the problem ones. In recent years this analysis has been accomplished with the aid of an integrated computerised programme in 101 registered herds milking about 30,000 dairy cows.

MATERIALS AND METHODS

General assumptions

The analysis of the principal reproductive disorders was based on the following assumptions:

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†See Appendix

1. Under given conditions a certain average incidence of fertility disorders occurs:

   (a) General: geographic, climatic, seasonal etc.,

   (b) Genetic: breed, acquired hereditary traits etc.,

   (c) Management: (including nutrition and housing),

   (d) Production.

   This average incidence may be calculated when data from a sufficient number of herds totalling several thousands of lactations are pooled. In individual herds marked deviations from this average may occur, without influencing the overall mean. We were able to confirm this assumption from data on over 20,000 lactations during an 8 year period.

2. In many herds the incidence profile shows characteristic long-lasting deviations from the average (Francos, 1974). In these cases the existence of some specific management errors should be suspected, provided that no infectious factors are involved.

3. In an average herd, the incidence of certain reproductive disorders is different in the first-calved heifer groups and those of multiparous cows. The first-calved heifers usually have a higher incidence of endometritis and unobserved oestrus, the multiparous cows a higher incidence of retained placentae and repeat breeders. Marked deviations from these norms point to probable management problems existing in either one or both of the two groups.

4. In order to draw conclusions from the data on the incidence of reproductive disorders for analytical and comparative investigations, precise definitions of the incidence must be formulated and strictly adhered to.

These assumptions have been confirmed under conditions existing in Israeli dairy herds*. We believe them to be valid under different geographic, breed, management and production conditions, especially in herds kept on zero grazing.

The definition of reproductive disorders.

Retained placenta: the placenta or parts of it not expelled 24 hours post-partum.

Lochia: faulty discharge until 20 days post-partum in cows which did not retain their placenta.

Endometritis: purulent or muco-purulent discharge from 21 days post-partum and onwards, in cows having neither retained their placentae nor discharged lochia.

Unobserved oestrus: oestrus not observed more than 60 days post-partum in cows without a previous diagnosis of retained placenta, lochia or endometritis. Unobserved oestrus with a previous diagnosis is recorded separately.

Cystic ovaries: without signs of nymphomania in cows without previous diagnosis; the same in cows with a previous diagnosis (recorded separately).

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*See Appendix

Cystic ovaries: with signs of nymphomania in cows without previous diagnosis; the same in cows with previous diagnosis (recorded separately).

The diagnosis of cystic ovaries is made by the veterinarian after two consecutive rectal examinations, with at least a 7-day interval, if cysts occur more than 60 days post-partum.

Negative pregnancy examinations: (post-service anoestrus) cows without previous diagnosis found not pregnant on examination. Cows found not pregnant with a previous diagnosis are recorded separately.

Repeat breeders: cows without a previous diagnosis and having calved normally, inseminated 4 times or more, having regular interinsemination intervals (above 15 days). Two inseminations within a shorter interval (below 15 days) are considered for the purpose of this diagnosis as one insemination.

Problem cows: all cows in a herd not having conceived 150 days post-partum. This group is then subdivided into categories according to the initial diagnosis (all of the above). This group also includes cows presented late for insemination for management reasons, conceiving normally, later than 150 days post-partum. (The computer is programmed to pinpoint these.)

Cows are therefore classified according to the primary diagnosis. For example a cow with retained placenta, developing endometritis and showing no signs of oestrus 75 days post-partum conceiving after 4 inseminations 170 days post-partum is included in the group with retained placentae and in that of "problem cows", subdivision "initial diagnosis : retained placenta". This method of classification is indispensable, because its purpose is to analyse the influence of the incidence of the disorder on fertility in the herd. Otherwise the cow would appear 4 times in different incidence groups "retained placenta", "endometritis", "unobserved oestrus", "repeat breeder", and then 4 times in the subdivision of the "problem cows" group. In addition, the fertility indices of the same cow would be recorded 4 times too: an illogical situation.

As shown in the diagnostic groups "unobserved oestrus", "cystic ovaries" and "negative pregnancy examination" subgroups of cows with a previous diagnosis are recorded. Only numerical data about these subgroups are summed up (number of cows percentage from all calvings) without analysis of their influence on fertility indices. A similar solution can be programmed for other groups too (e.g. "endometritis" after "retained placenta").

The input system

Until 1981, the occurrence of reproductive disorders was recorded in selected herds by the veterinary surgeon during his routine visits using special charts; when retrospective analyses were required, the data were extracted from the individual cow cards. With the introduction of the integrated programme in all registered herds, the data relevant to fertility events are recorded chronologically by the herd manager in a special "notebook". These records are mailed each month to the central computer office and recorded there. The diagnoses of reproductive disorders are recorded in chronological order in the same notebook as directed by the consulting veterinarian at the end of his visit. The following diagnoses are recorded by codes: "retained placenta", "lochia", "endometritis", "unobserved oestrus", "cystic ovaries". Diagnoses of negative pregnancy examinations, repeat breeder and problem cows are recorded in the printout only through computer analysis. A considerable number of herdbook herds participate in on line local or regional computer programmes,

described elsewhere (Neria & Mayer, 1984).

The magnetic tapes of their records are sent to the central computer at monthly intervals.

THE PRINTOUT AND ANALYSIS SCHEME

The printout is distributed twice a year. The cows selected for the analysis are those which calve during a 6 month period (January-June; July-December). The printout is ready seven to eight months after the end of each period. This delay is necessary as data on problem cows (not conceiving 150 days post-partum) must be included. Because of the strong predisposition to persistency in the incidence level in the herd (Francos, 1974, Martinez & Thibier, 1984) this delay does not generally diminish the value of the data. In order to supply a current flow of information, three additional printouts containing all numerical data on the incidence of reproductive disorders occurring within consecutive 4 month periods, are prepared and distributed by the central computer office.

The full data on the incidence are presented in the "half-year printout" according to lactation groups, as the percentage of all cows that have calved during that 6 month period. For each case recorded, the data on days to first insemination, days to conception, number of cows pregnant, and number of "problem cows" are recorded. In those with a diagnosis of: "retained placenta", "lochia" and "endometritis", the number of cows culled during the month since the date of the diagnosis, and the number culled from the end of that month until 150 days post-partum are recorded. In the diagnosic groups of "unobserved oestrus" and "ovarian cysts", the number of cows culled until 150 days post-partum are recorded. (Table 1).

Table 1a. Half year report for veterinary surgeons, all herds. Calvings during the period 1.7.83 - 31.12.83. (Data on the incidence: percentage of all calvings)

	Primiparous	Multiparous	Total
Number of calvings	4272	8053	12307
Retained placenta	3.9	10.5	8.2
Lochia	9.2	5.9	7.1
Endometritis	3.7	4.0	3.9
Unobserved oestrus (U.O.)	19.1	16.4	17.3
U.O. after previous diagnosis	4.1	5.6	5.1
Cystic ovaries (C.O.)	2.1	2.0	2.0
C.O. after previous diagnosis	4.7	4.9	4.8
Negative pregnancy examinations	4.1	4.9	4.8
N.P.E. after previous diagnosis	9.2	10.6	10.1
Repeat breeder cows	5.3	6.4	6.0
Problem cows (not conceived 150 days p.p.)	25.9	27.9	27.0

Table 1b. Percentage of cows culled within 150 days post-partum (from all cows with post-partum diagnoses), days to first insemination, days to conception, and percentage of "problem cows" in each diagnostic group.

	Retained Placenta	Lochia	Endometritis	Unobserved Oestrus	Cystic ovaries without Nymphomania	Cystic ovaries with Nymphomania
% culled during 30 days from the diagnosis	6.2	4.0	3.3			
% culled during 31 days from the diagnosis till 150 days post-partum	11.3	10.7	13.9	3.5	3.1	1.0
Days to first insemination	83	85	90	95	97	82
Days to conception	119	117	129	129	140	151
% conceived from all cows in diagnosis group	64	66	68	79	68	60.8
% conceived from all cows with diagnosis having reached 1st insemination	76.8	78	74.3	81.6	70	60.8
% of "problem cows" from all cows with diagnoses	34.4	34.0	43.4	39.8	53.1	65.8

A separate analysis is performed for the group of "problem cows". Their total number, the percentage of all cows that have calved, and their subdivision according to previous diagnoses is recorded. (Table 2)

Table 2. The division of "problem cows" (not conceived 150 days post-partum) according to previous diagnosis. (Total of 3328 animals.)

Previous diagnosis	
Retained placenta	10.4%
Lochia	8.9%
Endometritis	6.3%
Unobserved oestrus	25.5%
Negative pregnancy examination	9.4%
Cysts	4.4%
Repeat breeder	18.8%
Others	16.3%

The printout of this analysis of reproductive disorders is sent to the veterinarian supervising the herd, together with a printout of the average incidence in all herds participating in the programme, for the same period. The analysis performed by the veterinarian includes not only the herd incidence for each diagnosis, but also the days to first insemination, the days to conception, the number of "problem cows" and other data, all compared with the relevant herd average of all herds.

The same data comparisons are made for the group of "problem cows" and their subdivision according to initial diagnoses. A deviation of one third or more from the area average is considered significant and merits special investigation.

Extensive epidemiological studies comprising large numbers of herds, were performed in order to elucidate whether an association between deviations from the average incidence and management, particularly nutritional factors, could be found. The summary of these studies, performed during the last 20 years was published by Francos and Mayer (1983). A detailed discussion of our findings is beyond the scope of this paper. Probably the most interesting of them is of the connection between energy deficiency and the rise in the incidence of repeat breeder cows in the herd. (Francos, 1974; Francos et al., 1980). They may well serve as evidence that problems which remained obscure after many years of investigations by the classical methods of controlled experiments, can be clarified by the use of epidemiological investigative methods.

The data on the incidence and on the fertility indices for each "reproductive disorder" group indicate the severity of the problem and the efficiency of treatments. A trend to an interaction between those two factors exists, therefore in those cases, where a high incidence following a delay in conceptions does occur, an effort should be made to reduce the incidence and to improve the treatment methods.

The printout summarising the data from all herds participating in the programme shows that the highest incidence was recorded for "unobserved oestrus" (Table 1a). This diagnosis also was connected with a considerable delay in conception and consequently with a high percentage of "problem cows" (Table 1b). The data enable us to evaluate the type of problem in individual herds; thus cows with a large number of cases of "unobserved oestrus" in herd Z (Table 3) have a reasonable level of fertility, while this same diagnosis may be connected with a considerable delay in conception and with a large number of "problem cows" (Herd RZ, Table 3). When this occurs, the existence of serious disturbances of ovarian activity should be suspected.

Table 3. Fertility indices in cows with a diagnosis of "unobserved oestrus" in two different herds.

	No. of cows having unobserved oestrus	Incidence %	Days to first insemination	Days to conception	% of "problem cows" out of "unobserved oestrus" cases.
Herd Z.	80	38.6	88	113	25%
Herd R.Z.	36	33.3	100	130	47.2%

"Retained placenta", "lochia" and "endometritis", can be grouped in a "uterine disorder" group. After the first two pathological events, days to conception are shorter and the percentage of "problem cows" is lower than after endometritis (Table 1b). As this group, by definition, includes only cows in which uterine discharge was detected and treated 21 days and later post-partum it seems reasonable that every effort should be made to diagnose and treat this uterine problem as close to the calving as possible, thus preventing this long lasting damage to the uterus.

The percentage of cows with uterine disorders culled during the first 5 months post-partum was high: 14.7% after lochia, 17.2% after endometritis, and 17.5% after retained placenta. The parallel data after unobserved oestrus and ovarian cysts were 3.5% and 3% respectively. Retained placentae and endometritis are associated with a high incidence of other diseases such as mastitis, ketosis, fatty liver syndrome (Sommer & Marx; 1969, Morrow 1976; Russell 1983). It appears that the cows with "uterine disorder" diagnoses are at high risk of being culled; they should therefore be put under strict veterinary supervision and intensive treatment.

According to our observations, the consequences of retained placenta treated identically are much more serious in herds with a milk yield above 6000 kg./year (Francos, 1976). Nevertheless, the analysis of the printout shows that, even at very high production levels, a herd factor seems to exist. The data on different influences of retained placentae on fertility indices in 2 herds producing above 9000 kg/yr. are depicted in Table 4.

Table 4. Fertility indices after retained placenta 2 herds with average milk yields of above 9000 kg.

	No. of cows with retained placenta	Incidence %	Days to 1st insemination	Days to conception	% of Problem cows out of "retained placenta" cases
Herd N.L.	13	8.6	74	112	15.4%
Herd H.H.	29	14.6	60	126	41.4%

In many herds there is a marked difference in the incidence level between the primiparous and multiparous groups of cows, usually kept in different feeding groups. The printout data help to diagnose these differences, and thus investigation of the factors involved may be focused on specific groups of cows. (These investigations should include a careful analysis of ante-partum and post-partum feeding practices). An example of this different incidence level is shown in Table 5.

Table 5. Differences in the incidence of various diagnoses between primiparous and multiparous cow groups.

The Herd	Diagnosis	Primiparous		Multiparous	
		No. of cases	Incidence %	No. of cases	Incidence %
E.S.	Repeat breeder	10	22.7	9	11.7
G.	Unobserved Oestrus	32	55	31	27.0
A.	Retained placenta	3	2.9	12	17.9

Analysis of reprodutive disorders will usually provide one of the following incidence profiles:

1. normal (near the average);
2. a high incidence of retained placenta and/or endometritis with a normal or low one of repeat breeders;
3. a high incidence of unobserved oestrus, sometimes accompanied by a high incidence of post service anoestrus (negative pregnancy examinations);
4. a high incidence of repeat breeder cows with a low one of endometritis (Table 6).

According to our experience, based also on data published by others; profile 2 generally indicates a probability of feeding errors committed during the later stages of the previous lactation and/or during the dry period, (high energy and protein level, lack of long stem roughage in the dry period, selenium deficiency). (Whitmore et al., 1974; Trinder et al., 1969; Romanink, 1978; Morrow, 1976; Francos & Mayer, 1983).

Profile 4 points to the probability of a border line deficiency of energy during the present period of lactation or during the last half of the previous gestation (phytooestrogen activity should be ruled out) (Adler & Trainin 1960; Francos & Mayer 1983). When Profile 3 occurs, a thorough investigation of herd management practices, particularly those of heat detection, has to be carried out, all aspects of the feeding regime should be carefully examined, and an examination to differentiate between true anoestrus and silent heat should be attempted. Prolonged periods to first insemination and to conception and a high percentage of "problem cows" after a diagnosis of unobserved oestrus point to the possibility that nutritional deficiencies are involved. A low incidence of "retained placenta" and/or "endometritis" in herds with a high one of "unobserved oestrus" gives the same indication. (Wiltbank 1970; Nakao et al., 1984; Francos & Mayer 1983).

It should be emphasised that in the analysis of the herd incidence profile, the composition of the "problem cow" group, subdivided according to previous diagnoses, should be included. This permits attention to be focussed on the major factors connected with the delay in conception.

Table 6. Examples of herd incidence profiles - a 6 months period.

	Uterine disorders	Unobserved oestrus	Repeat breeder
Herd code	K.G.	M.	G.O.
No. of Calvings	181	171	154
Incidence from all calvings, %:			
Retained Placenta	14.5	2.3	2.0
Lochia	25.6	4.7	7.1
Endometritis	6.7	1.0	1.0
Unobserved oestrus	18.2	28.7	4.0
Negative pregnancy examination	8.0	10.5	5.0
Ovarian cysts	2.8	1.8	1.0
Repeat breeders	3.3	5.8	16.9
Problem cows	34.3	33.3	28.0
Subdivision of the Problem Cows according to the main diagnostic groups each group as percentage from all "problem cows".			
Uterine disorders	50.1%	10.6%	8.1%
Unobserved oestrus	21.0%	42.1%	7.0%
Repeat breeder	11.6%	3.0%	51.2%

The method presented here has proved very helpful in the identification of the fertility problems determining the incidence of reproductive disorders. It gives guidelines as to their probable causes, and permits the use of these data for research purposes, on a herd, regional and national level. In our opinion the method may be applied effectively in herds milking 50 cows and more, provided that computerised recording of general fertility data is practised.

APPENDIX

The "Hachaklait" Veterinary Clinical Services (founded in 1919) is a farmer owned and directed, non-profit-making organisation, employing about 70 veterinary surgeons. Payment is per head per year and covers all veterinary clinical diagnostic and therapeutic work, as well as all drugs, laboratory analyses, X-Ray, surgery, consultations etc. except prophylactic treatments and vaccinations, (the latter carried out by the government veterinary service). The clinical veterinary service is based on regular (weekly or more frequent) visits and urgent calls. No payment per visit being required, urgent calls by the farmer are made on time, permitting early intervention and thus a higher rate of success that is necessary in these very high producing cows.

The Israeli dairy herd is composed of Israeli-Holsteins only. They are all artifically inseminated, physiologically adapted to the climate and kept on zero-grazing (absence of natural pasture, high alternative value of water), fed a ration containing about 30% of roughage. Being, for the above reasons obliged to produce a maximum yield from a minimum number of cows, they were genetically upgraded in a manner permitting them to hold the world record for milk production for over 25 years. They have produced in 1984 (1.10.83-30.11.84) 8000 kg/cow/year, 260 kg/fat/year; registered cow averages: 8730 kg/cow/year, 280 kg/fat/cow; industrial sized (Kibbutz) herds: 9050 kg/cow/year, 297 kg/fat/cow/year.

REFERENCES

Adler, J.H. and Trainin D. (1960). A hyperoestrogenic syndrome in cattle. Refuah Vet. 17, 115-116.

Francos G. (1974). The occurrence of disturbances of reproduction in dairy herds in Israel. Deutsche Tierarzt. Wochenschrift 81, 135-138.

Francos G. (1974). Possible effect of different nutritional planes on the incidence of reproductive disorders in Israeli dairy herds. Refuah Vet. 31, 27-32.

Francos G., Mayer E. and Davidson M. (1980). Nutritional influence on first insemination conception and the repeat breeder syndrome - an example of the efficacity of epidemiological investigative methods. Proceedings of XI International Congress on Diseases of Cattle, Tel Aviv, October 1980, pp. 833-839.

Francos G. and Mayer, E. (1983). The association between nutrition and fertility in Israeli kibbutz herds. Refuah Vet. 40, 19-29.

Martinez J. and Thibier M. (1984). Reproductive disorders in dairy cattle, Theriogenology 21, 569-582.

Morrow, D.A. (1976). Fat cow syndrome. J. Dairy Sci. 59, 1625-1629.

Nakao, T., Yoshimeki, S., Moriyoshi, M. and Kawata K. (1984). Effect of energy and protein intake on ovarian activity in postpartum high producing Holstein cows. Proceedings of XIII International Congress on Diseases of Cattle, Durban, September 1984, pp. 803-808.

Neria A., and Mayer E. (1984). Dairyman computerised health and management system for dairy cows. Proceedings of XIII International Congress on Diseases of Cattle, Durban, September 1984, pp. 135-141.

Romaniuk, J. (1978). Fertility indices in dairy cows following retained placenta. Bull. Vet. Institute Pulawy 22, 54-59.

Russell, A.M. (1983). Cosreel-Computer system for recording events in economically important livestock. Proceedings of the Society for Veterinary Epidemiology and Preventice Medicine, Southampton, April 1983, pp.29-36.

Sommer H., and Marx, D. (1969). Fertility of cattle and its relationship to metabolism. Berliner und Munchner Tierarzt Wochenschrift 82, 201-204.

Trinder N., Woodhouse D.D. and Renton C.P. (1969). The effect of vit. E and Selenium on the incidence of retained placenta in dairy cows. Veterinary Record 85, 550-553.

Whitmore H.L., Tyler W.J. and Cassida L.E., (1974). Effects of early post partum breeding in dairy cattle. J. Anim. Sci. 38, 339-343.

Wiltbank, J.N. (1970). Nutrition and reproductive expectancy in beef cows. Proceedings VI International Conference on Cattle Diseases, Philadelphia, August 1970, pp. 206-214.

MASTITIS CELL COUNT SURVEYS IN ENGLAND AND WALES: 1971-83

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SUMMARY

Comprehensive national surveys of mastitis cell counts in the dairy herds of England and Wales began in 1971. The cell counts were carried out by Coulter Counter on a representative sample of bulked herd milk. Initially the surveys covered 10,000 herds each month, but since 1977 all dairy herds have been included.

Over this period the national average cell count for England and Wales has been reduced by 32% from 573 thousand cells/ml in 1971 to 390 thousand cells/ml in 1983. The greater part of this reduction occurred in 1975/76, at a time of increased culling of the national dairy herd, and in 1983, immediately following the introduction of a payment scheme for total bacterial counts.

A wide seasonal variation exists, with the highest counts occurring in August and the lowest around January. There are also large and consistent differences between different regions; in 1983 the mean for south Wales, traditionally the area of highest cell counts, was 447 thousand cells/ml, whereas in north west England, where counts have always been lowest, it was 317 thousand cells/ml. A highly significant correlation has been demonstrated between the average age of the dairy cows in a region and the mean cell count.

INTRODUCTION

The Milk Marketing Board began to accumulate data on mastitis cell counts in 1968 following the temporary employment of the author, who was located at a large dairy at Bamber Bridge in Lancashire. Cell counting at that time was carried out by the direct microscopic method originally described by Prescott and Breed (1910). A report on the cell count aspects of the work of the first year concluded:

1. A survey on 407 herds in Lancashire over the 12 month period March 1968 to February 1969 inclusive revealed a geometric mean cell count for bulk herd milk of 528 thousand cells/ml. This figure compares with 460 thousand cells/ml for Denmark (1963), 372 thousand cells/ml for Bavaria (1968), and less than 300 thousand cells/ml for Switzerland (1969).

2. Forty-eight (11.8%) of these herds consistently produced milk containing over one million cells/ml.

3. Milk from herds with bulk tanks had a mean cell count which was 12% higher than that of herds using churns.

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4. Parlour-milked herds had consistently higher cell counts than herds milked under a round-the-shed (RTS) pipeline system. Parlour-milked herds of all sizes had similar group mean cell counts, whereas mean counts were higher in RTS pipeline herds over 75 cows.

5. The mean cell count of milk-recorded herds was 21% lower than that of non milk-recorded herds.

6. Winter milk producing herds, ie more than 55% of milk produced in October to March, had a mean cell count which was considerably below other groups.

7. The mean cell count of herds re-established after slaughter due to foot and mouth disease was lower than the overall mean.

8. Herds of the Channel Island breeds had a mean cell count 60% higher than the overall mean.

9. The mean cell count of quarters infected with Strep agalactiae, which has been the predominant pathogen to date, was three times higher than that of quarters infected with Staph aureus.

10. All herds so far examined with bulk milk counts over 500 thousand cells/ml have had more than 30% of the cows infected at bacteriological examination.

11. There have been considerable variations between the geographical areas examined in England and Wales, with the lower mean cell counts coming from the northern counties of England. The mean cell count of 3,000 herds examined in September/October 1969 was 535 thousand cells/ml.

Following further work on both mastitis and brucellosis the research unit was transferred to Worcester in 1970.

MATERIALS AND METHODS

A sample of bulk herd milk has been received at the Worcester laboratory from every dairy herd in England and Wales since January 1971. The samples are taken for milk-ring testing for brucellosis and more than 97% of herds are included each month. Originally the samples were taken from the bulk samples received for milk compositional testing at 250 dairy laboratories around the country but, since July 1982, they have been submitted direct from the six MMB central testing laboratories. Samples are preserved, and the cells fixed, by the addition of eosin-stained formalin at a final concentration of one part to 500 parts of milk. The preserved milk samples are transported to Worcester and tested in an average of four days.

A random selection of approximately 10,000 samples was cell counted each month for the first six years of the surveys, and the cell count information was reported to the herdowner once a year. For the second half of the survey period, from April 1977, all herds were cell counted and this information was sent each month to the herdowner. A Coulter Counter model F was used initially for all cell counting, supplemented with a model ZF after the first three years. Once all herds were cell counted from 1977, this testing was carried out using five automated Coulter Milk Cell Counters (MCC).

RESULTS AND DISCUSSION

When the surveys were extended to cover the whole of England and Wales it was found that the area of original operations in Lancashire had mastitis cell counts which were appreciably lower than the national average. Nevertheless the majority of points made in the first annual report in 1969 have held true, although naturally there have been substantial changes in dairy farming practices over the intervening years.

(i) Annual variations

Table 1 lists the annual geometric mean cell counts for England and Wales over the 13 years of the surveys. The table shows relatively little change over the first four years during which time the mean was 568 ± 9 thousand cells/ml. Following substantial reductions in the national mean during 1975 and 1976, the mean over the next seven years became fairly constant and almost 100 thousand cells/ml lower at 473 ± 6 thousand cells/ml. The final year 1983 saw another marked reduction.

Table 1. Geometric mean cell counts in England and Wales

Year	Annual geometric mean cell count (thousand cells/ml)
1971	573
1972	543
1973	570
1974	586
1975	508
1976	467
1977	468
1978	503
1979	485
1980	469
1981	465
1982	456
1983	390

Herd levels of subclinical mastitis have been shown to be the major influence on bulk milk cell counts, although other factors will also have an effect (Booth, 1974). It might have been expected therefore that large reductions in the national mean would have coincided with the increased application of mastitis control measures, but no such relationship can be demonstrated. Throughout the 1970s figures from the Ministry's Dairy Husbandry Advisory Service showed increases in the application of teat dipping, of dry period antibiotic therapy and of milking machine testing, but these increases were relatively uniform throughout the decade.

A retrospective examination of the cell count records of 1,500 herds which ceased milk production during the period June to September 1975 revealed that their geometric mean counts in 1973 and 1974 were 57 and 122 thousand cells/ml higher than the respective national annual means. This would appear to indicate that improvement in the national cell count situation may have come about because of the elimination of some of the worst herds. However, between 1970 and 1980 the number of dairy herds in England and Wales was reduced from 81,520 to 45,885, a reduction of over 35,600 herds (44%), so that, on this basis, one might have expected to see a continuous reduction in the national

cell count during the period (United Kingdom Dairy Facts and Figures, 1971 and 1981). Nevertheless, over 10,000 of these herds were lost in 1974 and 1975, so this could have been a factor in the 1975/76 reduction in cell count. Investigations of the herds going out of milk production during 1982 and 1983 have shown that their cell counts have averaged 64 thousand cells/ml above the national mean in their last year of production, having risen by 20 thousand cells/ml during the previous year.

The years 1975 and 1976, when the national cell count fell substantially, also saw a decline of 150,000 in the number of dairy cows in the national dairy herd, and it is logical to expect that a proportion of these would be infected with mastitis. Exeter University showed that some 16,000 more cows were culled because of mastitis in 1976/77 compared to 1972/73, which goes some way to confirming this.

An interesting suggestion from one farmer was that there appeared to be a relationship between the price for cull cows and the national cell count level. This was examined over a four and a half year period from January 1974 to June 1978 and a correlation coefficient of -0.91 was found; as the cull cow price went up by £100 so the national cell count declined by 82 thousand cells/ml. However, this seems unlikely to be a direct cause and effect relationship.

The second period of marked reduction in the national cell count was throughout 1983. This appears to be associated quite clearly with the introduction of a payment scheme for total bacterial counts (TBCs). Farmers and herdsmen were made aware that the milk from cows infected with mastitis could have extremely high numbers of bacteria, which could quite easily move their classification out of the bonus band, sometimes even to a penalty band. Since the premium for remaining in the top TBC band was over £1,000 per annum for a 100 cow herd, the financial incentive was immediately apparent.

Over the first half of 1982 monthly cell counts were on a rising trend, but this was abruptly reversed in June when TBCs were first introduced on an advisory basis. The payment scheme became operationable in October 1982 and the monthly cell counts for the next five months averaged 111 thousand cells/ml lower than the same period in the previous year. This effect appeared to wear off to a certain extent, so that over the quarter July to September the average reduction was only 37 thousand cells/ml, but nevertheless the year as a whole showed a reduction of 66 thousand cells/ml.

Several investigations have shown a relationship between herd size and cell count. In a random selection of 1,937 milk-recorded herds the lowest mean cell count was for herds of 30 to 59 cows (Table 2). As the table shows, the groups of herds with 90 and more cows had above average mean counts, and this became more marked in the largest herds. It is possible of course that this increased cell count in larger herds may not reflect a higher prevalence of subclinical mastitis but may instead be due to greater problems in detecting clinical mastitis in larger herds. Larger herds have been shown to fail the test for antibiotic residues in milk more frequently (Booth, 1982); this could be interpreted as confirmation of either hypothesis.

Table 2. Cell counts and herd size, 1977/78 (Booth, 1980)

Herd size (cows)	No. herds	Mean cell count (thousands/ml)
<30	106	370
30-59	497	358
60-89	628	368
90-119	409	387
120-149	159	375
150-179	70	391
180+	68	422
	1,937	373

During the period of the surveys the average herd size in England and Wales has almost doubled from 35 cows in 1971 to 67 cows in 1983. Over the same period the proportion of herds with more than 100 cows has increased from 4.9% to 17.3%. On the basis of the surveys which have shown higher cell counts in larger herds one might have expected the national mean cell count to have increased substantially also. From the fact that it has not, and has actually declined, one could infer that measures for the control of mastitis in dairy herds in the country must have been applied increasingly effectively.

A series of surveys has confirmed the original finding of higher cell counts in herds of the Channel Island breeds. It has been suggested that this is more a measure of age, in that Channel Island herds tend to contain more old cows and that older cows are more frequently infected with mastitis, but this has not so far been established. However, small surveys have indicated that cell counts in the uninfected quarters of Channel Island cows are similar to those of Friesian cows, which indicates that the age factor may be involved.

Over the three years 1981 to 1983, herds of predominantly Channel Island cows have averaged 89 thousand cells/ml (20%) higher than the national average, although this has tended to fall over the period and was 79 thousand cells/ml higher in 1983, but was still 20%. The Channel Island proportion of the national herd has declined from 9.0% in 1970 to 3.5% in 1983, so this could have assisted the downward trend in national mean cell count over the period of the surveys.

As might be expected, there remains a consistently wide variation in the mean cell counts of individual herds (Table 3). In 1983 the range in annual means, with herds having eight or more monthly counts, was from 74 to 3,379 thousand cells/ml. EEC co-responsibility funds were granted in 1983 for a service to visit the majority of herds with cell counts over 400 thousand cells/ml in order to advise on the application of a basic mastitis control programme, so some improvement may perhaps be expected in these herds in the future.

Table 3. Distribution of herds by cell count, 1983

Cell count range (thousands/ml)	Herds	
	No.	%
<100	43	0.1
100-199	3073	7.6
200-299	9486	23.4
300-399	9370	23.1
400-499	6551	16.2
500-599	4230	10.4
600-699	2750	6.8
700-799	1697	4.2
800-899	1062	2.6
900-999	710	1.7
1000-1499	1290	3.2
1500-1999	183	0.5
2000+	65	0.2
	40,510	100.0

(ii) Regional variations

From the first surveys in 1968, as noted in the introduction, differences in mean cell counts have been observed between different regions. These differences have consistently favoured the north of England, with south Wales and south western England having the highest mean cell counts of all regions (Table 4).

Table 4. Mastitis cell counts by region

MMB region	Mean cell count (thousands/ml)		
	1978	1983	Change (%)
North Western A	413	317	-96 (23)
Northern	450	339	-111 (25)
East Midland	453	380	-73 (16)
West Midland	486	376	-110 (23)
North Western B	499	380	-119 (24)
Southern	506	400	-106 (21)
Eastern	513	401	-112 (22)
South Eastern	518	402	-116 (22)
Mid Western	526	419	-107 (20)
North Wales	529	408	-121 (23)
Far Western	555	440	-115 (21)
South Wales	578	447	-131 (23)
England and Wales	503	390	-113 (22)

The table shows the wide differences between regions and that there has been virtually no movement in the ranking over the five years 1978 to 1983, despite the large reduction in national cell count. Apart from the East Midlands, every region had a drop of 20 to 25% over the period, corresponding to a reduction of between 96 and 131 thousand cells/ml.

Several investigations have been carried out to attempt to explain these

regional differences. An obvious one was to examine the possible influence of herd size. One investigation in 1979 produced a non-significant correlation coefficient of +0.30 between the mean cell counts and herd sizes of the regions. However, three regions were very obvious outliers (they were the Celtic fringes of Wales and south western England) and when these were excluded the correlation rose to +0.82; as average herd sizes per region increased by ten cows so the mean cell count increased by 18 thousand cells/ml. The only explanation so far offered regarding the outlying regions was that these tend to be areas of non-specialist dairy farms, frequently with large numbers of sheep and beef cattle, so that the control of mastitis in the dairy herd may play a rather less prominent role.

The possible effect of average herd age has also been examined on both a county and a regional basis. Unfortunately the only data available on average herd ages derive from milk-recorded herds which, as already shown, are a biassed group, although they do reflect the same regional cell count differences found in the whole population. Comparing the regional mean cell counts for the year to September 1979, with the milk-recorded herds' average lactation number on a regional basis over the same period, gave a very highly significant correlation coefficient of +0.90. As the average herd life increased by half a lactation, so the mean regional cell count increased by 102 thousand cells/ml; the range was actually from 3.6 lactations in north west England to 4.3 lactations in south west England and south Wales. This may well be a more acceptable, and perhaps more accurate, explanation for the consistent differences noted in the regional mean cell counts.

(iii) Seasonal variations

Throughout the period of the surveys mean cell counts have always been significantly higher in late summer and early autumn, especially in July to September, declining rapidly thereafter, the lowest levels being found during the period January to March. This does not correspond to most farmers' experience of clinical mastitis and, although there is no reason why the peaks of clinical and subclinical mastitis should coincide, there is no immediately apparent reason why cell counts should be at their lowest in the winter. Table 5 shows the fairly typical year of 1980, a time when the national mean varied very little.

Table 5. Mean cell counts in 1980

Month	Mean cell count (thousands/ml)	% difference from median
January	423	-6
February	425	-5
March	431	-4
April	448	0
May	434	-3
June	443	-1
July	471	+5
August	515	+15
September	476	+6
October	446	-1
November	429	-4
December	442	-2

One investigation did show that there appeared to be a relationship with the proportion of first lactation cows in the milking herd. However, subsequent investigations have not so far confirmed this; a herd of 200 cows had correlation coefficients of +0.41 and +0.08 between the percentage of first lactation cows and the monthly cell count in 1982 and 1983 respectively. According to another small investigation, the explanation did not appear to lie in the dilution effect of more milk being produced at certain times of the year.

It may be that the seasonal cell count variation observed is a true measure of the proportion of milking cows with subclinical mastitis, but this has yet to be demonstrated. Certainly it has been shown many times that late lactation milk contains more cells, and in particular a higher proportion of cells other than leucocytes. For these reasons therefore it is normal practice to average the monthly counts over the year so that seasonal effects will be reduced and any change in the rolling annual mean cell count is likely to be of significance.

CONCLUSIONS

Over the 13 year period surveyed the national mastitis cell count has declined from 573 to 390 thousand cells/ml, a reduction of 32%. There is some corroborative evidence, not discussed here, to indicate that this is a true reflection of the national mastitis situation over the period. The two main periods of decline, in 1975/76 and in 1983, can be shown to have been significantly associated with factors not directly related to the increased application of mastitis control measures on the farm. Nevertheless, the fact that cell count levels have not returned to previous levels after these periods of reduction would indicate that the adoption of mastitis control measures on a wide basis has had a considerable effect upon retaining these lower levels. There is some evidence that larger herds, especially those over 90 cows, have higher cell counts, and the proportion of these herds has more than trebled during the period under review, which may be further confirmation of the general effectiveness of present mastitis control measures.

REFERENCES

Booth, J.M. (1974). Cell counting in milk. Veterinary Annual 15, 47.

Booth, J.M. (1980). The role of mastitis cell counts in a national control programme. Proceedings of a conference on resistance factors and genetic aspects of mastitis control, Jablonna, Warsaw, Poland. p20.

Booth, J.M. (1982). Antibiotic residues in milk. In Practice, Supplement to Veterinary Record 4, 100.

Prescott, S.C. and Breed, R.S. (1910). The determination of the number of body cells in milk by a direct method. Journal of infectious diseases 7, 632.

United Kingdom Dairy Facts and Figures (1971), Table 18.

United Kingdom Dairy Facts and Figures (1981), Table 12.

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