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OPEN SESSION

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Constitution of the Society

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GENETICS AND THE APPLICATION OF NEW TECHNOLOGIES

PROSPECTIVE USE OF RECOMBINANT DNA METHODS IN ANIMAL DISEASE CONTROL

*GEOFFREY GOLDSPINK and **GERALD-F. GERLACH

Recombinant DNA methods probably represent the definitive way of diagnosing virtually all veterinary diseases. These include methods of detecting the presence of a particular pathogen or an hereditary defect in the host's own genome. In addition they offer the only safe and sure way of determining the genetic background of the animal.

It is just about a quarter of a century since Crick and Watson working in Cambridge and Wilkins at the University of London began to publish their papers on the structure of DNA. Molecular Biology has now "come of age" and has given rise to the new applied science of biotechnology. It is reasonably safe to predict that our knowledge of the structure and function of DNA will be further used to revolutionize the way in which diseases are diagnosed, the way in which vaccines are produced, and the way in which growth, disease resistance, fertility and sex are manipulated in agricultural animals.

Advances are occuring at a very rapid rate as the technology is being developed in human medicine, and this has a "knock on" effect as far as veterinary medicine is concerned. The latter, however, differs in two respects from human medicine in that the manipulation of the germ line to impart disease resistance and growth potential will no doubt be permitted in agricultural animals but probably not in man. Also in veterinary medicine there must be a cost effective approach, and methods used for the screening of large herds must be cheap as well as accurate. Therefore, veterinary biotechnology needs to have a development separate from that in human medicine. Nevertheless, the mapping of the human genome, which is being carried out in several countries in the world, will have an enormous impact on

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our understanding of the mammalian genetic information. It will allow us to establish the genetic profile of any cow, pig or sheep, and this could be done at the pre-implantation stage by removing a few cells from the early embryo. Using pre-implantation diagnosis, it should be possible to assess the animal's susceptibility to contracting certain diseases. Therefore, it seems that the area of veterinary diagnostics is going to change vastly during the next quarter of a century or so.

The basis of recombinant DNA technology: In vertebrate animals the genetic information is coded for by approxiamtely 3×10^9 nucleotides and these represent a catalogue of its genetic characteristics. There are four different nucleic acid bases that make up DNA and these spontaneously anneal into pairs by hydrogen bonding to form a double helix (A with T and G with C). The linear arrangement of these bases dictates the amino acid sequence of the proteins that are synthesized. However, it is important to realize that much of the DNA is not translated into protein but is involved in the regulation of gene expression. This ensures that a given gene is expressed in a specific cell type at the correct time during development, and has an appropriate physiological response. Also, it should be appreciated that some genes present in the genome are not expressed and are apparently ancestral or pseudo gene sequences, presumably left overs from evolution.

Each gene is flanked by untranslated DNA sequences with the promoter which drives the transcription at the 5' end. Some regulatory sequences (enhancers) may occur many thousands of bases away from the gene, although their linear distance may belie the fact that they are probably in juxtaposition with the gene when the coiled DNA adopts the appropriate three dimensional configuration. Both promoters and enhancers are apparently binding sites for factors which are usually proteins and which facilitate or repress gene expression. In addition to the untranslated regions of the 5' and the 3' end of the gene there may be regions within the gene that are not translated into protein. These are called introns and they are particularly important in genes which produce more than one protein as a result of alternate splicing of the RNA transcript. Some introns may also include a regulatory sequence which is essential for high levels of expression of that gene.

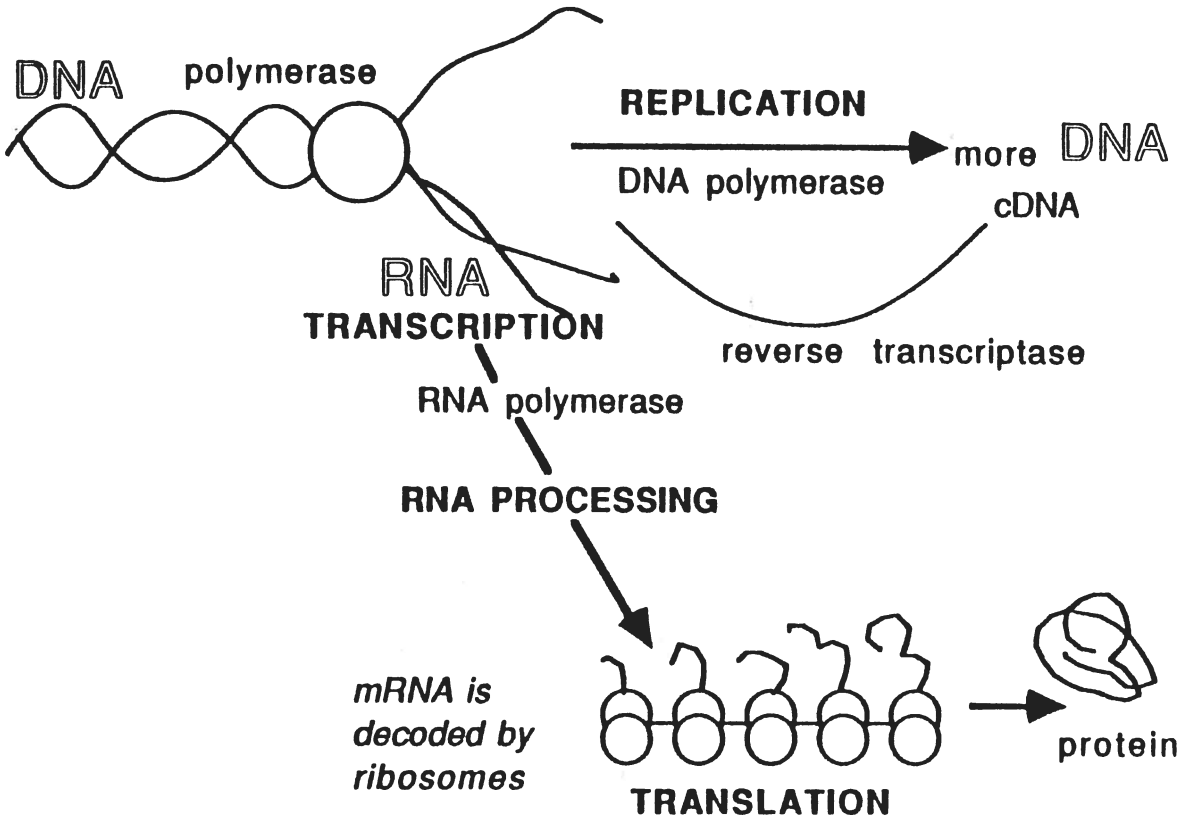

Figure 1 A summary of the main terms involved in DNA replication, transcription, and translation of the message into protein.

1. RECOMBINANT DNA TECHNOLOGY IN DIAGNOSIS

There are several thousand different genes and each one has a unique sequence so that the presence of the gene and the expression of the gene may be detected if a specific probe with the complementary sequence is available. These unique DNA sequences can therefore be used for diagnostic purposes whether it be to detect the presence of an invading organism or to probe the genome of the host for the presence of desirable or undesirable sequences. These probes may be complementary DNA (cDNA) probes or they may be genomic DNA probes. Also, an oligonucleotide probe can be synthesized if the sequence is known. As more and more genes are being cloned and sequenced, the data base of DNA sequences is growing in an exponential manner and sometimes it is possible to look up the sequence for a gene from a related species and, provided this has a high percentage of homology, it is possible to use it in a first approach in the development of a diagnostic procedure.

Preparation of gene probes: The key to developing a good diagnostic

procedure is to obtain the appropriate specific probe. The strategy adopted depends on whether the DNA sequences are known for that species or a related species. If the sequence is known then the probe can be synthesized using an oligonucleotide synthesizer. If the sequence is not known then it is necessary to clone an appropriate DNA fragment from either a cDNA library or a genomic library. In the latter case, the total DNA is extracted from any tissue of the body and then cut into fragments using a specific restriction endonuclease. This yields thousands of gene fragments which can first be separated according to size by agarose gel electrophoresis. These fragments are then ligated into a vector which can be a Lambda phage or a cosmid vector and then put into E.coli cells to mutiply the number of copies of the fragments. The bacterial clones are screened using a probe from a related species. An alternative method which is more selective involves preparing cDNA probes using RNA extracted from a tissue which is known to have a high level of expression of that gene. For instance, if one wishes to prepare a probe for growth hormone then it is sensible to use pituitary cells where high levels of the growth hormone message are known to be expressed. In this approach, the RNA is isolated and then passed down an oligo dT column which extracts the messenger RNA which invariably has a poly A tail. The messenger RNA is then converted into cDNA using the enzymes reverse transcriptase and DNA polymerase. These cDNA-fragments can then be cloned into an expression vector, which allows the subsequent screening of the gene library with a specific antibody. Having once obtained the specific gene probe it is then possible to use this to detect the corresponding DNA sequence in the genome of the animal or its transcription product (RNA) in a particular tissue.

Detection methods

A. Dot blots hybridization: Hybridization of DNA or RNA fragments can be carried out without first separating them electrophoretically. This approach is commonly referred to as the dot blot or the slot blot system. The RNA or DNA is extracted from the infected tissue or body sample and, after concentration, it is strand separated and dotted onto nitrocellulose paper. A labelled DNA probe is then added so that hybridization with complementary DNA fragments takes place. A simple piece of equipment with twenty or thirty wells is used so that many samples can be tested simultaneously. The label commonly used is ^{32}P or the avidin-biotin complex. This is a rapid diagnostic method which is used for total DNA or RNA. However, there are

pitfalls as it is necessary to carry out controls particularly if quantitative or even semi-quantitative data are required.

B. Southern and Northern blot hybridization: This approach involves separating DNA fragments by agarose gel electrophoresis and transferring the DNA to a nitrocellulose or nylon membrane. A labelled probe that is known to be complementary to a region of the gene is then hybridized to the DNA immobilized on the membrane. The band of DNA where the gene fragments are located is identified by the presence of the label. In the case of a radioactive label such as ^{32}P this is seen by carrying out autoradiography. This approach applied to DNA was developed by Professor E.M. Southern who is now in the Department of Biochemistry at Oxford University, hence the term "Southern Blotting". A similar approach to identify RNA species has been termed "Northern Blotting".

There are variations on Northern blot hybridizations which involve the removal of non-hybridized RNA species. Enzymes such as S1 nuclease can be used to remove any non-hybridized nucleotides so that only double stranded forms remain. For these protection assays it is necessary to have a high degree of homology between the probe and the gene sequence in question whereas, for Southern and Northern blot hybridization, this is not essential because they can be done under different stringency conditions. In our opinion, therefore, there is no real alternative to Southern and Northern blot hybridization to initially establish that a probe will hybridize to the gene in question.

C. In situ hybridization: As the name implies, this method involves hybridizing labelled probes to DNA or RNA in situ (Haase and Oldsdone 1989). This approach can be applied to bacterial colonies, tissue sections, blood or chromosomal smears. It is a more difficult technique; however, the advantages are that it permits the localization of hybridization. Thus, certain bacterial colonies can be identified or the expression of an RNA in question can be confined to one tissue or even to a certain cell type. Also, a given gene can be located on a particular chromosome. For the detection of gene expression it is preferable to use an RNA probe rather than a DNA probe, because RNA-RNA duplex molecules have a higher stability than RNA-DNA hybrids. Therefore unspecific reactions can be minimized by using more stringent hybridization conditions.

D. Polymerase Chain Reaction: Although cloning and hybridization testing is routinely carried out in many laboratories, it is relatively time consuming and expensive. At the present time it would take at least one week to carry out a batch of tests. The introduction of enzymatic gene amplification (Erlich et al. 1985) using the Polymerase Chain Reaction (PCR) has meant that recombinant DNA diagnostic methods can be carried out within one day. The principle of this method is that a specific DNA fragment is amplified in a test tube using a mixture of enzyme, label, bases and probes. The enzyme is a special type of polymerase (TAQ 1) which was originally isolated from a bacterium which lives in hot springs, Thermus aquaticus. A sample of tissue or tissue fluids containing DNA is subjected to a regime of temperature change using a programmable incubation system. The incubation is first carried out at about $90^{o}C$ to cause separation of the double stranded DNA. The next step involves annealing specific gene probes (which act as primers) to the DNA at temperatures of about $50^{o}C$. The temperature is then raised to about $70^{o}C$ which is the optimum for Taq polymerase activity. If two probes are used, one for each end of the gene, the amplification that takes place is geometric and results in millions of labelled copies of the gene within a time as short as two hours (Wong et al. 1987). PCR can in principle be used to detect the presence of any specific DNA sequence, whether it be an hereditary defect or a latent viral infection. It could also be used to distinguish between virulent and non-virulent bacterial strains. The rapidity and sensitivity of the Polymerase Chain Reaction could make it an ideal way of carrying out pre-implantation diagnosis in conjunction with embryo transfer. It could also be used in situations where the breeding stock have to be shown free of a specific disease.

E. Restriction fragment length patterns: One method of detecting altered gene sequences is to cut the DNA with restriction enzymes and to compare the pattern of the fragments following electrophoretic separation. A particular restriction endonuclease will cut DNA at only those sites where a specific combination of bases occurs. A mutation involving just one base may therefore result in a different restriction fragment length pattern. This approach is therefore more appropriate for detecting inborn errors, such as muscular dystrophy, than the presence of a pathogen.

F. Possible developments in the future: To date, diagnostic DNA probes have been constructed to detect para Tbc, (Whipple et al. 1989) and Leptospirosis

(Zuerner and Bolin, 1988). However, the methods mentioned so far would cost a minimum of five pounds per assay. This level of expense may be justified if one was screening elite breeding stock for the presence of specific diseases. In contrast, in order to carry out testing on a wide scale, a cheap but accurate method is required. Within the next decade it is likely that a dipstick approach will be used in which a specific gene probe together with a cascade reaction mixture will be incorporated into a thin film which will be used to coat dipsticks. The presence of an endogenous gene or a pathogen gene could therefore be detected by placing a drop of tissue fluid on the dipstick. These could be mass produced and the cost of the test reduced to a few pence rather than pounds.

2. THE USE OF RECOMBINANT DNA TECHNOLOGY TO PRODUCE NEW VACCINES

A good vaccine should, at best, give life-long protection at least against the clinically manifest infection by the wild type agent. It should not cause significant ill-effects, and it should be cheap. In veterinary medicine such vaccines are used, namely against canine distemper, rabies, and swine erysipelas. Other live vaccines, for example against Aujeszky's disease or canine hepatitis, give a protective immunity, but the animals shed live virus over prolonged periods of time. Some important infections of animals such as foot and mouth disease or equine influenza are prevented by the yearly application of inactivated virus. A large number of veterinary infectious diseases, however, can to date not be effectively prevented by appropriate vaccines, such as Maedi/Visna, shipping fever, BVD (bovine virus diarrhea), TGE (transmissible gastro-enteritis), African swine fever, and most parasitic diseases. Reasons for this inability to vaccinate are the inappropriate presentation of protective antigenic epitopes in inactivated or attenuated vaccine strains and/or the occurence of antigenic shift in the infectious agent. These problems can be tackled by the use of molecular biology techniques. Thus, genes encoding defined immunogenic regions of the infectious agent can be cloned and expressed in suitable vectors to either construct a live vaccine or to produce the immogen in large amounts without having to deal with the infectious - and thus dangerous - pathogen.

To construct recombinant live vaccines, the genome of several viruses has been used. The best known is vaccinia virus, an orthopox virus with a wide host spectrum (Moss and Flexner 1987). More recently, the use of other

viruses,such as fowl pox-, capripox-, herpes-, entero-, and adenoviruses, has also been suggested (Arsenakis and Roizman 1985; Burke et al. 1988; Gershon and Black 1988; Morris et al. 1987; Taylor et al. 1988). Furthermore, non-pathogenic Salmonella strains which have maintained their invasive abilities, have been constructed (Curtiss et al. 1989). For vaccine production DNA-fragments which are not necessary for viral replication have been removed from the viral genome. In the case of Salmonella, the shifting of essential metabolic genes onto plasmids, which are subsequently used as cloning vehicles, now allow the stable expression of foreign genes without the presence of artificial selection markers such as antibiotics.

The commercial use of these approaches, however, is hampered by several problems. Thus, viruses can exchange DNA with one another during the cause of infection. This leads to the fear of evolution of a "monstervirus". These objections do not take into consideration that similar risks are taken by using attenuated live vaccines. A second problem, especially with vaccinia virus is that immunocompromised hosts can develop systemic infections (Redfield et al. 1987). This problem is being counteracted by two approaches. Firstly, similar viruses with a narrower host spectrum, such as capripox and fowlpox viruses,are used. These, nonetheless,grow sufficiently in non-permissive cells to elicit an immune response. Secondly, the vaccine strain is made to simultaneously express a lymphokine such as interleukin 2 (Flexner et al. 1987). This leads to a loss of pathogenicity with reduction in immunogenicity due to yet unknown mechanisms.

These problems have also been tackled by using molecular biology to cheaply produce the immogen in question in large amounts in order to then use it as an inactivated vaccine. For this purpose several expression systems have been developed, such as viral surface protein genes (Valenzuela et al. 1982) and the yeast transposon TY (Kingsman and Kingsman 1988). Both of these systems induce the production of proteins which self-assemble into microspheres even after the insertion of foreign DNA, and both can be used in yeast. Furthermore, the microspheres in both systems appear to contain little, if any nucleic acids. Another approach which is currently used employs insect cells and Baculovirus (Miller 1988). The DNA encoding the immogen in question is fused to a baculovirus gene which is expressed in very high quantities. The latter system has the advantage that post-translational modifications (glycosilation, phosphorylation), which can be important for the

antigenic properties,appear to be identical in mammalian and in insect cells. A third approach uses bacterial plasmids encoding for an outer membrane protein. To date, the lamB and the phoE genes of E. coli (Agterberg et al. 1987) and the flagellin gene of Salmonella have been used (Newton et al. 1989).

Not all vaccines prevent the colonisation of the host with the pathogen. Some merely prevent the onset of clinical symptoms. A typical example for this situation is Aujeszky's disease. Thus, vaccination used to cause the problem that carrier animals which shed the pathogen could not be distinguished by immunoassay from immunized individuals which had not been in contact with the pathogen. This problem has also been overcome by recombinant DNA technology. Thus, a DNA fragment, encoding a protein which induces a nonprotective immune response, has been deleted from the genome. Therefore, vaccinated and latently infected animals can be distinguished and this recombinant live vaccine is currently commercially available (McGregor et al. 1985).

The production of vaccines using recombinant DNA technology therefore seems to hold great promise for the future. However, the technology is hampered by the complexity of the immune response and by our still incomplete understanding of the underlying mechanisms. A good and lasting immune response requires a local and a cellular response, and these responses are triggered by different regions of the antigen. Furthermore, the development of a protective immune response also depends on the genetic makeup of the host (Major Histocompatability Complex, MHC). Therefore, much research is still necessary before the technical possibilities of recombinant DNA technology can be fully applied in the prevention and therapy of infectious diseases in veterinary medicine.

3. The use of transgenic animals as a method of disease control

Transgenic biology offers a means of changing the genome of the animal more rapidly than it has ever been changed before by selective breeding. The outcome of this approach is, at the present time, unpredictable and it is possible that the restructuring of the genome may produce animals which have different disease susceptibilities. One method of producing transgenic animals is by micro-injection of DNA into the pro-nucleus of recently

fertilized ova (Palmiter et al. 1982). Another method involves transfecting novel genes into embryonic stem (ES) cells which can be screened before placing them into the early blastocyst. The former method has the advantage that it immediately introduces the desired DNA into the germ line without causing mosaic animals, although at present the success rate of gene integration and expression is low for agricultural animals. Thus, when genomic DNA is introduced into the one-celled ovum, its incorporation tends to be random. It appears that several gene copies are incorporated in tandem into any chromosome. This means that the locus of insertion and the expression of the extraparental gene is unpredictable.

There are various possibilities for disease control with the use of transgenic animals. One approach is to improve the disease resistance of animals by employing the individual genetic makeup of the host. It had been recognized early on, that certain breeds of cattle and certain inbred strains of mice did have a higher specific disease resistance than others. Special breeding programmes were then established to exploit the difference in disease susceptibility. Molecular biology should allow us to very much increase the speed of these developments. The genetic loci which are responsible for general disease resistance are being characterized and located. Among others, in cattle, goat and mice, strong evidence for a correlation between the MHC complex and the susceptibility to various viral infections has been found (Chesebro 1988; Lewin 1989 Ruff and Lazary 1988;). In addition, several non MHC-related disease resistance loci have been identified by infection studies (Bumstead et al. 1989, Albritton et al. 1989). Several disease resistance phenomena have been further investigated, and two general mechanisms seem to be employed. One is the presence of receptors which allow the infection with certain pathogens on the cell surface of the host (Albritton et al. 1989, Gaastra and DeGraaf 1982). If, as in the case of the E. coli K88 fimbriae receptor of pigs, no other vital function is associated with the receptor structure then disease resistance is conferred by its absence or alteration. However, the introduction of specific deletions into the host genome is to date not possible even though it is a standard technique in today's bacteriology research.

Other disease resistance phenomena are based on the presence of host proteins which are either directly inhibitory for the pathogen (Mx-protein; Hug et al. 1988) or which enhance the immune responses of the host (acute

phase proteins). Several of the encoding genes which impart disease resistance have been cloned and characterized. Also, transgenic mice, carrying one of these genes have been constructed and increased disease resistance has been shown (Arnheiter et al. 1989; Chen et al. 1988).

A second approach for the construction of transgenic animals with enhanced disease resistance uses artificial genes aimed at blocking the amplification of the pathogen. One possible way involves the introduction of DNA sequences coding for the activating factor which binds to the viral DNA activating transcription. However, the engineered sequence lacks the domain involved in activation, so that binding to the DNA still takes place but transcription does not commence. Hence this activation factor competes with the wild type factor and blocks the regulatory sequence. This method has recently been reported by Friedman et al. (1988) as a way of conferring intracellular immunity against the herpes virus. It has been speculated that this approach will be applied to HIV infection,as the engineered virus with the deactivated trans-acting factor can be introduced into haemopoietic stem cells. However, it is foreseeable that in the long term these DNA sequences can be introduced as transgenes so that a line of animals would have a built-in cellular immunity to specific viral diseases. A second way is to introduce genes which produce anti-sense RNA. This hybridizes with the mRNA which one wishes to deactivate. This approach has been used successfully to inhibit viral replication (Neinan et al. 1988) and clearly represents a powerful approach to controlling viral infections.

Molecular biology will have a profound effect on animal disease management in the future by its employment in three different areas. Firstly, it will change the conventional ways of diagnosis of microbial and parasitic diseases and it will, for the first time, allow the dependable and early detection of recessive hereditary defects. Secondly, it will improve our ability to construct vaccines not only against viral and bacterial but also against parasitic diseases. Furthermore, it may allow the development of a new class of so-called therapeutic vaccines. Thirdly, molecular biology may allow us to prevent diseases on the genetic level. Thus, the foundation of a breed with certain disease resistance characteristics could be done within a couple of years, rather than through an elaborative program of long-term cross-breeding.

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THE AETIOLOGY OF CANINE ELBOW OSTEOCHONDROSIS

S. GUTHRIE*

Osteochondrosis is a failure of subchondral ossification resulting in an area of retained thickened cartilage. Deepest layers do not receive adequate nutrition and become necrotic. These areas of cartilage are then subject to detachment, either partially to form a dissecting flap or totally to form joint mice.

The condition is very breed specific. The elbow joint is most commonly affected in Labradors, Retrievers and Rottweilers. The condition in this joint was first reported by Olsson (1974). Despite increased awareness of osteochondrosis, the diagnosis and treatment of the condition in the elbow remains problematical. There is considerable variation in the degree of lameness, radiographic change and severity of lesions; and there is no correlation between the three (Guthrie, 1989). The elbow develops secondary osteoarthrotic changes very early in the course of the disease. This arthrosis is irreversible and progressive, regardless of the line of therapy adopted. A significant proportion of affected dogs remain intermittently or persistently lame.

AETIOLOGY

Genetic factors

Earlier studies and observations in a number of different species suggested that genetic factors could contribute to the condition. Several aspects were investigated in swine, as osteochondrosis is an important cause of leg weakness in intensively raised pigs. A heritability of 0.14 to 0.30 was calculated (Reiland et al, 1978). Osteochondrosis dissecans of the stifle was recorded in two Bull Terrier litter mates (Woodard, 1979). Elbow dysplasia (ununited anconeal process) is most commonly seen in German Shepherd Dogs and genetic factors have been suspected (Hayes et al, 1979). Polygenetic traits such as growth rate, conformation and weight gain may also influence the development of the condition (Milton, 1983). A genetic study was carried out in a group of Swedish horses that had developed osteochondrosis (Hoppe & Philipsson, 1985). Some stallions had a significantly higher incidence of hock osteochondrosis among their progeny compared with the progeny of other stallions. Unfortunately the sample was too small to allow detailed analysis or a calculation of heritability. A study was carried out using data supplied by the

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Bernese Mountain Dog Club of Great Britain (Pidduck, 1987) and the heritability of osteochondrosis of the humeral head was found to be 0.55 to 0.70 in this breed.

Heritability of canine elbow osteochondrosis: It was decided to investigate the incidence and possible inherited basis of elbow osteochondrosis using the population of dogs owned by the Guide Dogs for the Blind Association (G.D.B.A). The G.D.B.A. is the largest single dog-owning body in the United Kingdom. It is responsible at any one time for over five thousand dogs. This includes breeding stock, working Guide Dogs, dogs in training and puppies under one year old that are 'out at walk'. The Association has controlled its own breeding programme for many years and produces its own replacements from a closed population of breeding animals. The majority of stock are Labradors, Retrievers and their various crosses. Records were available on the health and breeding of each dog owned by the G.D.B.A.

All dogs within the current G.D.B.A. population that were affected with elbow osteochondrosis were identified. In order to be confirmed as an affected, a dog had to fulfill the following criteria:-

i) exhibit foreleg lameness while less than one year old
ii) have radiographic signs of elbow osteochondrosis
iii) have lesions confirmed at exploratory arthrotomy.

The parentage of these dogs was then ascertained. The number and sex of litter mates from the litter in question and from any identical earlier or repeat matings were recorded. The health status of all these offspring was checked to identify any siblings that had developed the disease.

The total current G.D.B.A. population of Labradors, Retrievers and their crosses was calculated.

A chi-squared test (X^2) showed that there was a significant difference in the incidence of the condition between males and females (Table 1). This meant that for all the following analyses the sexes had to be considered independently (Bailey, 1964).

Table 1. Chi-squared Test

	Males	Females	Total
Osteochondrosis	31	15	46
Normals	2458	2626	5084
Total	2489	2641	5130

$X^2_{(1)} = 5.878$
$P<0.05$ and >0.01

A comparison of the incidence of a disease in the general population with that in relatives is a useful pointer to whether or not genetic factors are involved. The ratio of these incidences can then be used to fit the most likely model of inheritance (Table 2). The incidence in siblings was higher than that in the general population, showing that genetic factors were involved. A multifactorial mode of inheritance showed the best fit.

Table 2. Relative frequencies

			Relative frequencies Observed	Expected		
	general population q	siblings s	s/q	dominant 1/2q	recessive 1/4q	multi-factorial $1/\sqrt{q}$
Males	0.01245	0.10743	8.6289	40.160	20.080	8.962
Females	0.00568	0.0289	5.088	88.028	44.014	13.268

The heritability was calculated according to a method by Falconer (cited by Emery, 1986) where heritability

$$(h^2) = 2r$$

r being the correlation between the population and incidence and sibling incidence. The heritabilities were found to be as follows:-

$$h^2 = 0.77 +/- 0.12 \text{ (males)}$$
$$h^2 = 0.45 +/- 0.21 \text{ (females)}$$

The recurrence risks were calculated and the results are shown in Table 3 (Smith, 1970).

Table 3. Recurrence risks

	Recurrence R[a]	risk
First-degree male relatives of affected males	0.5	14%
Second-degree male relatives of affected males	0.25	5%
Third-degree male relatives of affected males	0.125	2.5%
First-degree female relatives of affected females	0.5	10%
Second-degree female relatives of affected females	0.25	3%
Third-degree female relatives of affected females	0.125	1.38%

[a] R is the coefficient of the relationship and is a measure of the probability that the two related individuals will both possess an identical gene because of a common ancestor.

Nutrition

Dietary influences were suspected as having a role to play in the aetiology of osteochondrosis because the condition was seen originally in the most rapidly growing animals that were on a high plane of nutrition, often receiving additional vitamin and mineral supplementation.

A study was carried out on eight Thoroughbred yearlings (Glade & Belling, 1986) that were randomly assigned to diets that provided either 100% or 130% of the National Research Council (N.R.C.) recommendations for energy and protein. Bodyweight was recorded twice a week and the diets adjusted accordingly. Cartilage samples were removed from the distal radius after 0,3,6 and 9 months of the study. This was carried out under local anaesthesia. The cartilage from the horses on the higher plane of nutrition had a reduced hexosamine and hydroxyproline content but an elevated DNA content. These changes were consistent with an increased cartilage cellularity and decreased matrix production.

Several studies have been carried out in pigs because, with intensification of farming methods, leg weakness in pigs has become an important cause of wastage. The effects of dietary levels of calcium, phosphorus, protein and energy have been investigated (Grondalen, 1974). There were no significant differences in the gross lesions found at post mortem between groups of pigs fed different mineral levels (all levels were at or above the recommended norm). Protein levels also had little effect on the occurrence of osteochondrosis.

Chronic calcium excess (3 times recommended levels) was found to give rise to several abnormalities in 11 growing Great Danes (Hazewinkel et al, 1985). Experimental dogs were fed ad libitum and controls were given 2/3 of the amount that the experimental dogs consumed. Abnormalities were more common in the experimental group.

Hypercalcaemia, hypophosphataemia, hypermineralisation and reduced osteoclast activity led to disturbances of endochondral ossification. Clinically animals were found to suffer from osteochondrosis, retained cartilage cores, angular limb deformities, stunted growth and Wobbler Syndrome.

In another study twenty four Great Dane pups were allocated to a variety of dietary regimes. They were paired so that one dog received ad libitum nutrition and the other received 2/3 of this amount (Hedhammar et al, 1974). Overnutrition accelerated growth and led to a number of skeletal abnormalities.

Conformation

The lesions associated with canine osteochondrosis occupy very specific sites within particular joints. The same is also true in Man. However in pigs the condition is considered to be a generalised skeletal problem and lesions can be demonstrated in most of the long bones.

Grondalen (1974) measured several different characteristics of the elbow joint, back, pelvis, femur and tibia in swine cadavers. Elbows with distinct ridges, steep joint surfaces and large angles of articulation were found to show the least number of lesions.

In Man, lesions with similar radiographic appearances can be divided into the following categories (Smillie, 1960):-

i) ossification anomaly (child)
ii) osteochondritis dissecans (teenager)
iii) adult osteochondritis dissecans
iv) tangential osteochondral fractures

It was postulated that repeated trauma played a secondary role in the first two groups, following on from some ischaemic insult or developmental anomaly. Trauma was thought to be of major importance in adult osteochondritis dissecans. The stress pattern across an articular surface in vivo would depend on the shape of that surface, ligamentous support, muscle and other soft tissue structures, joint angulation and general conformation.

A small study has been carried out by the author. A series of 28 Labrador and Retriever puppies of 6-12 weeks of age were photographed whilst standing squarely. Coloured dots had been placed on the point of the shoulder, lateral humeral epicondyle and styloid process of the ulna. The angle of the elbow joint was measured from the photographs by joining up these landmarks. The degree of angulation showed wide variation (110 to 159 degrees). The mean was 136.6 +/- 11.27 SD. These dogs were followed to 9 months of age and any that developed clinical osteochondrosis were identified. Only one dog was affected, with the condition occurring in both elbows. The joint angulations were 132 and 134 degrees respectively. Thus it was impossible to associate the lesion with a particular joint angle.

Exercise:

Overexercise at an early age has been suggested as a possible factor in the aetiology of osteochondrosis in the dog. There is little work to support or refute this idea. A project to investigate this aspect of the condition is shortly to be carried out by the author.

DISCUSSION

For the calculation of elbow osteochondrosis in the dog, strict criteria were used to classify a dog as affected. Thus the true number of affecteds is likely to be higher as some dogs will have been managed successfully on conservative treatment and we now have some evidence that the condition can occur subclinically. The chi-squared test showed that maleness itself would increased the likelihood of an animal developing osteochondrosis. The particular characteristics associated with maleness that predispose to the condition remain unclear. The heritability (h^2) is a measure of the proportion of the phenotypic variance which is due to additive genetic factors. There is a danger that the heritability will be overestimated if only sibling data is used as the animals will have shared common prenatal and postnatal environments. Unfortunately in this study only sibling data were available. This error was minimised by separating the pups at weaning and placing them in a wide variety of homes to be puppy-walked.

The heritability of osteochondrosis in both males and females is high. The recurrence risks calculated are the upper limits as they assume a heritability of 1.0. It can be seen from Table 3 that if an affected dog was used at stud, up to 14% of his male offspring would be expected to develop the condition. Suitable breeding policies, if adopted, should lead to a lowering of the incidence of osteochondrosis. Practical steps to take would be as follows:-

1) Never breed from affected dogs
2) Never breed from siblings of affected dogs
3) Never breed from offspring of affected dogs
4) Do not breed from the sires or dams of affected dogs (repeating the same mating or putting them to different partners).

Being multifactorial, the adoption of these guidelines will not eliminate the condition but would reduce the incidence over a period of time.

The effects of diet are very difficult to investigate. Because the condition is seen mainly in the large/giant breeds, trials involving statistically significant numbers of animals are expensive to run. N.R.C. recommendations may not be optimum for all dogs and nutritional requirements may vary between breeds and individuals.

The effects of conformation and exercise need further clarification. The elbow has particularly congruent articular surfaces and lesions at one site can cause secondary changes

elsewhere. The histories of affected dogs show considerable variation in the levels of exercise. When these dogs are examined, there is also an apparent wide variation in stature and conformation.

The effect of many environmental factors in the aetiology of elbow osteochondrosis could be investigated. Because the heritability is high, the incidence should be amenable to some control by adopting suitable breeding guidelines.

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ZOONOSES

SALMONELLA ISOLATES FROM HUMANS AND PRODUCTION ANIMAL SPECIES IN NORTHERN IRELAND FROM 1979-1988

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Salmonellosis is a zoonosis of worldwide economic importance in humans and animals. Infection of animals with various species of Salmonella sometimes results in serious disease and always constitutes a potential reservoir for the disease in humans. The interplay of Salmonella with its host takes a variety of forms including remarkable host specificity, inapparent infections, recovered carriers, enteritis, septicaemia, abortion and combinations of disease syndromes. Salmonella are readily transferred from animal to animal, animal to humans, and human to human by direct or indirect pathways. (Blood and Radostits, 1989).

The genus Salmonella is a member of the family Enterobacteriaceae and shares characteristics of the family (LeMinor, 1984). Typically, Salmonella are nonlactose fermenters and most schemes for their detection are based on this property. Serotyping is used to identify the organism beyond the level of genus; thus serotype is equivalent to species (LeMinor, 1984). Approximately 2,000 serotype have been described on the basis of somatic (O), flagellar (H), and capsular (Vi) antigens, but less than 50 of these are encountered at significant frequency in disease. Phage-typing, biotyping, drug resistance and plasmid profile analysis have all been used to identify isolates beyond the level of serotype and are useful for epidemiological studies (Clarke and Gyles, 1986).

Salmonella may be considered in 3 groups based on their association with human and animal hosts (Smith and Halls, 1968; Wray and Sojka, 1977; Turnbull, 1979; LeMinor, 1984). One group, which includes S. typhi and S. paratyphi, is characterised by specificity for the human host. A second group of Salmonella consists of organisms that are more or less adapted to specific animal hosts such as S. cholerae-suis in pigs, S. pullorum in poultry, S. abortus-ovis in sheep and S. dublin in cattle. The third group consists of unadapted Salmonella that cause disease in humans and a variety of animals. Most Salmonella fall into this group, but Salmonella typhimurium is the most frequent cause of disease.

This paper describes the establishment of a computerised database of information on all Salmonella isolations in cattle, sheep, pigs, poultry and humans in Northern Ireland between 1979 and 1988. The paper highlights the frequency distribution of individual serotypes in each species and the presence

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of statistically significant trends and seasonal patterns in the prevalence of Salmonella within the human and animal population of the country.

DATA COLLECTION SYSTEM

In Northern Ireland, microbiological examination of samples from production animal species is carried out by the laboratories of the Department of Agriculture. All isolations of Salmonella from such animals and their products are reported to the Department's Veterinary Division under the Zoonosis Order (Northern Ireland, 1976). Recently, isolations from the animals environment and feed are also similarly reported (Zoonosis Order NI 1979). Veterinary Division communicates information on Salmonella isolates to the Department of Health and Social Services (DHSS). Information on all isolations of Salmonella from cases of food poisoning in humans are sent to the four Area Health Boards and the Communicable Disease Centre of DHSS (NI).

COMPUTER SYSTEM

In January 1979, information on Salmonella isolates from production animal species in Northern Ireland was logged onto an ICL 2986 mainframe computer. Initially, a FORTRAN 77 program was used to produce statistical summaries on a routine basis. This computer system was initiated and has been subsequently managed for administrative and surveillance purposes. Thus, no database retrieval software was available for more complex statistical and epidemiological analyses. In 1988, a computer system was established to enable such analyses and facilitate the detailed investigation of the epidemiology of Salmonella isolates from production animals in Northern Ireland.

The Salmonella database was transferred via magnetic tape from the ICL computer to a VAX 6020 computer. This is a supermini computer, incorporating a virtual memory enhancement system and has available 64 megabytes of main memory, 5 gigabytes of hard disk backing storage and 1600 b.p.i. magnetic tape unit for routine archiving. The system supports 128 visual display terminals on a local area network (LAN).

The main software packages available for data analyses are the relational database management and retrieval system, Oracle, and the statistical package SPSS. This latter package enables basic statistical summaries of data and also more advanced statistical analysis such as generalised analysis, multiple regression and complex time series analysis. The software also facilities the production of tabular and graphical outputs, the latter enabling routine exploratory analysis of data on an interactive basis.

SALMONELLA DATABASE

Information associated with individual Salmonella isolates since 1979 from cattle, sheep, pigs and poultry and their products, is held in the database. The information recorded is:

1. Reference No of isolate.

2. Species of animal or bird.
3. Sample type from which isolate recovered (eg liver, faeces etc).
4. Date sample submitted.
5. Name of producer.
6. Name of veterinary surgeon in attendance.
7. Name of serotype and phage type.
8. Antibiotic sensitivity pattern.

The above information relating to individual Salmonella isolates is collated by incident. Each incident incorporates one or more Salmonella isolates of the same serotype from a single, or group of animals in direct or indirect contact. The termination of an incident is determined by the presence of a 30 day period in which the same serotype is not isolated from the particular animal or group of animals. Each defined Salmonella incident is given an unique reference code which is stored as an additional field in the relevant records of the database.

Information relating to Salmonella isolates from cases of food poisoning in humans are also logged onto the computer system. The information is limited to date of isolation, serotype and phage type. Individual cases of food poisoning due to specific Salmonella serotypes are defined using epidemiological selection criterion similar to those utilised in the clafficiation of individual Salmonella incidents in production animal species.

DATABASE ANALYSIS

Preliminary analysis of the database involves the investigation of annual trends in the prevalence of individual serotypes in any of the species. The statistical significance of any trends is assessed using both linear regression analysis and analysis of correlograms. The next stage in the analysis involves the assessment of the presence or absence of seasonal patterns in the monthly prevalence of serotypes, both for individual years and over all years. If seasonality patterns and/or statistically significant trends are identified, complex statistical detrending and deseasonalisation techniques are performed on the original time series. Correlograms are produced in both graphical and tabular format to assess the stationarity of the detrended, deseasonalised series. The software facilitates the investigation of cross correlations between the prevalence of serotypes in more than one species. Such cross correlations can be performed on data recorded at coincident time intervals or data lagged at any specified time interval. All subsequently computed correlation co-efficients are again available in both tabular and graphical format. Individual serotypes can be listed in chronological order to facilitate the investigation of epidemiological associations between Salmonella incidents, due to exotic serotypes in more than one species. Such epidemiological links are almost invariably not identified by routine statistical analysis of the database due to the relatively small number of incidents caused by exotic serotypes. However, the identification of such chronological links in more than one species is important in the understanding of the dissemination of Salmonella serotypes.

RESULTS AND DISCUSSION

ALL ANIMAL SPECIES

The relative frequency distribution of the principal serotypes isolated from Salmonella incidents in production animal species in Northern Ireland between 1979 and 1988 is given in Table 1. The overall upward annual trend for all serotypes is shown in Fig. 1 and is statistically significant ($p < 0.05$). The two predominant serotypes over the 10 years are S. typhimurium and S. dublin. Notably, these two serotypes account for 55% of all incidents. The peak prevalence attained in 1986 is accounted for by a substantial increase in the occurrence of S. typhimurium and the appearance of S. enteritidis as a major cause of Salmonella incidents. However, it is interesting and encouraging to note that there is a substantial reduction in the prevalence of Salmonella incidents in the latter two years of the time series.

A total of 2,028 Salmonella incidents occurred in the 4 animal production species over the 10 year period. The percentages attributed to each of the 4 species are shown in Fig. 2. Approximately equal percentages were recorded in cattle and poultry and incidents in these species accounted for 85% of the total. Investigations have identified that the overall statistically significant upward trend recorded in all production animal species is attributable to a significant upward trend in cattle, principally due to S. dublin. Furthermore, the peak prevalence attained in 1986 is accounted for by a substantial increase in the occurrence of S. typhimurium and S. enteritidis in poultry.

BOVINE

The relative frequency distribution of the principal serotypes isolated from Salmonella incidents in cattle in Northern Ireland between 1979 and 1988 is given in Table 2. The overall upward annual trend for all serotypes is shown in Fig. 3 and is highly statistically significant ($p < 0.01$). The two predominant serotypes in cattle over the 10 years are S. typhimurium and S. dublin accounting for 89% of incidents. Notably, S. dublin occurs twice as frequently as S. typhimurium and accounts for over 60% of all incidents. Investigations of the seasonality of individual serotypes demonstrated a distinct and consistent pattern in the occurrence of incidents of S. dublin. The seasonal pattern is shown in Fig. 4. The maximum prevalence is invariably recorded in the late autumn and early winter. A similar seasonal pattern is evident in the prevalence of incidents due to S. typhimurium. Both of these serotypes can cause late stage abortion and enteritis in young calves, and the observed seasonality may reflect the known calving pattern of Northern Ireland dairy herds with the majority of calves being born in the late autumn and early winter.

OVINE

The relative frequency distribution of the principal serotypes isolated from Salmonella incidents in sheep in Northern Ireland between 1979 and 1988 is given in Table 3. The overall annual prevalence for all serotypes is shown in Fig. 5 and no statistically significant trend was found. The two predominant serotypes in sheep over 10 years are S. typhimurium and S. dublin, accounting for 71% of all incidents. The prevalences of these serotypes are

TABLE 1. NUMBER OF INCIDENTS OF SALMONELLA INFECTION IN ALL ANIMAL SPECIES IN N. IRELAND (1979-1988)

Serotype		1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	TOTAL
S. typhimurium	No.(%)	27 (22.2)	27 (20.0)	29 (19.9)	63 (34.4)	42 (25.1)	42 (20.6)	70 (26.3)	123 (36.2)	77 (24.7)	22 (12.7)	522 (25.7)
S. dublin	No.(%)	17 (16.5)	25 (18.5)	32 (21.9)	46 (25.1)	49 (29.3)	80 (39.2)	85 (32.0)	52 (15.3)	120 (38.6)	86 (49.7)	592 (29.2)
S. virchow/infantis	No.(%)	21 (20.4)	14 (10.4)	31 (21.2)	32 (17.5)	20 (12.0)	9 (4.4)	29 (10.9)	21 (6.2)	6 (1.9)	10 (5.8)	193 (9.5)
Other serotypes	No.(%)	38 (37.0)	69 (51.1)	54 (25.3)	42 (25.0)	56 (33.6)	73 (35.8)	82 (30.8)	144 (42.3)	108 (34.8)	55 (31.8)	721 (35.6)
TOTAL NUMBER OF INCIDENTS		103	135	146	183	167	204	266	340	311	173	2028

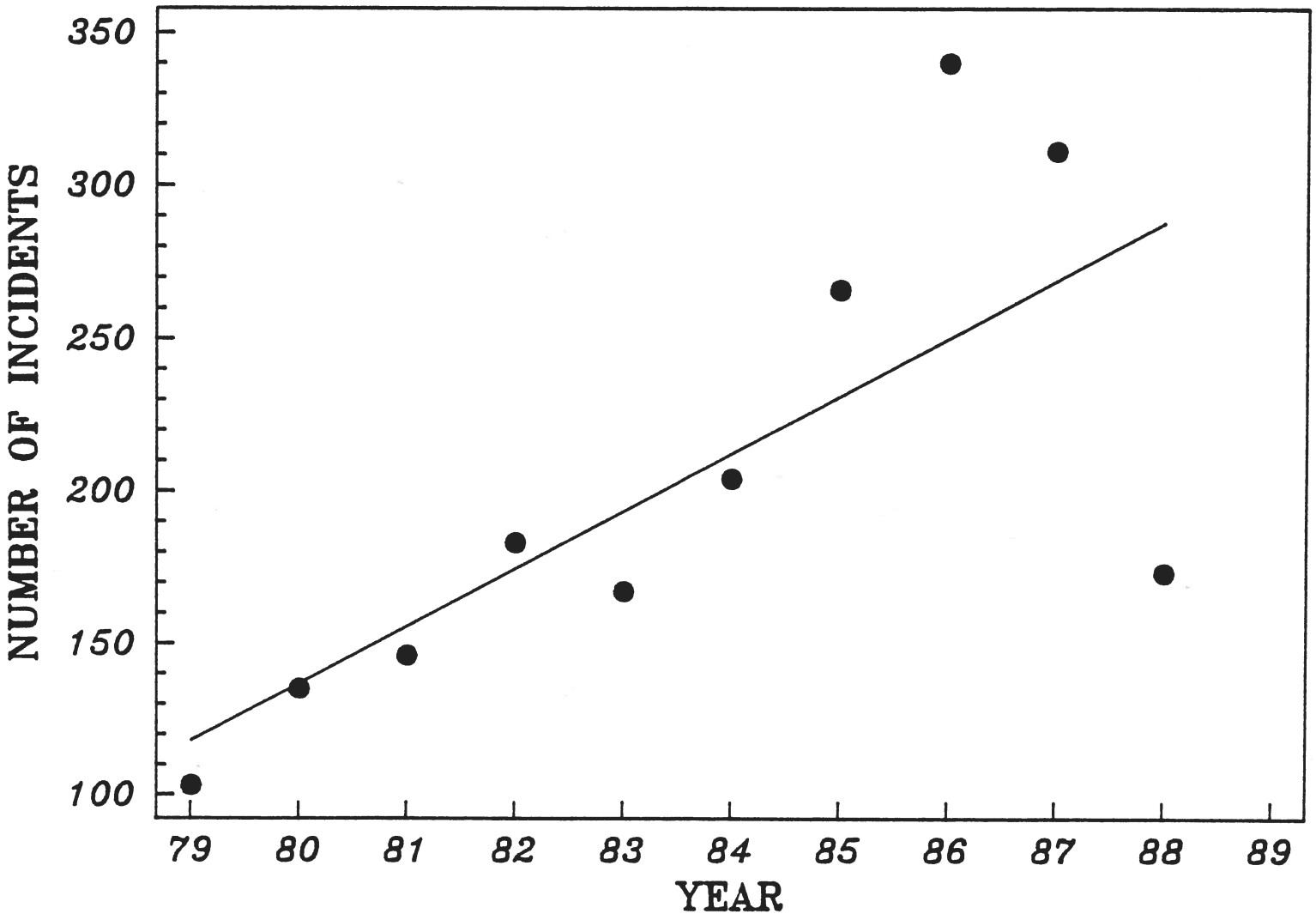

Fig 1. The annual prevalence of Salmonella incidents due to all serotypes in all animal species.

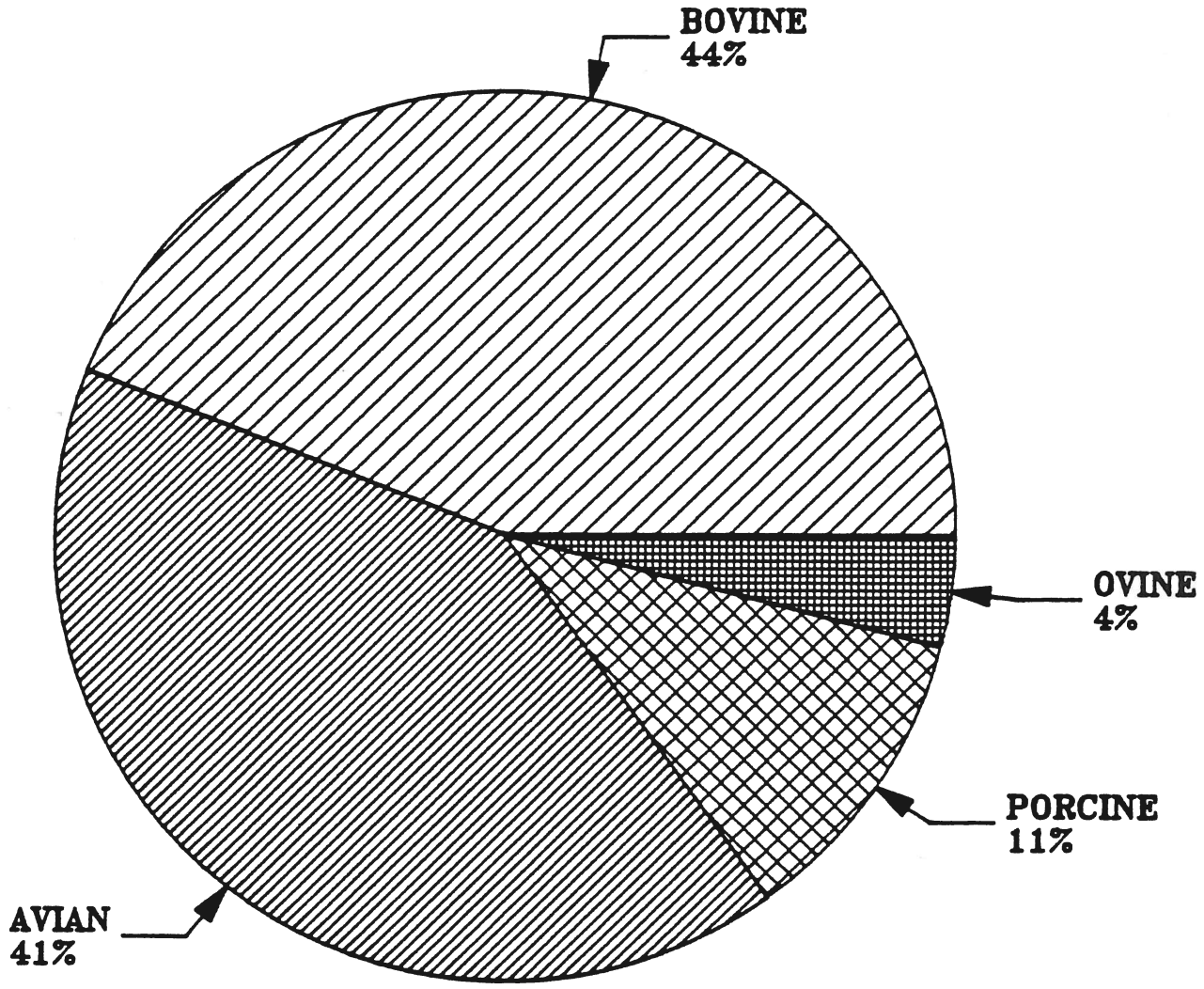

Fig 2. The frequency distribution of the principal serotypes in all animal species.

TABLE 2. NUMBER OF INCIDENTS OF SALMONELLA INFECTION IN CATTLE IN N. IRELAND (1979-1988)

Serotype		1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	TOTAL
S. dublin	No.(%)	17 (53.1)	21 (56.7)	28 (57.1)	41 (55.4)	42 (55.2)	79 (70.5)	80 (60.1)	49 (40.5)	103 (67.3)	80 (84.2)	540 (61.2)
S. typhimurium	No.(%)	11 (34.3)	8 (21.6)	15 (30.6)	29 (39.2)	30 (39.5)	25 (22.3)	37 (27.8)	46 (38.0)	33 (21.6)	7 (7.3)	241 (27.3)
Other serotypes	No.(%)	4 (12.6)	8 (21.9)	6 (12.3)	4 (5.4)	4 (5.3)	8 (7.2)	16 (12.1)	26 (21.5)	17 (11.1)	8 (8.5)	101 (11.5)
TOTAL NUMBER OF INCIDENTS		32	37	49	74	76	112	133	121	153	95	882

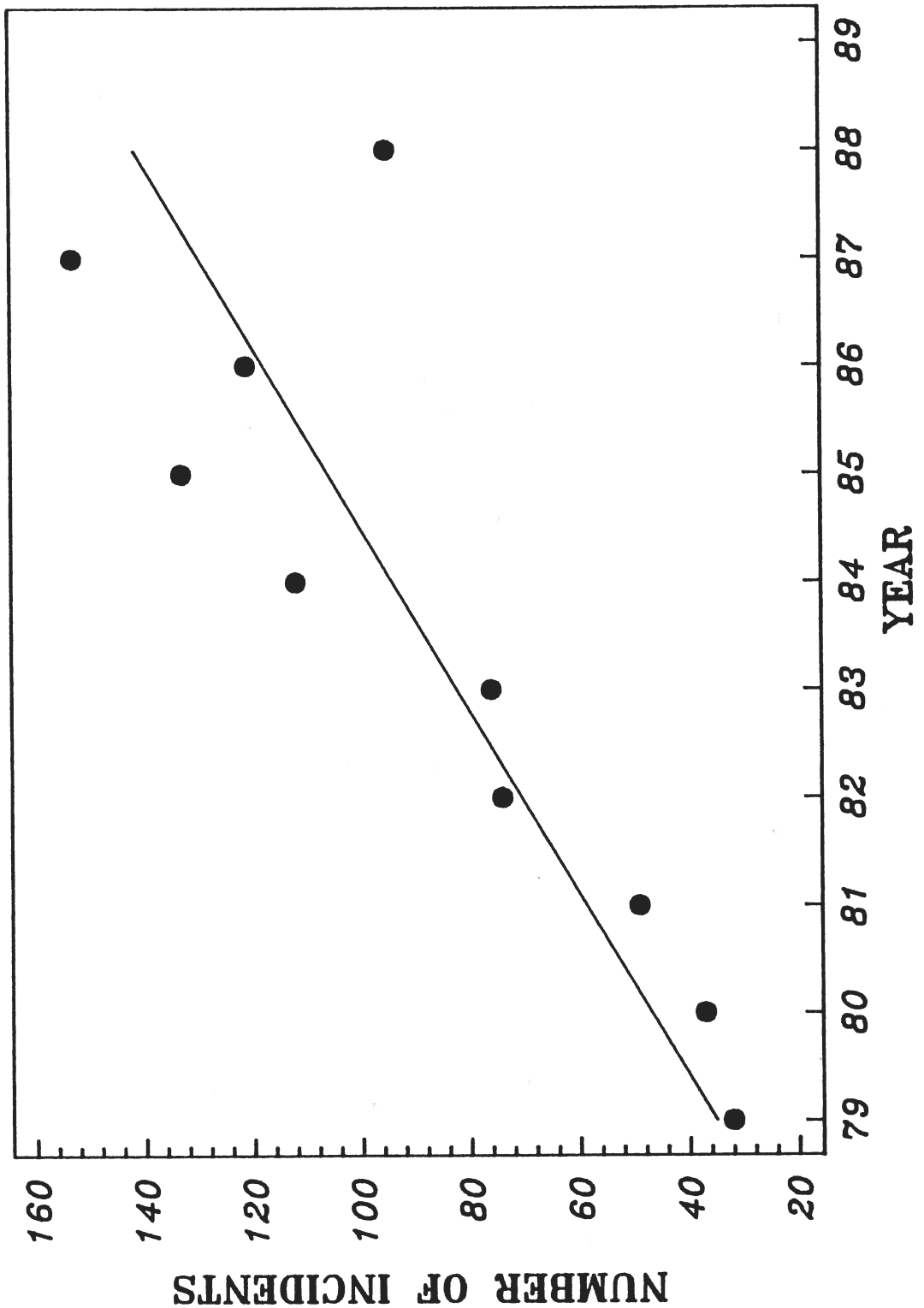

Fig 3. The annual prevalence of Salmonella incidents due to all serotypes in cattle.

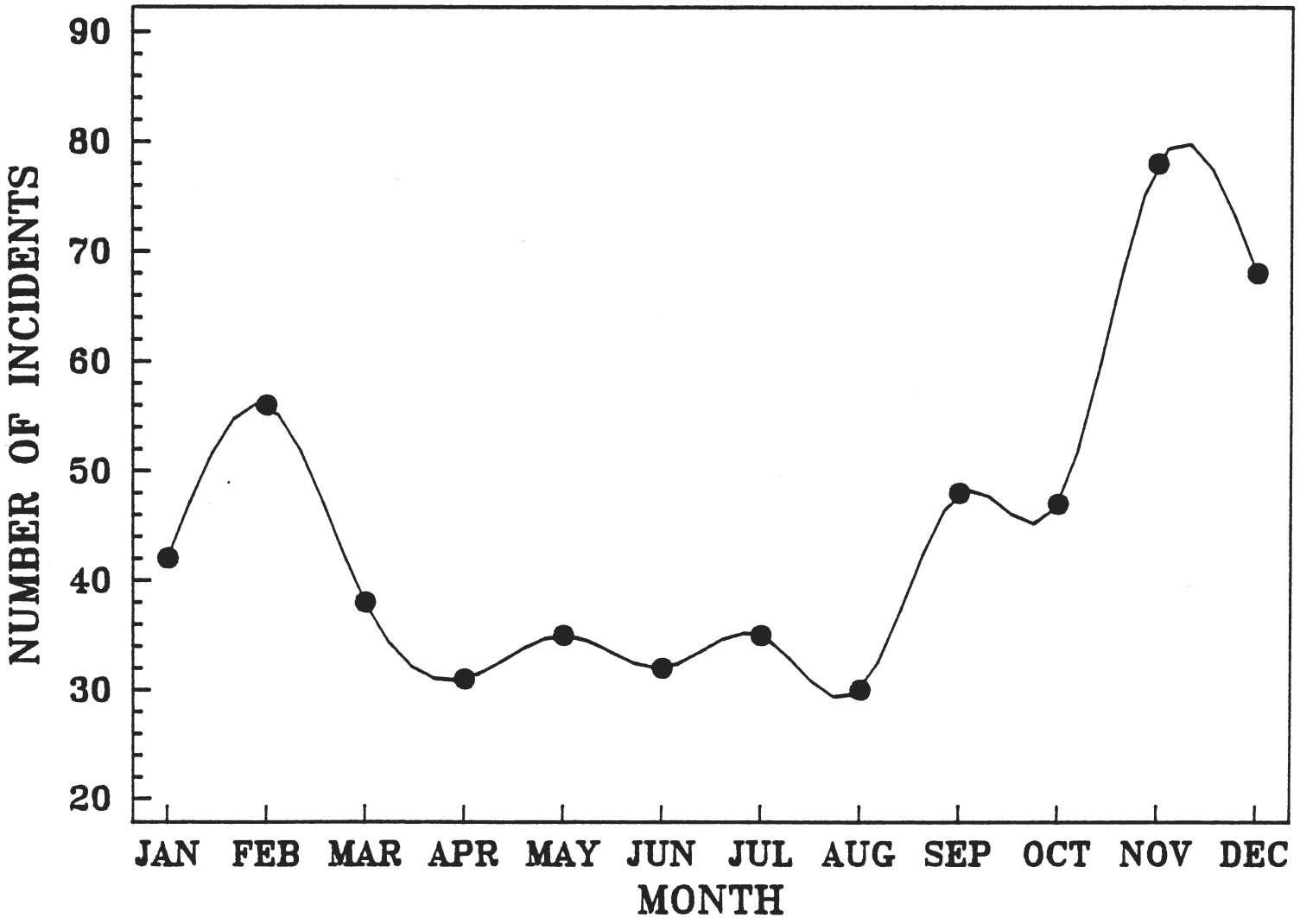

Fig 4. The average monthly prevalence of S. dublin in cattle (1979-1988).

TABLE 3. NUMBER OF INCIDENTS OF SALMONELLA INFECTION IN SHEEP IN N. IRELAND (1979-1988)

Serotype		1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	TOTAL
S. dublin	No.(%)		1 (50.0)	2 (66.7)	4 (23.5)	3 (60.0)		2 (25.0)	2 (13.3)	15 (55.5)	5 (55.5)	34 (36.1)
S. typhimurium	No.(%)	1 (100.0)	1 (50.0)	1 (33.3)	11 (64.7)	1 (20.0)	2 (28.6)	3 (37.5)	7 (46.7)	5 (18.5)	1 (11.1)	33 (35.1)
Other serotypes	No.(%)					2 (11.8)	5 (71.4)	3 (37.5)	6 (39.0)	7 (26.0)	3 (33.4)	27 (28.8)
TOTAL NUMBER OF INCIDENTS		1	2	3	17	5	7	8	15	27	9	94

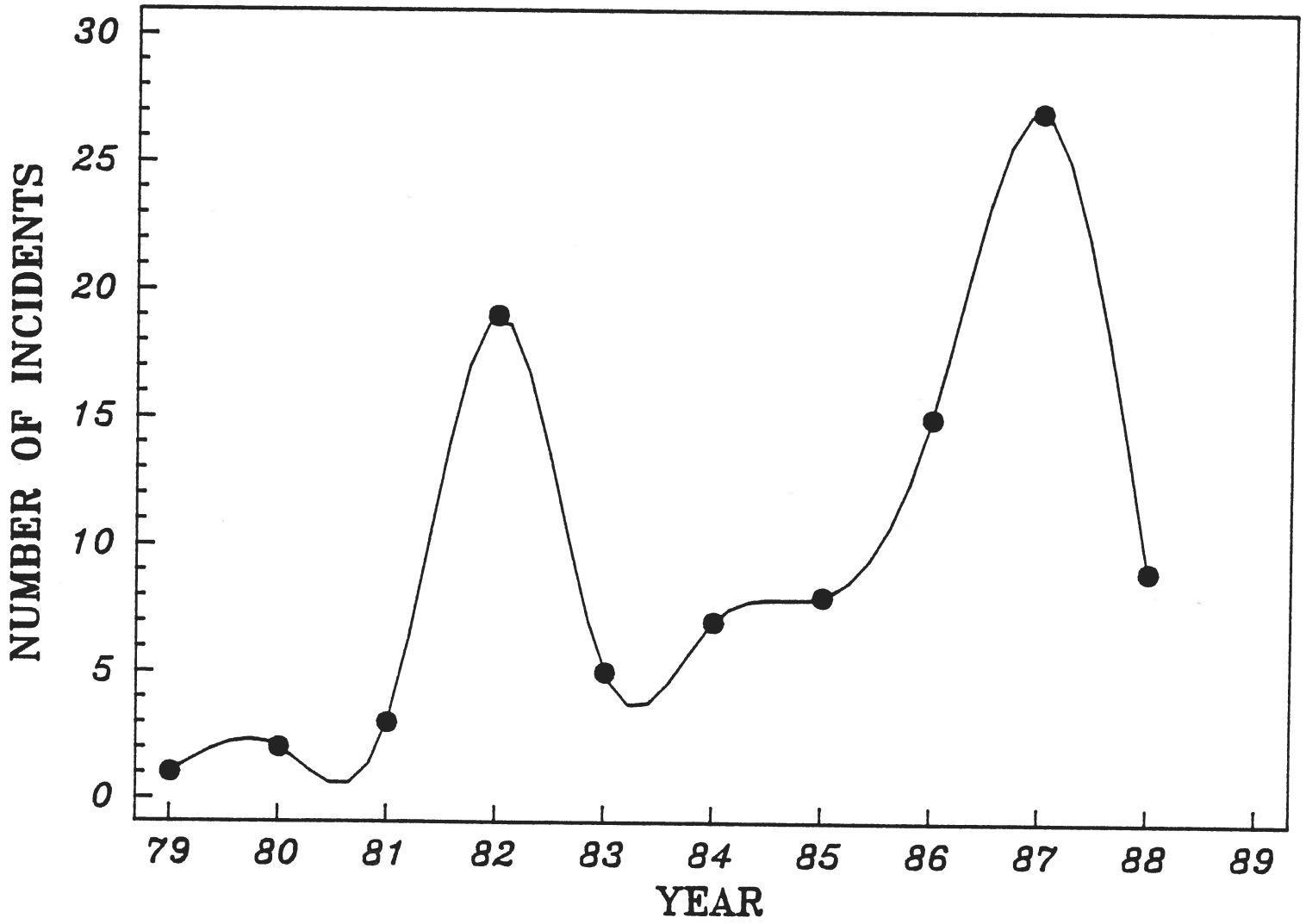

Fig 5. The annual prevalence of Salmonella incidents due to all serotypes in sheep.

approximately the same. Investigations of the seasonality of both serotypes demonstrated distinct pattern over the 10 years. The overall seasonality pattern of S. typhimurium and S. dublin incidents in sheep is demonstrated in Fig. 6. The maximum prevalence is recorded in the late winter and early spring. This pattern may again reflect the lambing cycle in Northern Ireland with susceptible adult ewes and young sheep being present at this time.

PORCINE

The relative frequency distribution of the principal serotypes isolated from Salmonella incidents in pigs in Northern Ireland between 1979 and 1988 is given in Table 4. The overall annual prevalence for all serotypes is shown in Fig. 7 and no statistically significant trend was found. The two predominant serotypes in pigs over the 10 years are S. cholera-suis and S. typhimurium, accounting for 50% of all incidents. It is interesting to note from Table 4 that although the annual time series for S. typhimurium is stationary, a highly significant ($p < 0.01$) downward trend is present in the prevalence of incidents due to S. cholera-suis. This is shown in Fig. 8. Notably, only one incident was recorded in the latter two years of the time series.

The dramatic decrease in the prevalence of S. cholera-suis in Northern Ireland can be attributed to restriction of swill and/or garbage feeding, restriction of pig movement and to the positive approaches adopted by the Northern Ireland pig industry including the development of a supply of S. cholera-suis free pigs and an all in all out policy in commercial fattening units.

AVIAN

The relative frequency distribution of the principal serotypes isolated from Salmonella incidents in poultry in Northern Ireland between 1979 and 1988 is given in Table 5. The overall annual prevalence of all serotypes is shown in Fig. 9 and no statistically significant trend was found. The two predominant serotypes in poultry over the 10 years are S. typhimurium (25%) and S. virchow/infantis (21%). The next most common serotype is S. enteritidis (13%). Notably, there were no incidents due to S. enteritidis in poultry prior to 1986.

Examination of the annual prevalences of the 3 principal avian serotypes, revealed no statistically significant trends. Individual serotypes attained a high prevalence in some years. Detailed epidemiological investigations of records submitted to the VRL and co-operative research work with the Northern Ireland poultry industry has identified that periods of high prevalence of individual serotypes over the 10 years, corresponded with the known or suspected infection of a parent broiler flock, with subsequent vertical transmission and dissemination throughout the pyramidal structure of an integrated poultry organisation. The peak annual prevalence of Salmonella incidents recorded in poultry in 1986 was due to infection with S. typhimurium and S. enteritidis in parent broiler flocks. Such flocks were voluntarily slaughtered and the infection controlled by strategic veterinary measures as described in detail by McIlroy et al. (1989). The voluntary eradication policy for S. enteritidis and S. typhimurium was adopted by the local poultry industry in 1986 approximately 3 years before compulsory legislation was introduced in both Great Britain and Northern Ireland.

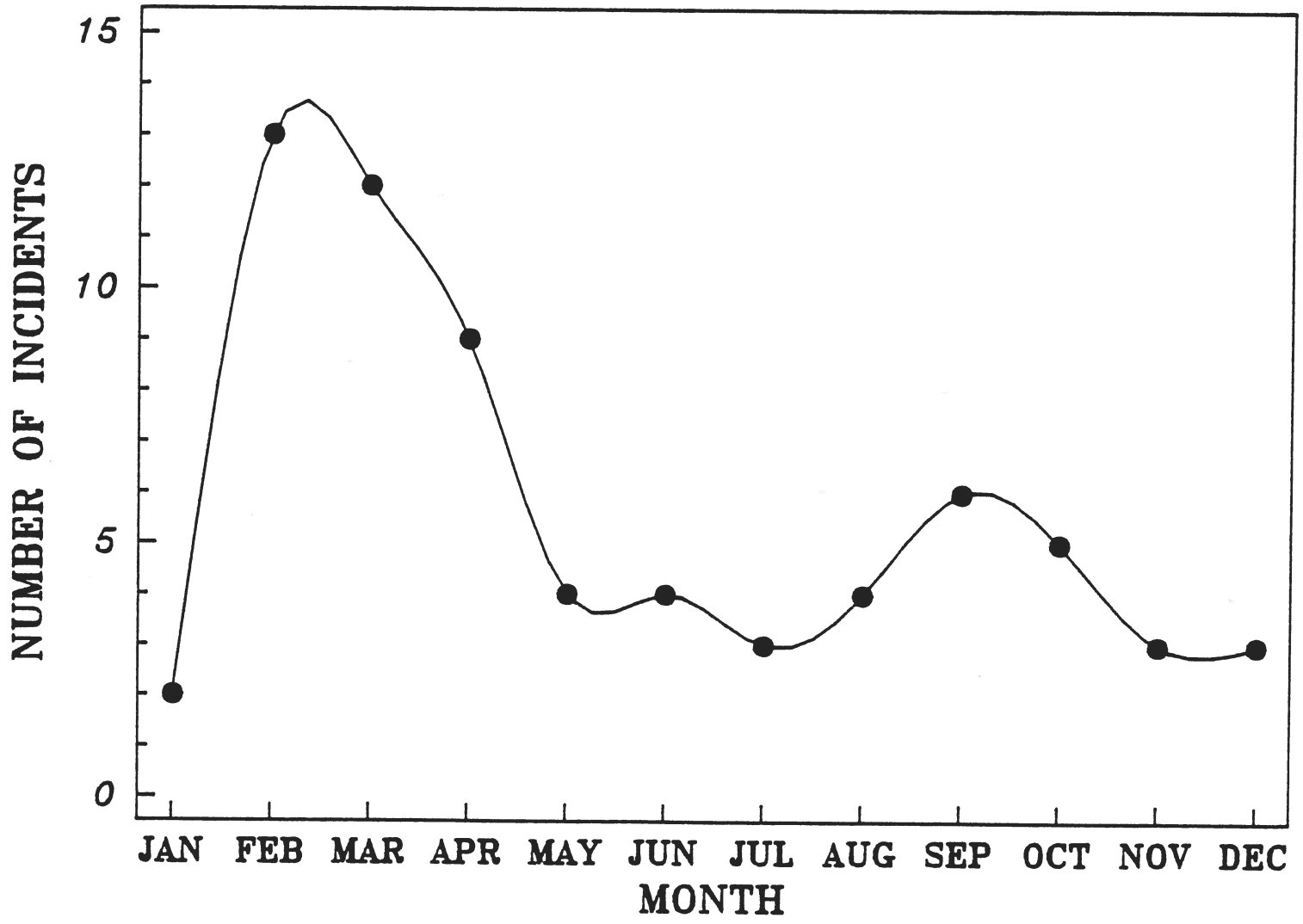

Fig 6. The average monthly prevalence of S. typnimurium and S. dublin in sheep (1979-1988).

TABLE 4. NUMBER OF INCIDENTS OF SALMONELLA INFECTION IN PORCINE IN N. IRELAND (1979-1988)

Serotype		1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	TOTAL
S. cholera-suis	No.(%)	13 (52.0)	16 (53.3)	10 (47.6)	5 (31.2)	3 (14.3)	8 (38.1)	10 (31.2)	4 (14.3)		1 (6.7)	70 (30.4)
S. typhimurium	No.(%)	7 (28.0)	3 (10.0)	3 (14.3)	5 (31.2)	5 (23.8)	3 (14.3)	3 (9.3)	4 (14.3)	8 (38.1)	4 (26.7)	45 (19.6)
Other serotypes	No.(%)	5 (20.0)	11 (37.3)	8 (38.1)	6 (37.6)	13 (61.9)	10 (47.6)	19 (59.5)	20 (71.4)	13 (61.9)	10 (66.6)	115 (50%)
TOTAL NUMBER OF INCIDENTS		25	30	21	16	21	21	32	28	21	15	230

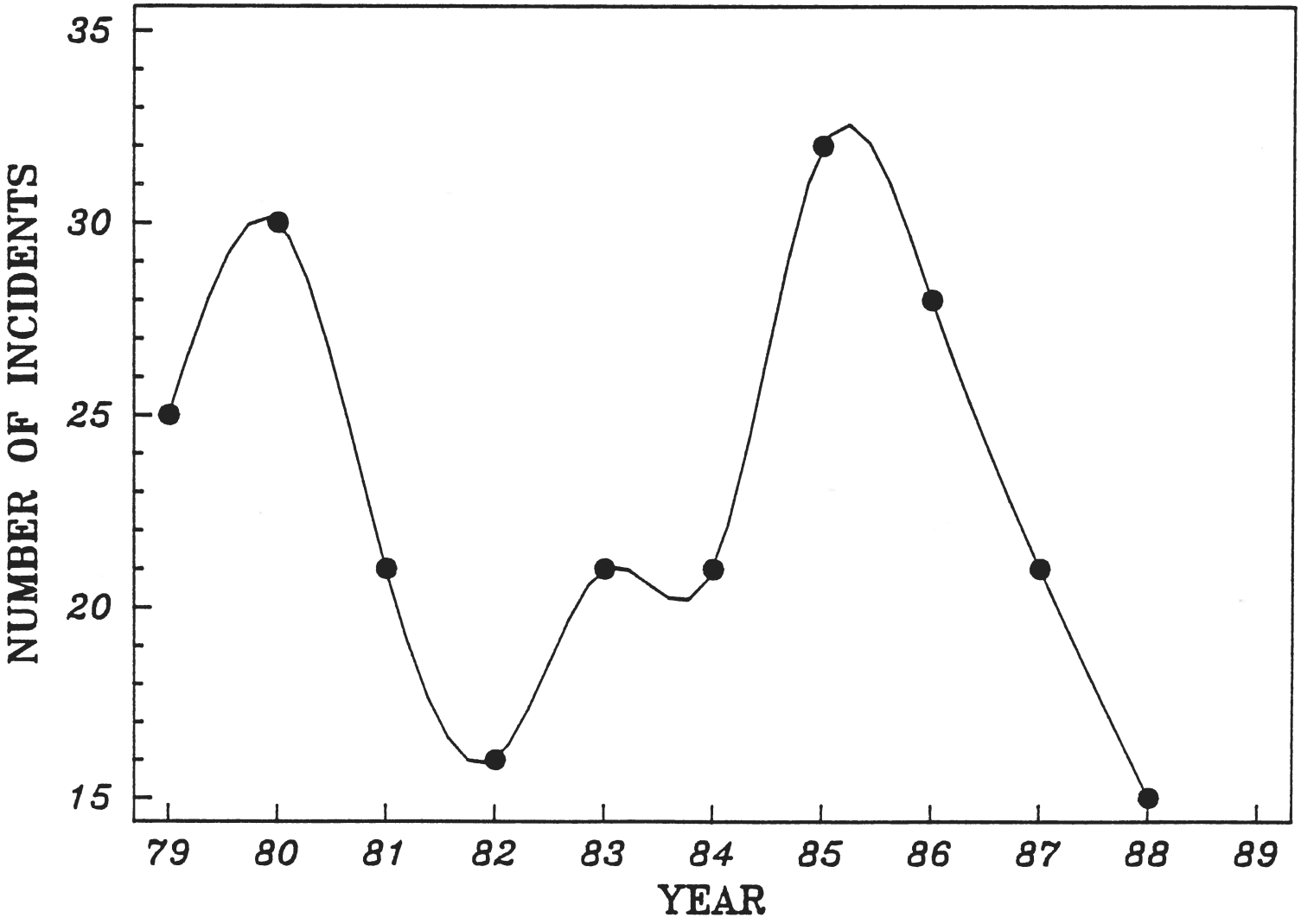

Fig 7. The annual prevalence of Salmonella incidents due to all serotypes in pigs.

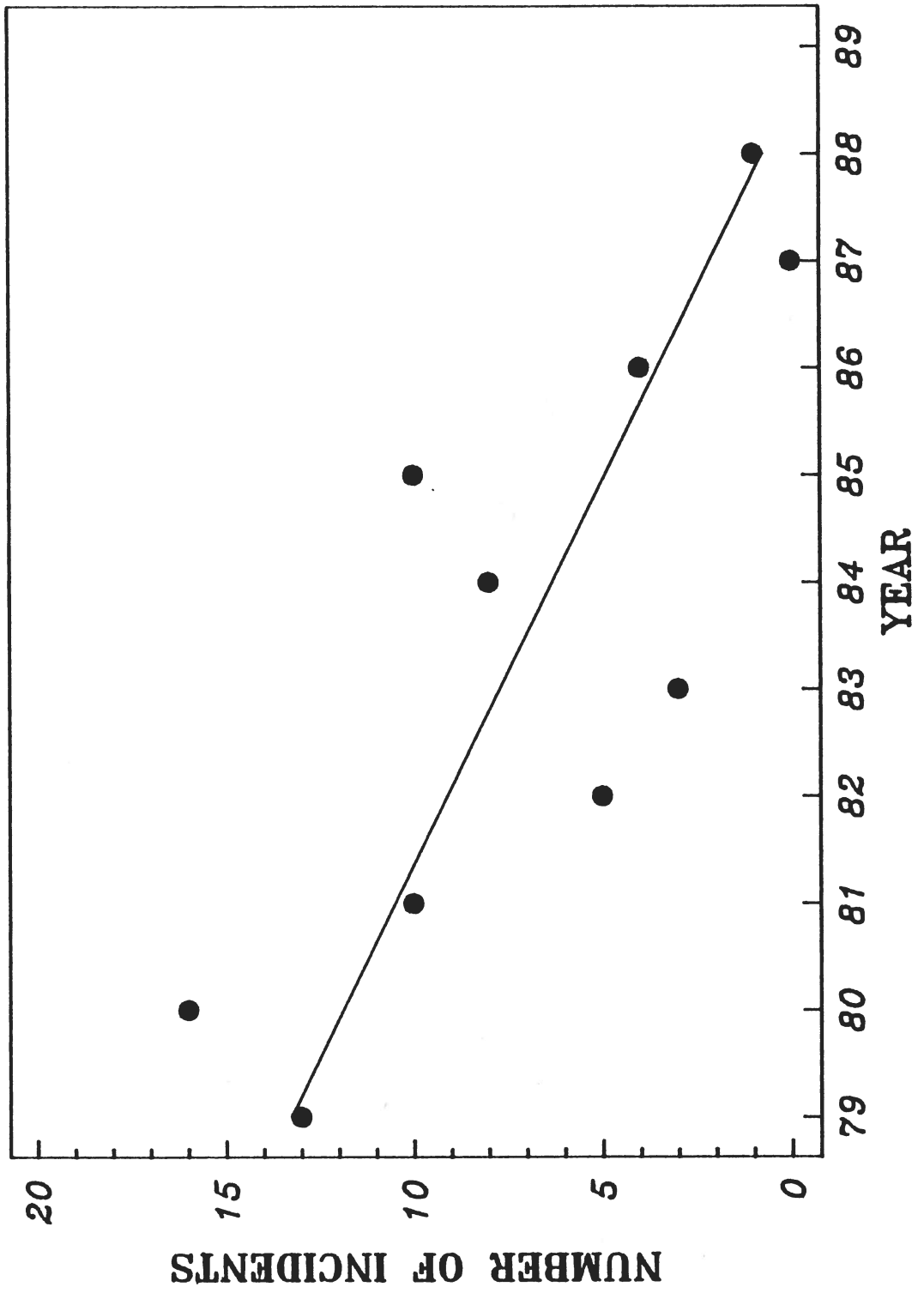

Fig 8. The annual prevalence of Salmonella incidents due to S. cholera-suis in pigs.

TABLE 5. NUMBER OF INCIDENTS OF SALMONELLA INFECTION IN POULTRY IN N. IRELAND (1979-1988)

Serotype		1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	TOTAL
S. typhimurium	No.(%)	8 (17.8)	15 (22.7)	10 (13.7)	18 (23.7)	6 (9.2)	12 (18.7)	27 (29.0)	66 (37.5)	31 (28.1)	10 (18.5)	203 (24.7)
S. virchow/infantis	No.(%)	21 (46.7)	13 (19.7)	30 (41.1)	32 (42.1)	19 (29.2)	8 (12.5)	23 (24.7)	19 (10.8)	3 (2.7)	8 (14.8)	176 (21.4)
S. enteritidis	No.(%)					1 (1.5)			50 (28.4)	45 (40.9)	9 (16.7)	105 (12.8)
Other serotypes	No.(%)	16 (35.5)	38 (57.6)	33 (45.2)	26 (34.2)	39 (60.1)	44 (68.8)	43 (46.2)	41 (23.3)	31 (38.3)	27 (50.0)	338 (41.1)
TOTAL NUMBER OF INCIDENTS		45	66	73	76	65	64	93	176	110	54	822

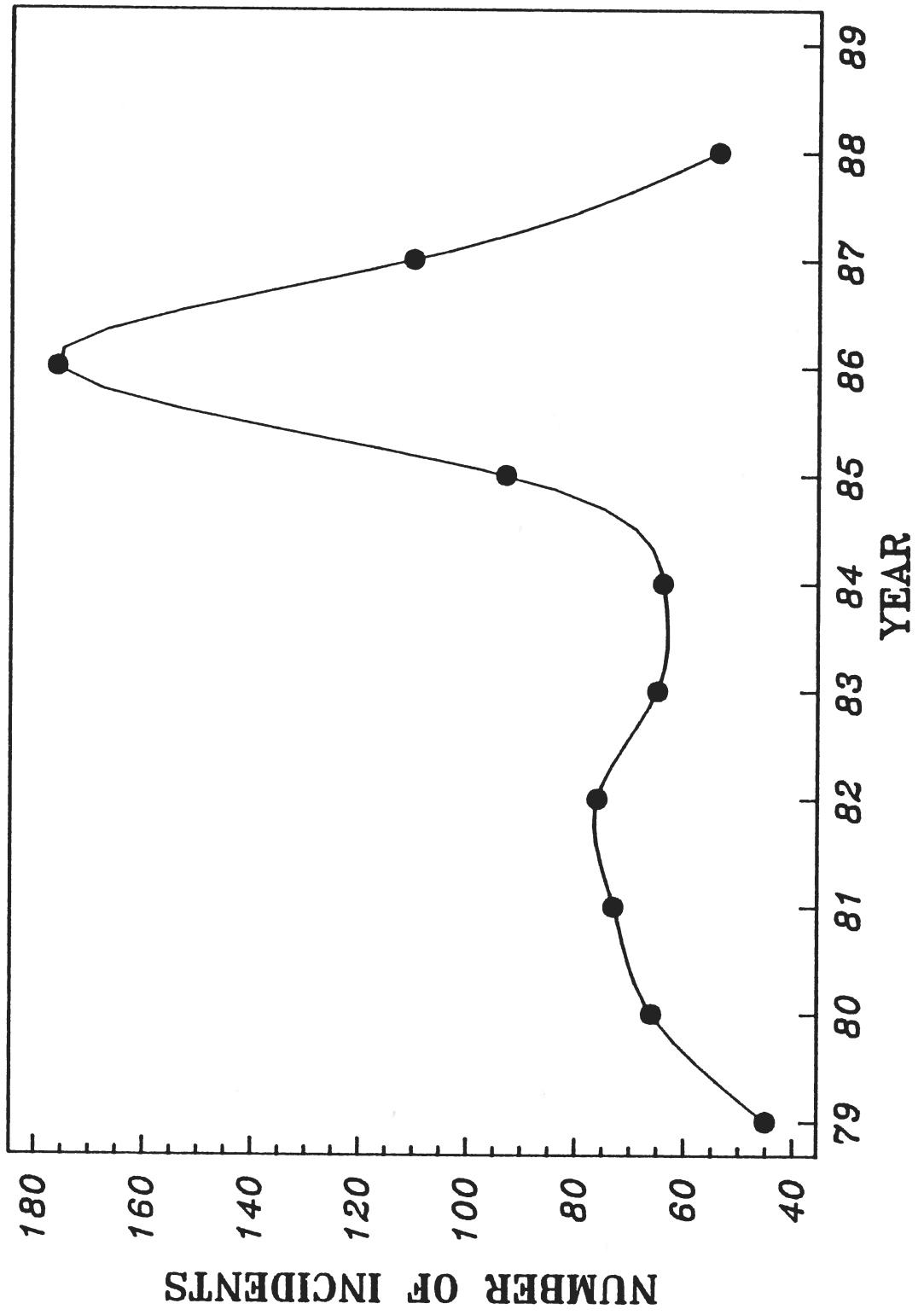

Fig 9. The annual prevalence of Salmonella incidents due to all serotypes in poultry.

HUMAN

The relative frequency distribution of the principal serotypes isolated from Salmonella food poisoning incidents in humans in Northern Ireland between 1979 and 1988 is given in Table 6. The overall upward annual trend of all serotypes is shown in Fig. 10 and is statistically significant ($p < 0.05$). The two predominant serotypes in humans over the 10 years are S. typhimurium and S. enteritidis, accounting for 55% of all incidents. Notably, the prevalence of S. enteritidis has increased dramatically since 1986 and in the latter two years of the time series accounted for over half of all food poisoning incidents. Detailed investigations have identified that no statistically significant upward trend in food poisoning incidents due to S. typhimurium has occurred whereas a statistically significant ($p < 0.05$) upward trend is present in the time series for S. enteritidis. Both serotypes demonstrated a consistent and distinct seasonal pattern with the maximum prevalence being recorded in late summer and early autumn and the overall pattern is demonstrated in Fig. 11. It is considered that a major epidemiological determinant in the occurrence of this peak prevalence at this particular time of the year is the large number of people who contact food poisoning whilst on holiday abroad and return to Northern Ireland over this time interval.

EXOTIC SEROTYPES

The 2,028 Salmonella incidents which occurred in all animal species over the 10 year period were collated in chronological order for each serotype. Serotypes identified in less than 10 incidents were extracted into a file for more detailed analysis. Several serotypes were identified which occurred for the first time in Northern Ireland simultaneously in more than one animal species. Such serotypes were invariably identified in several animal species within the same 4 week period. Detailed investigations of records submitted to the VRL identified that no geographical association existed between the production units in which the animal species were present. Furthermore, there was no discernible direct or indirect contact between such units. Details of 3 exotic serotypes which occured in more than one animal species within a 4 week period are given in Table 7. The only common epidemiological determinant for all incidents relating to individual serotypes was the supply of feedingstuffs from the same meal manufacture.

These results indicate that exotic serotypes may be imported into Northern Ireland in raw materials for inclusion in animal feedingstuffs. Furthermore, the dissemination of such serotypes to more than one animal species is readily achieved by the inclusion of contaminated raw materials in several feed types. Epidemiological investigations of exotic serotypes provide a useful marker for the important role of feed in the etiology of Salmonella incidents in animal species. Exotic serotypes are unlikely to be present in other domesticated or wild animals on production units.

It is well recognised that contaminated feed is an important and common source of infection for Salmonella incidents in all animal production species (Williams, 1981). Certainly, feed is considered to be the commonest vehicle for the lateral transmission of S. enteritidis and S. typhimurium into poultry flocks, especially laying flocks, and thus feed constitutes the greatest risk to that sector of the agricultural industry (Stuart, 1984; McIlroy et al., 1989). These results from the analysis of exotic serotypes confirm the important role of feed in the epidemiology of Salmonella incidents and

TABLE 6. NUMBER OF INCIDENTS OF SALMONELLA INFECTION IN HUMANS IN N. IRELAND (1979-1988)

Serotype		1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	TOTAL
S. typhimurium	No.(%)	17 (22.7)	32 (24.2)	42 (27.6)	19 (17.2)	49 (34.7)	31 (23.8)	37 (31.9)	106 (45.1)	133 (30.6)	43 (20.8)	509 (29.4)
S. enteritidis	No.(%)		7 (5.3)	8 (5.3)		12 (8.5)	8 (6.1)	8 (6.9)	59 (25.1)	225 (51.7)	115 (55.8)	442 (25.5)
S. virchow/infantis	No.(%)	38 (50.7)	15 (11.4)	26 (17.1)	25 (22.7)	10 (7.0)	10 (7.7)	7 (6.0)	13 (5.5)	15 (3.4)	9 (4.3)	168 (9.6)
Other serotypes	No.(%)	20 (26.7)	78 (59.1)	76 (50.1)	66 (60.0)	70 (49.6)	81 (62.3)	64 (55.2)	57 (24.3)	62 (14.3)	39 (18.9)	613 (35.4)
TOTAL NUMBER OF INCIDENTS		75	132	152	110	141	130	116	235	435	206	1732

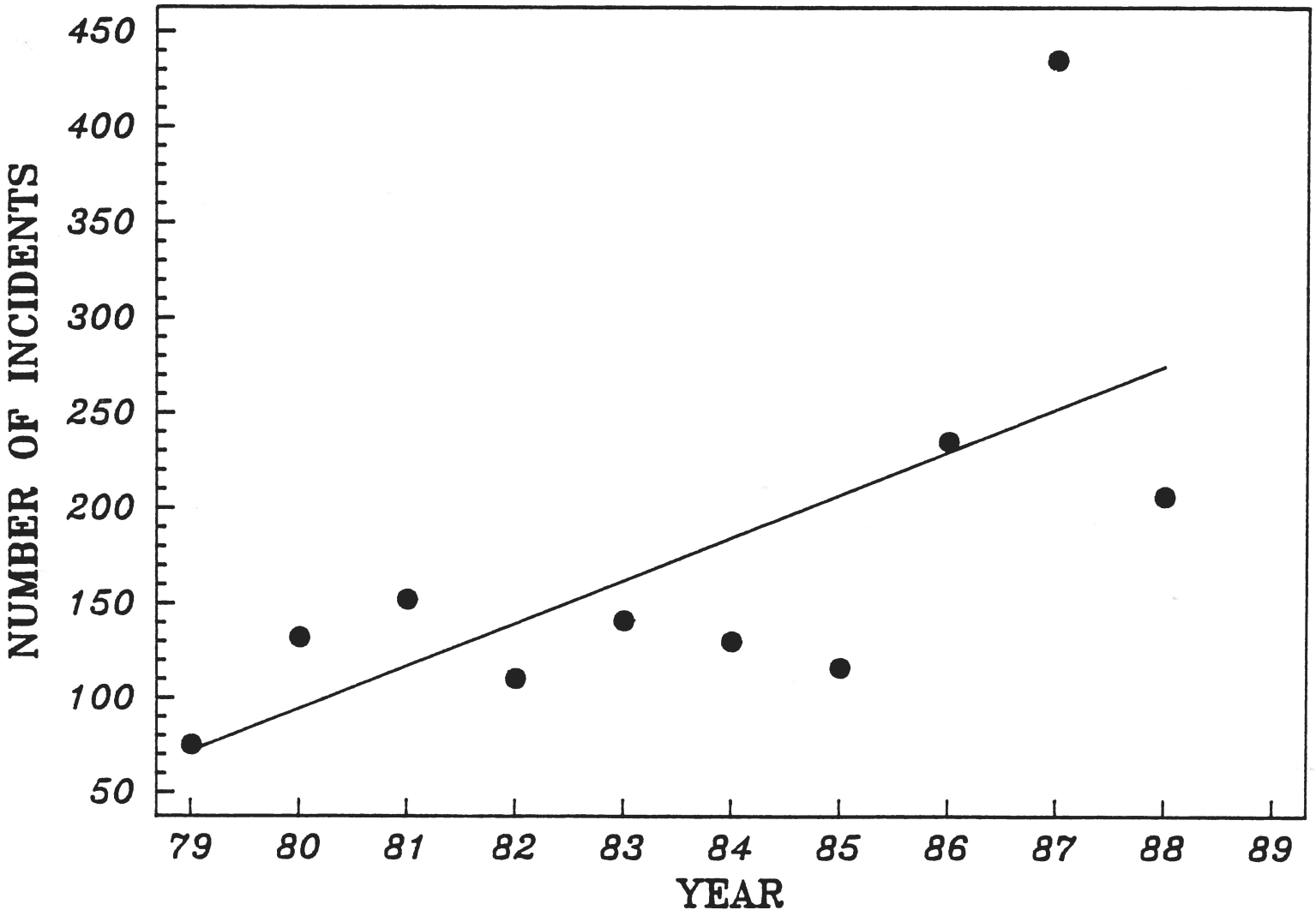

Fig 10. The annual prevalence of Salmonella incidents due to all serotypes in humans.

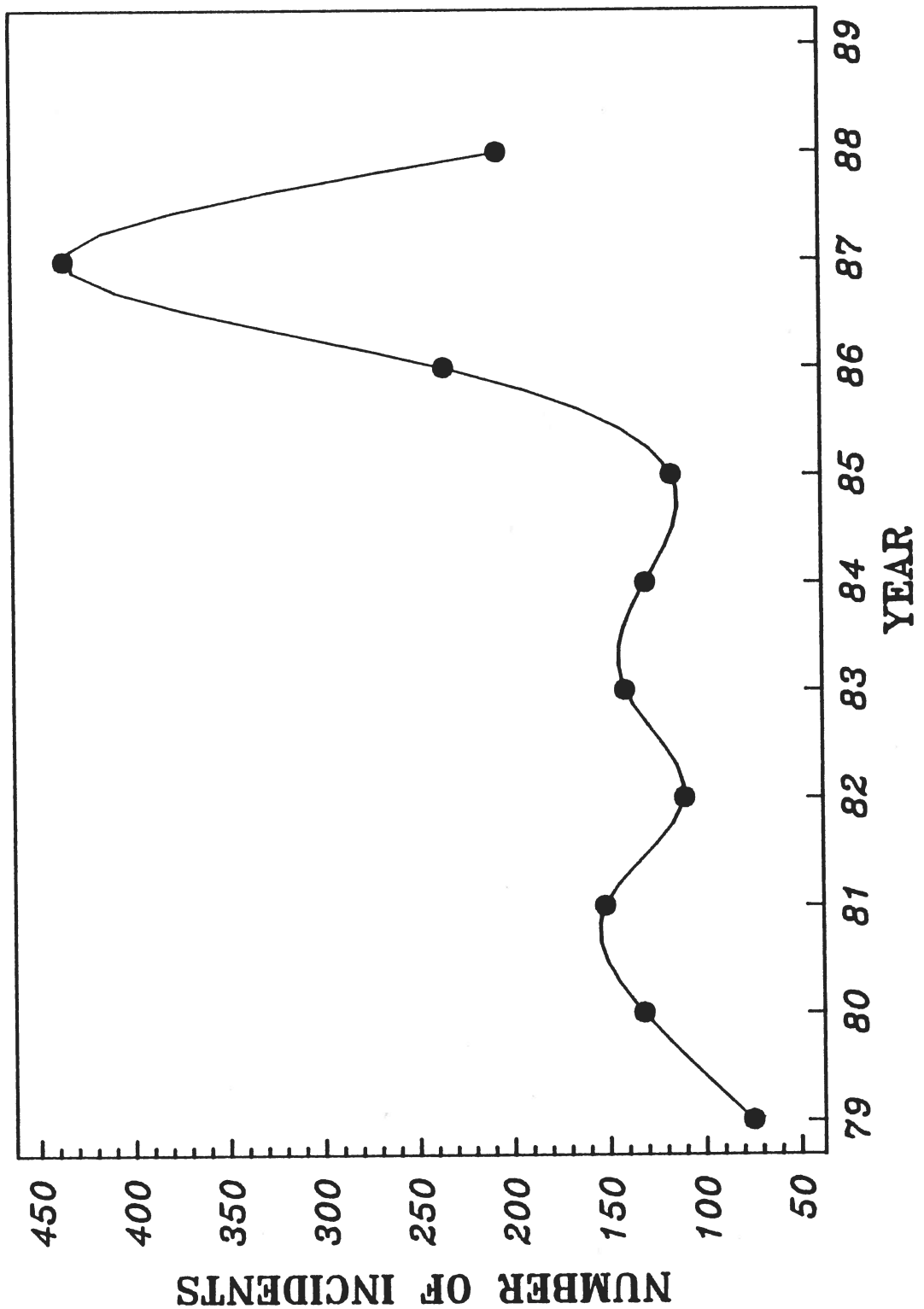

Fig 11. The average monthly prevalence of Salmonella incidents in humans due to S. typhimurium and S. enteritidis (1979-1988).

highlight the importance of such a source to all production animal species. Since 1987 in Northern Ireland all feed to parent broiler flocks is subjected to heat treatment and transported by a dedicated handling system to ensure a decontaminated, Salmonella-free product. The dramatic reduction in the number of Salmonella isolates from poultry in 1988 in Northern Ireland is considered to be directly attributable to the protection of parent broiler flocks by the supply of Salmonella-free feed.

TABLE 7. TEMPORAL AND SPATIAL DISTRIBUTION OF SALMONELLA INCIDENTS DUE TO EXOTIC SEROTYPES

Month	Year	Animal Species	Serotype
April	1988	Ovine	Elizabethvilly
April	1988	Porcine	Elizabethvilly
April	1988	Avian	Elizabethvilly
February	1985	Avian	Give
February	1985	Bovine	Give
February	1985	Porcine	Give
March	1985	Ovine	Binza
April	1985	Bovine	Binza

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LISTERIA MONOCYTOGENES -

ITS OCCURRENCE IN FOODS AND METHODS OF CONTROL

T A ROBERTS* and P J McCLURE*

The genus Listeria contains six species viz L.monocytogenes, L.innocua, L.welshimeri, L.seeligeri, L.ivanovii and L.grayi (syn. L.murrayi). L.monocytogenes is the principle pathogen in humans (McLauchlin, 1987) and animals, although L.ivanovii (also a pathogen in animals) has occasionally been reported to cause disease in humans (Busch, 1971).

Listeria monocytogenes is distributed widely in the environment (reviewed by Brackett, 1988). It has been isolated from uncultivated and cultivated soils, plants, silage, the faeces of animals, raw and treated sewage, and from surface water. Domesticated and wild animals serve as reservoirs of L.monocytogenes. Stool-carriage studies in humans (Kampelmacher & van Noorle Jansen, 1969; Ralovich, 1984) support the view that many people harbour the organism but remain asymptomatic.

LISTERIOSIS

Reports of food-borne transmission of L.monocytogenes as a cause of human infection and disease have increased in recent years. Sporadic cases of human listeriosis were reported as early as 1929-1939 (Nyfeldt, 1929) and the first description of foodborne listeriosis in humans directly linked to animals was by Potel (1953/54). Between 1949 and 1980 several outbreaks were reported with milk suspected as the vehicle, but not proven. Foodborne listeriosis was first conclusively documented after an outbreak in Nova Scotia in 1981 and implicated coleslaw as the vehicle of transmission (Schlech et al., 1983). A second major outbreak occurred in Massachusetts in 1983 (Fleming et al., 1985) identifying pasteurized milk as the vehicle of infection, and a third in southern California in 1985 when 142 cases of human listeriosis occurred in an eight month period. Mexican-style cheese was implicated as the source of infection (Linnan et al., 1988).

Large outbreaks are fortunately very rare, the majority of human listeriosis cases occurring sporadically. Implicating food as the vehicle is extremely difficult because the incubation period may be as long as several weeks (Linnan et al., 1988). The point of exposure to a contaminated product is therefore difficult to determine, and by the time symptoms appear, the contaminated product is rarely available for microbiological analysis. Recent evidence suggests that the primary means of transmission to humans is via foodstuffs contaminated during or after production and processing (WHO, 1988).

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L.monocytogenes IN FOOD

These outbreaks have resulted in surveys of a wide variety of foods, to determine the extent to which L.monocytogenes is present. As a consequence it has been detected in raw milk (Liewen & Plautz, 1988) pasteurized milk (Fleming et al., 1985), soft and hard cheeses (Pini & Gilbert, 1988) ice cream (Farber et al., 1989a), raw liquid whole egg (Leasor & Foegeding, 1989), raw poultry (Bailey et al., 1989), raw red meats (Skovgaard & Morgen, 1988), cured meat (Farber et al., 1988, 1989a), shellfish (Lennon et al., 1984), and pre-packed salads (Sizmur & Walker, 1988). In most instances numbers were very low and it was detected only after enrichment, but occasionally high numbers were present e.g. in some mould-ripened soft cheese and some pâté. Surveys have also been carried out in dairy processing plants (Gabis et al., 1989) and food processing, non-food and domestic environments (Cox et al., 1989), emphasizing the importance of including such environments in epidemiological studies.

The widespread distribution of L.monocytogenes makes it difficult to imagine by what means it might be excluded entirely from raw agricultural products. It is slightly more tolerant to heating than e.g. salmonellae but many meats and meat products can be heat processed to above 70°C to guarantee its inactivation. In some cases, recontamination of the cooked product has occurred, and very strict attention to hygienic practices is imperative if this is to be prevented. No outbreaks of listeriosis have been conclusively linked to meat or poultry products although there is a report suggesting an epidemiological association between eating uncooked hot dogs or undercooked chicken and listeriosis (Schwartz et al., 1988).

Identification of animals carrying L.monocytogenes without symptoms of listeriosis is impossible. Identification by bacterial isolation (reviewed by Cassiday & Brackett, 1989) is impractical because of the cold enrichment techniques used, requiring several weeks to isolate a pure culture. Improved selective media and isolation techniques are being developed rapidly (Klinger, 1988). Methods of rapid detection, based on the use of monoclonal antibodies (Mattingly et al., 1988) and nucleic acid hybridizations (Klinger et al., 1988; Notermans et al., 1989), are also becoming available.

Listeria infections of food animals, especially cattle, sheep and goats, are common (Blenden & Szatalowicz, 1967). There is a clear association of infection with the feeding of poor quality silage (Wilesmith & Gitter, 1986, Low & Renton, 1985), but in some areas of the world where silage feeding is not practised, listeriosis of livestock still occurs. Better control of listeriosis in the animal population would reduce the numbers in animals at slaughter but not eliminate them entirely from the slaughter environment. The prevention of contamination of carcasses by faeces during slaughter has always been a part of the hygienic practices, but slaughter hygiene may need reappraisal if contamination by L.monocytogenes is to be reduced. Attempts to reduce the contamination of slaughterhouses and meat processing plants have demonstrated the difficulty of eliminating L.monocytogenes from the environment.

CONTROL

In food processing areas the aim should be to control and, wherever possible, eliminate L.monocytogenes from any cooked product environment. The problem is mainly recontamination after cooking, so control must

prevent it re-establishing and growing in that environment. A critical factor is the motivation of personnel and their commitment to the application of more intensive than normal hygienic measures, some of which differ greatly from those that have been applied previously. Raw meats must be separated from cooked; equipment and personnel must not be interchanged; cooked product equipment and raw processing equipment must be washed in separate areas.

A new attitude must be instilled into cleaning crews. The overall objective must be to clean and dry because L.monocytogenes is able to multiply in damp patches, condensate on chill room walls and ceilings, in hoses left on the floor and even in soiled clothes in lockers and in brushes/squeeges/wipes used to clean. Drains must be designed and maintained to avoid back-up. If back-up occurs, production must cease, the drain cleaned. High pressure hoses must not be used to clear drains, because an aerosol will be produced and this will spread contamination through the room.

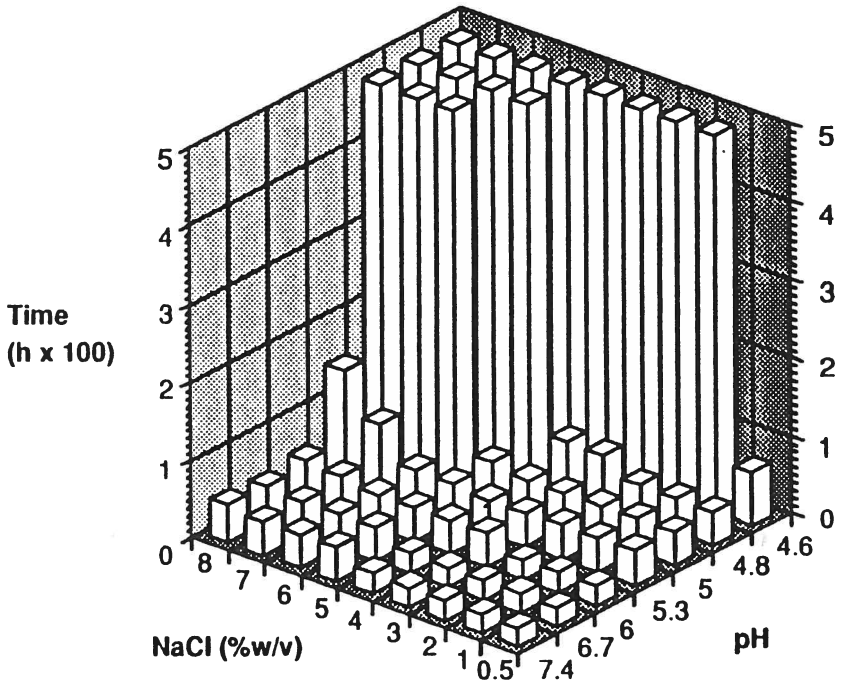
FACTORS CONTROLLING GROWTH

L.monocytogenes can multiply slowly under some conditions previously believed to prevent growth of pathogenic bacteria, e.g. at 1°C, so refrigeration alone may not be adequate to ensure that it does not multiply in stored foods. In addition to the temperature of storage, the pH and the water activity of the food product are important factors in determining the microbial growth response. The minimum pH that allowed growth of L.monocytogenes in 28 days was pH 4.2-4.4 at 20-30°C, pH 4.4-4.6 at 7-10°C and pH 5.0-5.2 at 4°C (Farber et al., 1989b).

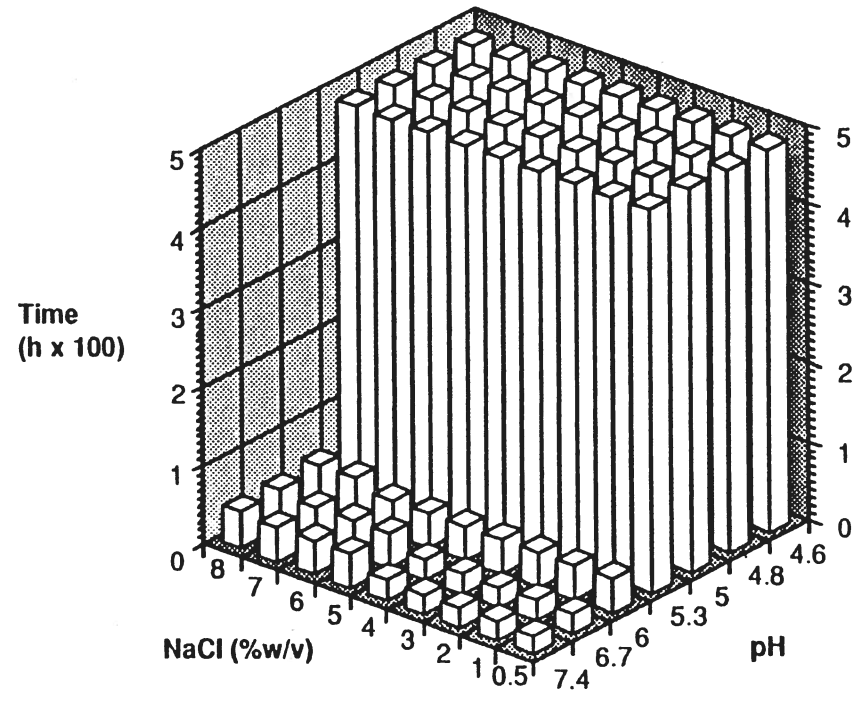
Figure 1 illustrates that control of growth of L.monocytogenes can be achieved by factors acting in combination. Growth responses were measured turbidimetrically, the highest columns indicating that no growth was detected after 20 days (483 hours) incubation. At 20°C no growth occurred at pH 4.6, or at pH 4.8 and 5.0 (Fig 1a) if rather high concentrations of sodium chloride were present. Adding 100 μg/ml sodium nitrite (Fig 1b) prevented growth at pH 5.3 and below at all salt concentrations. Comparing Figs 1a and 1c shows the effect of reducing the incubation temperature from 20°C to 5°C, increasing the number of circumstances where no growth was detected from 15 (Fig 1a) to 44 (Fig 1b). If 100 μg/ml sodium nitrite was included at 5°C growth was prevented in 53 of 63 treatment combinations. It is important that we understand more fully the effects of preservative factors acting in combination if growth of L.monocytogenes in foods is to be controlled. It is evident from the figure that in acid foods such as sauerkraut, yoghurt or many salamis growth of L.monocytogenes would not occur. However, many meat products fall into the pH range 6.4-7.4, where growth is not prevented by sodium chloride and sodium nitrite at levels currently used in cured meats.

PREDICTIVE MODELLING

In laboratories across N.America and Europe many foods have been inoculated with L.monocytogenes in an effort to determine their relative safety, particularly whether numbers of L.monocytogenes increase during storage at temperatures representative of distribution and retailing practices.

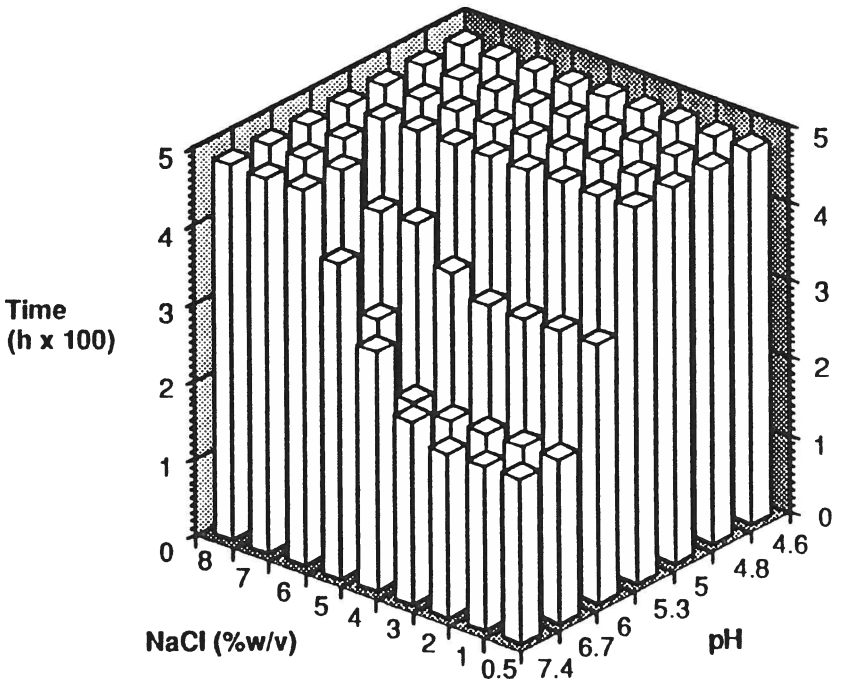


a) 20°C and Sodium nitrite absent

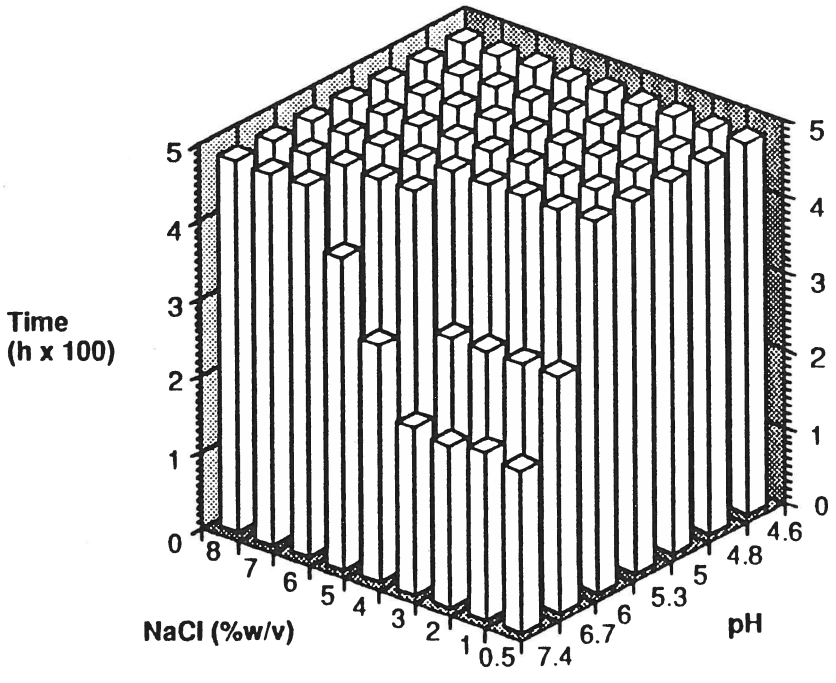


b) 20°C and Sodium nitrite 100 µg/ml

Figure 1. Effect of preservation factors on time to detect growth of L.monocytogenes



c) 5°C and Sodium nitrite absent



d) 5°C and Sodium nitrite 100 µg/ml

Such a piecemeal approach has been the response of food microbiologists whenever a new hazardous bacterium is identified. However experience with other hazardous microbes (e.g. Clostridium botulinum) shows that data accumulated in that way are largely relevant only to the food inoculated. In addition it is difficult, even impossible, to combine data from different investigations. As a consequence in recent years the concept of "predictive modelling" has been developed (Robinson et al., 1982; Roberts & Gibson, 1986). By modelling mathematically the growth responses of food-borne pathogens with respect to pH, water activity and temperature, a basic model of growth responses can be developed (Gibson et al., 1988; Roberts, 1989). If those factors are monitored during food storage, the growth response can be computed from the model in a few seconds. Such models must be validated in food products before being relied upon, so there will still be an element of inoculating foods with pathogens until models for the common food-borne pathogens have been validated. The MAFF is funding research at Research Institutes and Research Associations to develop a database for the bacteria currently associated with food-borne illness in the UK, and for "emerging pathogens".

Examples are given in Tables 1 and 2 computed from the IFR model for L.monocytogenes. Such a matrix of predicted values can be generated quickly and illustrates the consequences of manipulating the formulation or storage conditions. The estimates of lag and growth rate can be integrated across a profile of storage temperature with time. Predicted values for growth parameters (lag time, generation time) from models for salmonellae and L.monocytogenes compare very favourably with values published by workers from different countries after growth in various foods.

Such models will allow foods to be formulated, and storage and packaging systems to be designed, to prevent multiplication of particular pathogens. There is a need for clearer definition of what is necessary to improve food safety and what can be achieved, particularly in foods that are not subjected to a process that inactivates L.monocytogenes. Foods that are heat processed, either in the factory or the home, should not be a risk. Trends toward the consumption of unprocessed or minimally processed "whole foods" may pose a new problem, particularly to susceptible groups (WHO, 1986).

The extent of the problem of food-borne listeriosis is still poorly understood and only coordinated monitoring and systematic data collection will enable us to judge the extent of the problem. Meanwhile, food processors must ensure that, wherever possible raw materials are as free from L.monocytogenes as is reasonable to expect, that appropriate processes are applied and controlled, that recontamination of cooked product is avoided. Consumers can also play a part by observing "use by" dates, ensuring that foods intended to be reheated are heated throughout, and that "ready-to-eat" foods are stored under good refrigeration and used within their allotted shelf-life.

Table 1: Predicted lag times (h) for L.monocytogenes

NaCl (% w/v)	pH	°C	$NaNO_2$ (µg/ml) 0	100	200
2.5	6.0	5	188	281	401
2.5	6.0	10	73	103	140
2.5	6.0	15	34	45	57
2.5	5.3	5	352	627	1030
2.5	5.3	10	136	227	350
2.5	5.3	15	61	96	140
2.5	4.6	5	702	1477	2793
2.5	4.6	10	267	525	926
2.5	4.6	15	119	218	359
1.5	6.0	5	156	217	294
1.5	6.0	10	62	81	104
1.5	6.0	15	28	35	43
1.5	5.3	5	296	493	760
1.5	5.3	10	114	178	259
1.5	5.3	15	52	76	104
1.5	4.6	5	594	1163	2030
1.5	4.6	10	226	411	667
1.5	4.6	15	101	170	257

Table 2: Predicted generation times (h) for L.monocytogenes

NaCl (% w/v)	pH	°C	$NaNO_2$ (µg/ml) 0	100	200
2.5	6.0	5	15	33	55
2.5	6.0	10	6	13	20
2.5	6.0	15	3	6	9
2.5	5.3	5	20	57	120
2.5	5.3	10	8	22	45
2.5	5.3	15	4	10	19
2.5	4.6	5	33	116	310
2.5	4.6	10	14	47	118
2.5	4.6	15	7	22	52
1.5	6.0	5	16	35	58
1.5	6.0	10	6	13	21
1.5	6.0	15	3	6	9
1.5	5.3	5	22	61	127
1.5	5.3	10	9	24	47
1.5	5.3	15	4	11	20
1.5	4.6	5	37	128	334
1.5	4.6	10	15	50	125
1.5	4.6	15	7	23	54

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DATABASE SYSTEMS FOR DISEASE RECORDING

ANIMAL HEALTH MONITORING SYSTEMS IN CANADA

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Canadian agriculture, like agriculture everywhere, is increasingly subject to the influence of global trading patterns as evidenced by the US-Canada Free Trade Agreement, events in the European Economic Community, development patterns in third-world countries, and recent political and economic changes in the Eastern Block countries. One obvious implication of these influences is that production efficiency and quality of product will become more important aspects of the production and marketability of foods of animal origin, whether they are for domestic or export purposes. Another feature is that agricultural practices will have to stress sustainability and have a positive impact on the environment (Agriculture Canada, 1989). It is in this context that we wish to briefly discuss the monitoring of animal health as a component of efficient food production in Canada.

According to Schwabe, 1984, Alexander Langmuir, the former Chief of Epidemiology in the United States Public Health Service's Centers for Disease Control, developed the concept of active disease intelligence, including surveys and problem investigations, as a component of disease surveillance. Data collection and analysis, designed to produce information which is useful to guide subsequent decisions and actions, forms the basis of this intelligence (Schwabe, 1984). Generally, these surveillance activities are now called monitoring in the veterinary literature (Ingram et al, 1975).

The process of monitoring in populations, is analogous to taking the pulse of an individual patient. If the pulse deviates sufficiently from normal bounds (target values), then action needs to be taken to return the pulse (and presumably the patient) back to within the normal limits. This analogy fails, however, to point out some unique features of monitoring animal health, two of which will be mentioned briefly. First, the unit of concern is usually the herd, not the individual. In this regard, it needs to be recalled that herds have properties in addition to those of individuals. For example the phenomenon of herd immunity is one indication that straight line thinking from individuals to herds, or vice-versa, will not always be appropriate. Another example relates to testing groups of animals; tests that are nonspecific at the individual level will probably be very sensitive at the herd level, if the testing process is based on the results of testing a number of individuals (Martin, pers comm). A second feature is that the critical level of the outcome being monitored (ie. the pulse) that will trigger action in herd monitoring, is only partly dependent on biologic factors. Economic considerations and, to a large extent, the owner/manager's objectives for the herd play the predominant role in defining the "action level" for a parameter (Fetrow et al, 1987, 1988; Stein, 1986). As the nature of the dairy industry changes over time, so will the economic conditions, owner objectives and thus, by necessity, the action levels for many parameters.

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In food producing animals, what is monitored often depends on who, or what agency, is doing the monitoring (Ingram et al, 1975). For example, the producer probably has different objectives in mind when monitoring his/her herd than a publically employed veterinarian. Historically, owner supported production monitoring programs have been available, but few have also monitored disease. As mentioned, the target levels for the parameters being monitored are dynamic and the current move towards global markets demands that production efficiency, not production per se, be a key aspect of monitoring. Unfortunately, we are at the fledgling stage in this regard, and for practical reasons often end up measuring biologic outcomes that are correlated with production efficiency. However, because valid measures of production efficiency are not readily available under current industry environments, research is ongoing to identify additional production outcomes that can serve as valid indicators of the economic and biologic production efficiency of the herd or industry.

Monitoring can be performed at one or more levels of organization. For example, within any industry, one could monitor individuals, groups of individuals such as herds, or aggregates of herds in specified geographic areas, perhaps extending to the "national herd". Another approach to monitoring is related to the measurement of events in the pathogenesis of a specific disease, or set of diseases. The potential for monitoring, in the causal pathway (Martin et al, 1987), may begin with the identification of exposure to a sufficient cause, the identification of early pathologic changes (subclinical disease), or the recording of clinical disease. At the herd level, one could monitor exposure to defined components of sufficient causes for mastitis (eg Strep agalactiae in bulk tank milk), monitor subclinical changes (eg somatic cell counts), monitor clinical disease (eg percent of cows with mastitis), or monitor the effects of disease (eg the survivorship or productivity of dairy cows with mastitis, or the quality and safety of milk and milk products). Measuring the effect of disease on outcomes such as survivorship or productivity are not usually a component of ongoing monitoring programs (Martin et al, 1989). Rather, producers and practitioners depend on research findings to establish reasonably accurate estimates of the impact of disease on performance. These estimates may then be built into the monitoring system to predict future productivity.

Focusing monitoring at a specific disease, or set of diseases, has advantages in that it has the potential to prevent future production losses by identifying and correcting the problem in its early stages. However, our level of understanding of the effects of these diseases on the individual animal is significantly greater than our understanding of their impact on the herd. In addition, monitoring specific syndromes at the herd level may be a more sensitive, but perhaps less specific, indicator of current as well as future health problems and production efficiency losses. For example, monitoring the post partum status of cows can identify disease problems that, if not corrected, may reduce the reproductive performance of the herd and eventually impact on the overall production efficiency of the herd. Similarly, monitoring somatic cell counts can be a sensitive indicator of production inefficiencies in an individual dairy cow; its sensitivity and specificity at the herd level remain to be quantified.

Over-reliance on this approach can produce monitoring systems that identify "causes" (diseases) in search of a problem (production inefficiency). For this reason, and the recognition on the part of veterinarians of the strong interrelationships among production, nutrition and disease, practitioners have turned their attention towards incorporating production outcomes into their monitoring systems. In this manner, production becomes a surrogate indicator of the health status of the herd (Stein, 1986). As one aspect of monitoring production, one attempts to find reasons for the production deficit; these may be disease (in the traditional sense) or, as we are becoming more aware, they may be nondisease factors such as problems of management, housing, feeding, etc. Thus, deviations from expected levels of production signal "disease", regardless of whether the underlying causes are traditionally defined syndromes or not (Stein, 1986). Of course, it is also instructive to search for "causes" of efficiency (ie. health), not just causes of deficits (ie.

disease) (Evans, 1978). By combining traditional disease-oriented and more recent production-oriented monitoring activities in the same system, veterinarians hope to obtain some synergy in the monitoring system. Again, who you are, and the attributes of the industry being served, may well dictate both the nature of the monitoring system, and at what stages of disease causation and level of organization monitoring is directed.

The authors have the view that an integrated, formal monitoring system is an essential part of the health management programs of private practitioners. The system must allow for the monitoring of production data as well as clinical and subclinical disease, and if possible, exposure data. It should provide general information on the who (host factors), when (temporal factors), and where (geographic factors), for each what (disease or production parameter). These data should be sufficient to provide initial clues about the why (determinants) of health, at least in terms of the common climatic, demographic, management, and feeding factors (Martin et al, 1987). Further details on the objectives and principles to be incorporated into the design and implementation of monitoring systems are available elsewhere (Fetrow et al, 1987,1988).

To be successful, the system must, in the first instance, meet the needs of the individual producer and his/her veterinarian. Ideally, however, the system should have sufficient planning and data sharing capability to allow for the centralized manipulation and analyses of the large volume of data necessary to identify factors influencing health status, and/or to assist in identifying optimal health management strategies. In addition, the design of the system should reflect the recognition that many members of our food producing industries are not sophisticated data recorders or users. Others (Hardaker and Anderson, 1981) have discussed why recording systems may be doomed to failure. For example, many farmers have an aversion to data recording, most do not see value in historical data, and most tend to make decisions based on physical evidence rather than data. The system should be designed for the typical, not the advanced articulate producer. Information overload must be avoided, else the system will not be used. The caveat for successful monitoring, that we must be aware of the producers ability and objectives, is often made. We would stress that this should not be forgotten.

In Canada, to our knowledge, our federal veterinary infrastructure has not planned a Canadian version of the larger National Animal Health Monitoring Scheme (NAHMS) which exists in the United States of America (Farrar, 1988). Historically, like many coutries (Beal, 1983), we have had federal systems to monitor progress in brucellosis and tuberculosis eradication. Currently there is a Canadian Health Accredited Herd Program which certifies freedom from these diseases, and offers a voluntary leukosis testing and control scheme (Kellar, 1989).

At the provincial level, in Ontario, there are no mandatory health monitoring programs other than a milk quality program based at the Central Milk Testing Laboratory, in Guelph. However, there are a number of optional programs including a production and somatic cell count recording scheme (Ontario Dairy Herd Improvement Corporation), and production recording programs in the beef, sheep and goat industries. Veterinarians and producers have access to a diverse number of herd management software packages. The following are examples of such systems: producer-practitioner systems for monitoring individual beef feedlots (Jim 1989, Thorlakson B 1989), systems designed to prompt the veterinarian and farmer (Lemire, 1989), more sophisticated systems which to variable degrees include both individual animal and herd levels of monitoring(Eicker, 1989; Wiersma, 1989; MacKinnon and Peters, 1988; Williamson and Udomprasert, 1989), systems focused primarily at the herd level (Fetrow, 1988), as well as systems emphasizing surveillance of provincial animal industries (OMAF, 1989). To date, the most extensive and comprehensive provinicial program in existence is the Animal Productivity and Health Information Network (APHIN) in Prince Edward Island (Dohoo, 1988, 1989). This system ties in production, disease and abattoir, and diagnostic lab findings. Regular user-friendly reports, which make extensive use of graphics, are generated for the producer and his/her veterinarian.

The system is designed to help these individuals make better decisions with regard to disease control and herd managment. The system also will, with time, accumulate a vast amount of quality data which can be used for research purposes.

A more comprehensive review of computerized animal health monitoring systems, with emphasis on Canada, has recently been published (Lissemore, 1989). In order to assess the strengths of some of these diverse programs, some benchmark testing procedures need to be developed. The results of evaluating a few systems aimed at the dairy industry are available (Menzies et al, 1988), and other evaluations are currently underway.

So what is happening at the Ontario Veterinary College, in terms of monitoring? Two examples, one relating to the beef industry, and one to the dairy industry will be described.

We developed a system, based on the cooperation of farmers, private practitioners, government personnel, and university veterinarians to monitor the health and production of the beef cow-calf industry in the province of Ontario, Canada (Martin et al, 1989). The resulting project, called BENCHMARK, was a three year study designed to describe existing production and disease levels, the current management practices, and to examine the association between these management practices and health. The project was designed both as a research project combining descriptive and analytic epidemiologic features and as a vehicle to enhance the skills of collaborating veterinarians and producers in ongoing production and health monitoring. (In this sense, the project went beyond traditional monitoring approaches.)

Our study farms were selected from a sampling frame of approximately 1450 farms. In the initial stage, 300 volunteers were selected in a formal random manner. Of these, 50 were no longer in business, leaving 250 available for participation; 193 (77%) of these agreed to collaborate in the study, and, of these, 170 collaborated for two years. An additional 50 herds were selected purposively through private practitioners.

BENCHMARK was designed to supplement a government supported beef herd improvement program (BHIP), which concentrated on weighing calves at weaning so that future breeding stock could be selected on this basis (OMAF, 1985). Cows and calves were uniquely identified, and assistance with weighing provided by the BHIP. However, since the program focused primarily on weaning weights, data on cows that did not wean a calf, or cows that weaned a calf at a time outside of the "weighing period" were often not included in the data base. Thus our study began with a complete census of the herd, and this was repeated on an annual basis in order to detect and confirm any changes in herd membership and size.

With regard to disease recording, we relied primarily on farmer recorded diagnoses. Usually the diagnosis was specific only to anatomic site (eg. pneumonia, mastitis, sore foot, etc). If possible, the animal's identity was recorded to allow differentiation of a case from a treatment and for purposes of future decision making on an individual basis. Leech, 1971, eloquently discussed the strengths and weaknesses of similar approaches to monitoring. On many farms only herd level disease frequencies were recorded; that is, a count of disease frequencies which could be related to a population at risk, but not to an individual animal. Clinical disease was not a common event in our study. Given this low frequency and the fact that clinical disease may not provide useful clues about the true extent or nature of health problems, it is often desirable to incorporate measures of subclinical disease, such as serologic testing, as part of the monitoring system. Tissue or blood samples from a few (3-4) animals per farm will provide sufficient information about the prevalence of putative pathogens in the industry as a whole. More samples per farm will be required to provide firm estimates of prevalence, or incidence, on individual farms. Unfortunately, such sampling was not possible in BENCHMARK, but has been a central feature of a sister project, called SHEPHERDS, in the sheep industry (Menzies, 1989).

Data collection methods were adapted to both the need to collect information from a large number of producers not accustomed to data recording, and the level of intended decision making on each farm. The collection system was flexible so that a variety of producer recording schemes could be accommodated. Pocket and/or clipboard diaries were provided for on-farm recording and portable photocopiers were employed during farm visits to capture information from these diaries, government supplied weigh sheets, breeding charts and any owner designed recording system. Information on management was collected by formal surveys; short general surveys were conducted by mail, detailed surveys were conducted by personal interview and inspection (eg the latter included such items as inspecting and scoring the body condition of the breeding females, and inspecting the calving area for drainage, protection from the elements, etc). Because three veterinarians were involved in the study, considerable effort was expended to standardize recording and assessment of herd management factors. The surveys themselves went through many iterations, pilot trials, modification and reassessment before being widely used in the study. One cannot overestimate the time and effort required to produce a useful survey form/questionnaire for monitoring. In fact, the sensitivity, specificity, precision and accuracy of the survey questions should be determined when possible.

BENCHMARK is now complete, but two ongoing monitoring systems, one elementary and a central mandatory component of the new BHIP, and the other a more elaborate spreadsheet program, called BEEFWATCH, (available from the author (Martin, S.W.)) are available.
We foresee the situation where the solution of health problems identified through production monitoring will be the major activity offered to the cow-calf producer by the veterinary profession.

The second study relates to monitoring in the dairy industry. In Ontario, currently, there is growing consumer concern over the presence of drug and hormone residues in milk products, there is an aggresive penalty program for herds with high bulk tank somatic cell counts, dairymen are under increasing economic pressure to become more efficient in the production of milk products, and there is a perceived shortage of veterinarians serving the dairy industry. In response to these issues we have just initiated a study to collect herd level data on management, production, disease occurrence, economics and drug usage, in 100 purposively selected dairy farms. This pool of data will be used to develope a herd level monitoring and analysis program aimed to meet the demands of the Ontario dairy industry. The collaborating farms are all serviced by privately employed veterinarians who are currently enrolled in a new graduate diploma program at the Ontario Veterinary College. This applied training program, The Dairy Health Management Certificate Program, has linked our research efforts to a group of progressive dairy practitioners. This interface should help us obtain valid, detailed data from a large number of herds, and at the same time, assist the private practitioner to evolve a herd oriented health management service that is suited to the demands of the coming decade.

Data pertaining to the dairy enterprise can be accumulated and manipulated either on the farm or at a regional information processing center. Given the small (average herd size is approximately 47 milking cows) size of our dairy farms, it seems that a bureau system of record keeping is more likely to be implemented than a more elaborate on-farm computer based system. Hence, we will link with the major production recording system, the Ontario Dairy Herd Improvement Corporation, to access their data on production (herd and individual yields of milk, fat and protein) and udder health (herd and individual cow somatic cell count data). These data will be incorporated into our software program, where they will be supplemented by more detailed data on disease occurrence and reproductive efficiency collected on-farm, and then will be analysed and returned to the farmer and veterinarian in a highly palatable, and informative, manner. The basic concepts of the herd monitoring and analysis program are similar to those used by Fetrow et al, 1988, but his approach will be modified to better fit the Ontario dairy producer. Herd status with respect to infectious bovine rhinotracheitis will be determined on a

monthly basis. We are using an ELISA test developed by the Danish government(Nylin, 1989), and if it proves useful, we hope to adapt other validated ELISA tests in order to eventually offer testing for a number of viral agents (including bovine virus diarrhea virus and bovine leukemia virus). Of course, the possibility of monitoring bulk tank milk immunologically is being investigated by others, particularly given the recently documented shortcomings of routine bacteriologic monitoring for mastitis pathogens, and we will be anxiously looking for new technologies to incorporate into our program. With the highly automated milk quality testing system employed in Ontario, additional testing could be easily incorporated.

Given the short time frame for the study, we are unlikely to see major changes in the health status of the study herds. Hence, our outcomes will relate to measures of economic efficiency, the usefulness of the monitoring system as measured by the farmers "willingness to pay" for the system, other measures of production, and disease occurrence. Much descriptive data on the variations, within-farm and between-farms, for traditional and new measures of disease and production will be gathered. Farms that are clearly performing below expected levels with respect to selected parameters will be compared to those that are at or above expected levels of performance, so that potential "risk factors" can be identified, and eventually controlled. It is hoped that this program may serve as a model for a large scale disease and performance monitoring scheme for Ontario dairy herds.

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THE NATIONAL ANIMAL HEALTH MONITORING SYSTEM (NAHMS): EVOLUTION OF AN ANIMAL HEALTH INFORMATION DATABASE SYSTEM IN THE U.S.A.

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Since the early 1900's, livestock producer organizations and veterinary associations in the U.S.A. have encouraged the government to develop a national animal disease surveillance system (National Research Council, 1966). After several unsuccessful attempts with voluntary reporting systems, Veterinary Services of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) launched a major initiative which is now called the National Animal Health Monitoring System (NAHMS) (King, 1985). The NAHMS program, previously called the National Animal Disease Surveillance System (NADS) and later, the National Animal Disease Detection System (NADDS), utilizes the national infrastructure of government veterinarians and animal health technicians in the collection of data and biological specimens from a statistically based sample of U.S. herds and animals. Overall, the NAHMS program mission includes the protection and improvement of animal and human health while ensuring quality and abundance of food and fiber of animal origin and enhancing the competitiveness of U.S. agriculture (NAHMS, 1989).

NAHMS PILOT PROJECTS (1983-1989)

The NAHMS pilot projects were successful in demonstrating that government veterinarians could be used to collect valid information on animal disease occurrence and costs through a non-regulatory program. The pilot projects were implemented on a state by state basis and focused on a range of livestock classes. Within each state, the projects were designed and coordinated through a cooperative effort with a college of veterinary medicine. The pilot projects involved two major forms of data collection, administration of a general farm questionnaire on the first visit and maintenance of farm records of disease occurrences and their costs on subsequent visits over a year's time.

The disease events and their associated costs were summarized for each farm and within each state by herd size strata (Owen, 1987). The epidemiologic analyses of the early pilot project data focused on tabulation of disease events. Proportionate morbidity and mortality rates (PMR) were a standard part of the pilot summary reports as well as ratios of the number of disease events compared to the average total herd population. The early economic analyses followed a partial budgeting approach; however, only the cost side of the budgeting was considered. Producers recorded out-of-pocket expenses for drugs and veterinary services and also estimated the value of their own labor input and the replacement value of animals which died or were culled due to disease.

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Later pilot projects incorporated subsampling for the collection of biological specimens from individual animals and their environment for further diagnostic testing. Blood, feces, and milk were collected from animals along with necropsies of selected deaths. Water and feed samples were analyzed for both nutritional characteristics and contaminants. Most of the results of the subsampling were presented descriptively focusing on individual diagnostic results.

Another interesting development in the pilot phase of NAHMS was the use of NAHMS herds as the control group for specific case-control studies. In California's 3rd pilot round, fowl cholera outbreaks in turkey flocks were examined in this manner. The case population consisted of flocks with laboratory confirmed fowl cholera. The control population comprised a sample of 60 turkey flocks which was selected, interviewed, and subsampled using the NAHMS infrastructure. Epidemiologic analyses of the data sought to identify specific risk factors associated with the occurrence and severity of fowl cholera.

Although all the pilots succeeded in establishing the feasibility of a large scale disease surveillance system utilizing the infrastructure of government veterinary services, they also succeeded in identifying some major weaknesses in the program. First, as reflected in the earlier names of NADS and NADDS, the scheme focused principally on disease with only minimal production data collected. The interactions between animal health and productivity were not addressable. Secondly, the pilot forms required specific coding of each disease occurrence or prevention activity using the Standardized Nomenclature for Veterinary Diseases and Operations (SNVDO). Consequently, every clinical sign observed by the producer and/or the veterinarian required a specific diagnosis in order to be entered into the database. The general categories of NOS (not otherwise specified) were heavily used and some observations such as "off feed" and "decreased milk production" defied coding. Thirdly, the implementations varied sufficiently from state to state to preclude summarization of the data on a regional or national basis. Fourthly, the economic information generated through the pilot projects failed to consider either the resource requirements necessary to reduce the disease costs or the profitability of individual operations. No information was collected on the major variable cost of livestock production, namely feed. Lastly, the process of developing specific NAHMS objectives was orchestrated by the government and university specialists involved in the pilot projects, often failing to include information needs considered to be high priorities by other, much larger potential users and beneficiaries of NAHMS, specifically, producer groups and agribusiness.

TRANSITION TO A NATIONAL PROGRAM

In 1989, the NAHMS program entered its second phase, establishment of a national animal health information program. Animal health information is collected by both active and passive surveillance. The active surveillance component is accomplished through NAHMS on-farm surveys while the passive surveillance component involves the collection and compilation of animal health information available from existing sources such as the network of state and federal diagnostic laboratories, the meat and poultry inspection system, state and federal agricultural statistics agencies and the agricultural research services.

Swine was chosen as the first class to survey nationally and a Swine Advisory Group, comprised of representatives from throughout the industry, was convened

to identify and prioritize swine information needs. The Advisory Group recommended that the first national survey focus on one aspect of swine production, the farrowing to weaning period. Furthermore, they advised collection of baseline data on farrowing performance as well as morbidity and mortality among pre-weaned piglets. Information about management and husbandry practices, disease history and preventive medicine activities, feeding regimens and livestock facilities were encouraged so that health and productivity could be related to farm level independent variables. On the economic side, profitability projections were stressed as well as estimating both the costs associated with specific problems and the resource requirements necessary to reduce these costs.

While the NAHMS national swine survey is being implemented, methods development for a national dairy survey is progressing. A Dairy Advisory Group has met and reached a consensus that the most important information need of the dairy industry concerns neonatal calves and replacement heifers, specifically how management and environment interact with health, productivity, and profitability. A Dairy Technical Council has convened to evaluate alternative analytical designs and has developed a prioritized list of the independent variables which should be measured during the study. Development of the data collection instruments and the study design.are underway currently.

DESIGN OF THE NATIONAL SWINE SURVEY

The NAHMS National Swine Survey utilizes a multistage sampling design, incorporating selection of states first, then herds, then animals within herds. A total of 18 states are involved in the survey. Since a portion of the states were chosen by probability sampling, the data can be extrapolated to estimate the characteristics of 95% of the hog population and 84% of the swine operations in the U.S. Selection of the participating herds involves collaboration with the National Agricultural Statistics Service (NASS) using a multiple frame (list and area) approach. Herds were randomly sampled after stratification by herd size. Within the herds, up to 100 farrowings are monitored over a 3-month period of time. In large operations where more than 100 farrowings are expected within the 3-month period, specific farrowing facilities or sections within the farrowing facilities are chosen in order to limit the monitored farrowings to no more than 100 but include farrowings throughout the monitoring period.

The sample size for the number of herds was calculated on the basis of being able to describe pre-weaning mortality in baby piglets at +/- 1%. The estimated pre-weaning mortality was 12% of the pigs born alive. Allowing for some dropouts, the sample size was calculated at 1400 herds. Over sampling of the original NASS frames was utilized to assure adequate sample size while anticipating 30-50% ineligibility or refusal to participate. Through the collaboration with NASS, some general characteristics of the participating farms and the refusals can be compared in order to test for participation biases.

The survey design involves collection of retrospective, cross-sectional, and prospective data. Personal interviews are utilized to record producer's recollections of animal health problems and their assessment of current management practices. Animal inventory, facilities design, and feeding programs are documented through these questionnaires. A record keeping system involving farrowing and feeding diary cards is implemented at the beginning of the herd monitoring system. Individual farrowing and litter diaries are maintained for each sow farrowing during the 3-month monitoring period. The producer notes the birth statistics, morbidity and mortality experience of the sow/gilt and litter

between farrowing and weaning, and records the disposition of sow/gilt after weaning. A feed diary card is maintained for 2 weeks during this time to corroborate feeding practices and amounts. Samples of water are collected from the farrowing facility in which the sows/gilts are monitored and a sample of 10 farrowing sows/gilts is randomly selected for serologic screening. Finally, data on the economics of the operation are collected from existing records through a personal interview.

The infrastructure of NAHMS is also ideally suited for the collection of additional data to address emerging national or regional issues. Consequently, additional data collection and analyses have been planned in conjunction with the national swine survey in order to: (1) conduct a case-control study of swine reproductive failure syndrome using the NAHMS sample as the control herds and (2) examine air quality (relative humidity, ammonia levels, and dust) in farrowing operations in the midwestern states. Some state-level projects are planned to allow additional subsampling such as testing water samples for total dissolved solids in Ohio and screening fecal samples for Salmonella in California.

FIELD TEST RESULTS FROM SWINE NAHMS

Preliminary analyses of field test data from 127 herds in 8 states established wide variability in the herd averages of specific measures of physical performance such as farrowing outcomes and pre-weaning mortality (Table 1). These farm averages are similar to other assessments of productivity by Wilson, et al., (1986) and Duffy (1988), which lends support to the validity of this field test information.

Morbidity and mortality in baby pigs was monitored by the producer and observations of clinical signs allowed the calculation of specific incidence and mortality rates (Table 2). The morbidity and mortality parameters provide sufficient variability to allow for investigation of associations with management, environment, and agent characteristics. For example, preliminary analyses found no association between various biosecurity measures and observed diarrhea in pre-weaned piglets. However, more piglets were observed with diarrhea in farms utilizing total confinement as compared to all other types of housing ($p < .05$).

In all of the states included in the field tests, producers most frequently attributed piglet deaths to crushing by the sow while the most frequently observed clinical sign in sick piglets was diarrhea. Although the precise cause of death cannot be assessed through producer observations alone, recent validity studies by Vaillancourt (1990) provide estimates of the sensitivity and specificity of producer records of cause of death based on observed clinical signs. For instance, Vaillancourt found that the sensitivity of producers' evaluations of trauma as cause of death was high (96.7%) but the specificity of their evaluations was considerably lower (68.1%). As a result, while producers are likely to correctly identify most deaths associated with crushing, they also attribute additional deaths to crushing that were, in fact, due to other causes. These findings facilitate the adjustment of the cause-specific mortality rates derived from producer observations.

The collection and analysis of national swine health and productivity data began with the extensive on-farm survey described above. This National Swine Survey is scheduled to be completed in January of 1991, however, the collection of swine data will continue to evolve and expand.

Table 1. NAHMS Swine Survey - Preliminary Results[a]

Farm Averages for Sow[b] Farrowing Outcomes[c]

	Average	Range	
		Min	Max
Total Pigs Born per Litter	10.9	6	14.2
Born Alive (%)	91.5	53.5	100
Stillborn (%)	7.5	0	30.8
Mummies (%)	1.0	0	5.9
Pre-weaning Mortality (%)	15.7	0	47.4
Total Pigs Weaned per Sow	8.3	3.8	11.2

[a]Results from field tests in 8 states: Virginia, Maryland, Oregon, Wisconsin, Illinois, Tennessee, Alabama, Georgia.

[b]Excludes litters born to primiparous females.

[c]Includes data from 1546 litters monitored in 127 herds from farrowing to weaning.

Table 2. NAHMS Swine Survey - Preliminary Results[a]

Farm Averages for Piglet Morbidity and Mortality[b]

	Average Incidence Density[c]	
	Morbidity	Mortality
Clinical Sign		
Diarrhea	2.04	0.24
Neurologic Problem	0.01	0.02
Lameness	0.13	0.06
Deformities	0.06	0.05
Other Known Diseases	0.11	0.30
Unknown Diseases	0.07	0.30
Crushed	N/A	1.69
Starvation	N/A	0.57

[a]Results from field tests in 8 states: Virginia, Maryland, Oregon, Wisconsin, Illinois, Tennessee, Alabama, Georgia.

[b]Includes data from 1546 litters monitored on 127 farms from farrowing to weaning.

[c]Expressed as cases/100 piglets/week.

N/A - not applicable.

EVOLVING STRATEGIES - WEANING TO SLAUGHTER LOSSES IN SWINE

Evaluation of the cost of clinical and subclinical disease from weaning to slaughter is now under consideration and ideally the following information should be collected: (1) post-weaning mortality rate, (2) the incidence of clinical diseases (expressed as cases/1000 pig days), (3) the cost of mortalities, culls (lost premiums, condemnation, trimming), reduced weight gains, veterinary services, drugs not included in veterinary services, labor, etc., (4) the prevalence and severity of subclinical diseases, (5) the cost of subclinical diseases, i.e., poorer average daily gain (ADG) and feed conversion efficiency (FCE), and (6) the cost of minimizing subclinical diseases. Data on the first three components has been collected in pilot studies using on-farm surveys, however, consideration is now being given to incorporation of data from other sources as part of an expansion of NAHMS.

Slaughter checks are particularly suited for detecting and assessing severity of subclinical diseases in growing stock. They provide an objective measurement of disease prevalence/severity and can be used to monitor significant changes in health status as a result of disease control measures, thereby providing a basis for evaluation of the direct cost of subclinical diseases and the cost of their control. In the pilot studies, diagnosis of the cause of death and clinical disease has relied on producer observations of clinical signs. As inaccuracies in this approach have been reported by Salman et al., (1988) and Vaillancourt (1990), the degree of producer bias occurring in these post-weaning diagnoses should be established by similar validation studies using necropsies and laboratory testing. Consideration is also being given to the incorporation of post-weaning mortality data from existing industry data bases, e.g., PigCHAMP (University of Minnesota), or Pigtails. To assess how this data, from a biased herd sample, relates to industry, comparison can be made with data collected from randomly selected herds used in the pilot studies.

Economic data generated in the pilot projects (Miller and Dorn, 1987; Owen, 1989) is likely to be of better quality than the disease diagnoses due to the relative ease of recording mortalities, culls, cost of treatments, and preventive medications. Few of the pilot projects, however, addressed subclinical disease losses which are a large component of many diseases. As approximately 70% of the cost of producing a pig is represented by feed consumed or wasted, reduced feed efficiency and weight gain as a result of subclinical disease can inflict far greater financial losses than direct expenses such as mortalities or treating sick pigs. Much of the cost of these endemic subclinical infections is comprised of expenditures on facilities to improve the quality of the environment e.g., automatic ventilation and building modifications, however, the "whole farm" cost of disease control has not been addressed yet by NAHMS or other published reports. So far the cost-benefit of control has concentrated largely on performance improvements as a response to a preventive medicine program which has controlled infection with a specific agent. In reality, however, the incidence/prevalence of disease is also an indication of environment or management faults which are likely to be playing a larger role in disease losses than are specific infections. With a slaughter check program NAHMS will have
the opportunity to address subclinical disease losses and the cost of their control.

MONITORING PATHOLOGY AT SLAUGHTER

Extension of routine meat inspection to encompass diseases which limit growth efficiency was pioneered by Backstrom and coworkers in Scandinavia (Larsson and Backstrom, 1971; Lindqvist, 1974; Backstrom and Bremer, 1976; Willeberg et al., 1984/85). The approach aimed to provide information to producers to assist diagnosis of problems and encourage implementation of control strategies. Both producers and abattoirs benefited, especially through reduced condemnation of carcass components and improved feed conversion efficiency. Herd disease prevalence data was used to select herds for case-control studies aimed at identifying risk factors associated with major disease complexes (Aalund et al., 1976; Backstrom and Bremer, 1978). The development of this population management approach to disease has been a major advancement in herd health management, especially in the area of subclinical diseases.

The Scandinavian approach utilizes veterinarians who are employed to perform routine carcass safety inspections for classification of both infectious and noninfectious conditions. Results are collated on a central computer and herds notified when prevalences exceed targets. As producers own a large share of slaughterhouses, there is immediate collaboration which ensures that herd profiles can be reliably generated. The approach has continued and expanded over 20 years; in Denmark, veterinarians are also employed by the producer cooperatives to manage problems identified at slaughter. Factors which have ensured the success of the approach have been (1) the use of routine (veterinary) inspection staff, i.e., no additional costs at slaughter and (2) the use of veterinarians to accurately classify the type of lesions.

Establishment of this approach in countries where routine inspection is not done by veterinarians has been limited. In general, in these other countries, slaughter checks have been limited to practicing veterinarians performing inspections on behalf of consultancy clients. As this approach has not been coordinated, identification of industry problems or development of case-control studies from this data has not occurred. The Pig Health Monitoring Scheme developed in South Australia (Pointon et al., 1987) overcame limited resources to produce an industry profile of disease at minimal cost.

Features of the Australian Slaughter Check Scheme

The Australian scheme was, however, designed with the objectives of the Scandinavian schemes in mind, being to: (1) Improve disease diagnosis accuracy and identify industry problems, (2) assist cost-effective disease control, (3) identify risk factors associated with disease problems i.e., act as a research tool, (4) directly or indirectly benefit all of industry.

The main modifications incorporated into the scheme included: (1) monitoring a statistically derived sample of stock periodically, (2) monitoring a broad range of production limiting diseases including ileitis (Pointon, 1989) which had not been previously monitored, and (3) introduction of severity scoring systems for sarcoptic range and nephritis. With only 180-225 pigs being killed/hour an intensive and detailed inspection is performed by a veterinarian with a lay assistant on a sample of stock from an individual farm. The objective of the herd inspection is to provide a comprehensive assessment of subclinical disease prevalence and severity. Consulting veterinarians interpret reports in conjunction with knowledge of the age/weight of stock inspected, their clinical history, and farm management practices. Inspection results are entered on computer via PIGMON, a specific purpose program developed in collaboration with

Dr. A. Mercy (West Australian (W.A.) Department of Agriculture). As well as providing herd reports, in which sequential results are graphed, the program provides area, abattoir and state reports which assist the consulting veterinarian to manage herd health and production.

All herds with more than 200 sows joined the initial pilot scheme. Overall, the 196 member herds accounted for approximately 60% of pigs produced in South Australia (S.A.). The herd sample is biased toward large and progressive producers who undertook to use a consultant veterinarian as a condition of joining the scheme. This latter requirement was instituted to minimize failure to respond to identified problems; a concern raised by Willeberg (1984/85) in the Danish scheme. Industry problems identified in S.A. (Pointon et al., 1987) and W.A. (Mercy and Brennan, 1989) were sarcoptic mange, ascariasis, enzootic pneumonia, pleurisy, and leptospirosis. Ileitis was diagnosed in 15% of herds at their first slaughter check (Pointon, 1989; Mercy and Brennan, 1989) and was detected in a third of herds monitored quarterly for one year (Pointon, 1989).

When herds were stratified by size for analysis, an association between herd size and prevalence of enzootic pneumonia was found, as demonstrated in Scandinavia by Aalund et al. (1976). Smaller herds were found more likely to be infested by Ascaris suum; a result consistent with Mercy and Brennan (1989) in W.A. Since associations found between disease prevalence and herd size were consistent with other studies, limiting inspection to a sample of stock from each herd did not apparently limit detection of disease trends and associations.

The need to record severity of lesions was evaluated by determining the proportion of herd severity which could be predicted from prevalence alone. For diseases which are heavily dependent on environmental or management factors to cause infection and pathology, e.g., ascariasis, leptospirosis, and E.P., the association between prevalence and severity is low (r^2 = 0.23, 0.19 and 0.34, respectively), whereas for sarcoptic mange, where such environmental influences have not been demonstrated, a stronger association exists (r^2 = 0.52). All associations were highly significant ($p < 0.0001$), reflecting the large number of pigs included in the analysis. Therefore, both prevalence and severity scoring need to be used where practicable to accurately describe the pathologic status of a herd, particularly if the measurements are being used to determine if significant changes in health have resulted from disease control interventions.

Case control studies have evolved from the slaughter check data. The intriguing prevalence of ileitis led to the study of the epidemiology of this condition (Pointon, 1989). Control herds were selected on the basis of freedom from gross lesions over 18 months of quarterly inspections. Chronic ileitis (campylobacter-associated pathology) was found to be associated with acute hemorrhagic deaths in two-thirds of affected herds. Deaths predominantly occurred in stock 15-25 weeks of age, however, 10-15 week-old growers and selected gilts were occasionally affected. Feeding preventive levels of antibiotic continuously had not eliminated chronic lesions or mortalities (completely) in the positive herds. The method of effluent disposal (i.e., open drains versus partially slatted finishing pens) did not influence the presence of chronic ileitis. Clinicians reported ill thrift in growing pigs to be associated with a prevalence of chronic ileitis of 5-15%, while hemorrhagic deaths are seen at higher herd prevalence levels. Additional case-control studies are being performed to investigate the epidemiology of leptospirosis and the significance of herd scores for sarcoptic mange.

GUIDELINES FOR SELECTING SAMPLE SIZE AND INTERPRETING RESULTS

In countries where routine carcass inspection is performed by non-veterinary staff, monitoring of production-limiting pathology at slaughter has been performed on a subset sample of stock for expediency and to limit cost. Unfortunately, little attention has been given to defining the statistical basis for sample size selection and to relating requisite sample sizes to the number of pigs available on a weekly basis. Pointon et al., (1987) used guidelines presented by Cannon and Roe (1982) for selecting sample size to (1) detect minimum prevalence of disease and (2) estimate prevalence.

A detailed discussion of selecting sample size to monitor herd health status is provided by Pointon et al., (1990). Lesions monitored at slaughter should be related directly to the "at risk" population. In the case of conditions known to resolve, e.g., enzootic pneumonemia, pleurisy, and ascaris liver spots, lesions recorded in market swine should only be related to stock slaughtered within eight weeks of normal market weight. To detect a minimum disease prevalence of 10% at a 95% level of confidence using the normal weekly market group as the sample, the 8-week pre-market population would have to be at least 208 animals in order to market 26 pigs/week. This is equivalent in size to the production of a 90-sow herd marketing 15 pigs/sow/year or to a 64-sow herd marketing 21 pigs/sows/year. For herds producing less than 26 pigs/week, the sample size would require bolstering with stock from the subsequent weeks of production. In herds marketing >54 pigs/week a minimum prevalence of 5% (95% level of confidence) can be detected. The impact of these limitations in smaller herds means that only a minimum prevalence of 10% or 15% is detectable. Consequently the proportion of affected herds will be underestimated for conditions which commonly occur at a low prevalence.

When estimating prevalence, a precision of 10% is recommended to enable distinguishing mild, moderate, and severe prevalence of disease so that significant fluctuations can be detected at an early stage. Accuracies poorer than 10% (90% confidence limit of point estimate) will constrain the usefulness of slaughter check data for herd health management, however, they do not bias estimates of average prevalence for industry.

In the U.S., the number of pigs monitored by practicing veterinarians on behalf of practice clients is limited by abattoir procedures, particularly the requirements to maintain a chain speed of up to 1100 pigs/hour. Typically, sample sizes of 20-30 pigs are monitored with little adjustment for herd size. The system, as it currently operates, is best described as a "motivational tool" for producers. For herds with greater than 100 sows, sample sizes commonly utilized only provide an accuracy of 15% for high and low prevalence estimates and 20% for mid-range prevalence (90% C.L.). A paper discussing this limitation (Pointon et al., 1990) was written to provide veterinarians with a practical guide to enable an evaluation of the value of slaughter checks as they are currently performed, to establish functional targets which are achievable and to provide statistical tables to act as a basis for improvements. These guidelines will act as a cornerstone of the slaughter check evaluation to be coordinated by NAHMS in the U.S. during 1990.

U.S. SLAUGHTER CHECK SCHEME - CONSIDERATIONS AND DESIGN

A feasibility evaluation of a slaughter check scheme for production limiting diseases is underway in Minnesota as part of the NAHMS effort. Consideration of experiences obtained in the Scandinavian and Australian systems has been

incorporated into the design. The absence of veterinarians routinely performing extensive slaughter checks for production-limiting conditions is a major feature distinguishing the Scandinavian schemes from the U.S. and Australia. In the initial U.S. scheme, practicing veterinarians will have an opportunity to perform both the inspections and follow-up clinical work as developed in the Australian model.

The sample frame for selecting herds for monitoring will be to utilize herds currently using veterinarians on a regular consulting basis (and having slaughter checks performed). This biased sample has been chosen as (1) there is an increased likelihood that they will have adequate production/financial records for use in on-farm studies, (2) they will have a commitment to participating in slaughter checks performed by their consultant veterinarian, (3) they will be motivated to respond to identified disease problems, and (4) they will utilize veterinary services to participate in disease control initiatives.

Monitoring randomly selected herds at slaughter is logistically difficult as current circumstances require a high-level of producer commitment and expense/inconvenience to ensure a herd can be monitored. A large proportion of these herds are also unlikely to have adequate production/financial records to facilitate intervention/economic studies. Random sampling of pigs at slaughter enables detection of seasonal trends but prohibits relating disease information to environment and management conditions and herd size influences. Despite limitations of the random approach, Backstrom (personal communication) advises monitoring pigs on a random basis (i.e., non-herd based) seasonally. This data could than be used as a benchmark for comparison with industry figures extrapolated from the biased herd sample, thereby satisfying the NAHMS requirement of an industry prospective.

For example:

* sample prevalence compared with the pooled result of all pigs monitored from the biased herd sample, and,
* sample prevalence compared with a "calculated industry result" (= disease prevalence of stratified herd size groups x proportion of total pigs killed from each herd size group).

The source of pigs/herds is determined by the location of major abattoirs in southern Minnesota, consequently, pigs will be drawn from the mid-west corn belt of Minnesota and northern Iowa. Herd details to be requested initially from producers/veterinarians will be limited to those required for initial analysis of herd data as performed by Backstrom and Bremer (1976). Data from farrow-to-finish herds will be compared with feeder pig operations and continuous production with all-in-all-out systems. More detailed data collection will be left to case-control studies of specific conditions to ensure all relevant variables are evaluated. Biases in the data collected at slaughter in the U.S. may be expected from two sources. Pigs with obvious illness, e.g., abscessation, arthritis, or light weight, which are delivered to regional buying stations may be sent to other abattoirs. The proportion of pigs not sent to the major abattoirs will be assessed. Another bias may result from the need to remove the lung/heart/liver from the viscera table for later inspection. This requirement stems from the inability to accurately inspect viscera at fast speeds. Viscera stamped as condemned, therefore, cannot be handled or stored with "passed" organs as this would contravene meat hygiene protocols. An evaluation of reasons for viscera condemnation and the proportion condemned will be performed to determine if not monitoring these animals will result in a bias. As sick pigs are generally not

delivered to major abattoirs in the midwest, inspection of consecutive hogs from a herd is not considered to bias the result.

Conditions proposed for regular monitoring in this feasibility evaluation will include sarcoptic mange, ascaris, enzootic pneumonia, atrophic rhinitis, pleurisy, pericarditis, pleuropneumonia (Actinobacillus), lungworm, abscessation, and ileitis. Additional conditions are to be monitored on a random basis; peritonitis, arthritis, nephritis, and oesophogastric ulcers. Practical severity scoring systems outlined by Straw et al., (1986) and Pointon et al., (1987) will be used. For new conditions as ileitis, criteria to define gross lesions is required; these will be established by comparison with "gold standard" laboratory tests to support the specificity of classification. In the case of nephritis due to leptospirosis, validation of the specificity of lesions is required to ensure results are credible. Laboratory support will, therefore, play an important role in validation of the gross pathology classification systems used. Pathology other than conditions designated for monitoring will also be recorded to fully define the range of differential pathology which may be encountered. This information, along with the statistical guidelines for selecting sample size and interpreting results, will form the basis of a training manual for slaughter checks.

To implement such a scheme, the collaboration of practicing veterinarians, abattoir management, producers, and laboratory staff is required. This requires considerable consultation and coordination by key industry veterinarians; in Minnesota, Dr. Gary Dial, University of Minnesota, has provided that impetus. Results will be collated at the University using the PIGMON program developed collaboratively between the S.A. and W.A. groups. Graphed herd reports comparing sequential inspections will be available. These will be supplemented by summary reports for area, veterinarians, abattoirs, season, and industry.

Follow-up projects to assess the economics and epidemiology of problems identified can be implemented. It is envisaged that producers will be keen to collaborate in intervention/economic studies, collecting the necessary data to enable a comprehensive economic evaluation of all disease control procedures. As the producer will be the principle beneficiary of the exercise, there should be no direct on-farm cost to NAHMS; producers should be keen to involve NAHMS expertise to assess the cost-benefit of their own expenditure. For these economic studies it is proposed that only herds using computer records systems will be selected. After repeat inspections of herds (i.e., quarterly) it will be feasible to select herds for case-control studies to investigate risk factors associated with industry problems defined by the scheme. This is likely to require collaboration between NAHMS epidemiologists and statisticians, state-based NAHMS Coordinators, laboratory/university groups, and practicing veterinarians.

SUMMARY

The NAHMS program represents an evolving animal health information database system in the U.S. The NAHMS program has been designed to remain flexible in order to capitalize on other existing data sources and to develop new methodologies to gather data consistent with the program goals. The collection of swine data, beginning with the pilot projects and evolving through the current national swine survey, forms the initial swine health database. Slaughter surveillance, drawing upon the lessons learned in the Scandinavian and Australian systems, offers methods for expanding this database by including information on subclinical production-limiting diseases which are not currently monitored. The

feasibility of this approach is being evaluated in the Minnesota project just described. The resulting information will meet needs of producers, industry, and the veterinary profession as they search for more cost-effective methods of producing high quality livestock products which address consumers desires.

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EMERGING DISEASES

EPIDEMIOLOGICAL STUDIES OF BOVINE SPONGIFORM ENCEPHALOPATHY IN GREAT BRITAIN AND NORTHERN IRELAND

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Bovine Spongiform Encephalopathy (BSE) is a slowly progressive degenerative neurological disease of adult cattle, so called because of the spongiform appearance of affected brain tissue when viewed under the light microscope (Wells et al., 1987). BSE belongs to a group of transmissible encephalopathies which are generally fatal. These include scrapie of sheep, transmissible mink encephalopathy of mink and Creutzfeldt-Jakob disease and Kuru of man (Fraser, 1979).

This novel disease of cattle was first diagnosed in Great Britain in November 1986 (Wells et al., 1987), in Northern Ireland in November 1988 and in the Republic of Ireland in January 1989 (Bassett and Sheridan, 1989). Diagnosis was originally based on the presence of vacuolar changes in the neuronal perikarya and neuropil in various regions of the brain (Wells et al.,1987). Experience of a large number of cases of the disease has now indicated that pathognomonic vacuolar changes are almost invariably present (99.6% of confirmed cases) in the medulla oblongata sectioned at the level of the obex (Wells et al., 1989). These findings have resulted in a simplified and more rapid method of diagnosis.

The nature and distribution of the histological changes and the identification of fibrils similar to scrapie-associated fibrils (Wells et al., 1987; Wells and Scott, 1988) led to the suggestion that the aetiology of BSE was related to that of scrapie. This hypothesis was further supported by the finding of scrapie-associated protein in BSE fibrils (Hope et al., 1988). Subsequently Fraser et al. (1988) successfully transmitted BSE to mice by intra-cerebral inoculation of brain homogenates. The nature and distribution of the lesions were similar to those found in mice experimentally infected with the scrapie agent. The disease has also been experimentally transmitted, using affected bovine brain homogenates inoculated intracerebrally or intravenously to cattle (Dawson et al., 1990). Furthermore, the disease has also been reproduced in mice by the oral route (Barlow and Middleton, 1990).

Morgan (1988) postulated an epidemiological link between the sudden widespread appearance of BSE and the feeding of scrapie-infected offal to

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cattle. Subsequent extensive epidemiological studies (Wilesmith et al., 1988) indicated that the occurrence of BSE was consistent with exposure of cattle to a scrapie-like agent, via cattle feedingstuffs containing sheep-derived protein. The study concluded that the temporal and spatial distribution of affected cases was consistent with an extended, common source epidemic.

This paper reports the results of the analyses of epidemiological data from 33 confirmed cases of BSE in Northern Ireland from November 1988 to December 1989. The paper also compares and contrasts these epidemiological findings with those from 9,000 confirmed cases in Great Britain between November 1986 and December 1989.

NOTIFICATION OF CASES

All suspect cases of BSE must be notified to MAFF in Great Britain and DANI in Northern Ireland under the relevant BSE Orders introduced in June 1988 and November 1988 respectively. Prior to these Orders notification by veterinary practitioners was requested on a voluntary basis. Clinically suspect cases were defined as those which presented a syndrome consistent with the clinical signs observed in confirmed cases and described by Cranwell et al., (1988); Gilmour et al., (1988) and Wilesmith et al., (1988).

EPIDEMIOLOGICAL DATABASE

Subsequent to the identification of a confirmed case of BSE in a herd in Northern Ireland, a detailed epidemiological investigation was carried out by a veterinary officer of DANI. The following data relating to each affected herd was obtained: herd type; adult herd size and age structure; details of all cattle movements since the birth of the confirmed case and the presence of sheep on the farm since 1982. Details of the feedingstuffs used on the farm since the birth of the confirmed case were recorded and included the type, source and duration of all concentrate feeding. Details of the use of pharmaceutical products, vaccines, pesticides and herbicides were also noted.

The following data relating to each of the 33 confirmed BSE affected animals was obtained: date of clinical onset; age; sex; breed and pedigree where known; stage and number of lactations; stage of pregnancy; herd of origin; location and identification of all offspring; location and identification of all siblings with the same dam and the source and type of insemination by which the affected case was conceived. Additional information on the detailed parentage of all affected cases was obtained from farm, AI and breed society records where available. The presenting clinical signs and their progression were described in each case under the categories of general, behavioural and locomotory signs.

Similar herd and case epidemiological data was collected on the 9,000 confirmed cases in Great Britain by a questionnaire form described by Wilesmith et al. (1988). Analyses of the Northern Ireland and Great Britain databases was carried out as described by Wilesmith et al. (1988) to provide a quantitative epidemiological description of the disease.

RESULTS AND DISCUSSION

Clinical signs

General: The percentage frequency distribution of general clinical signs noted in the 33 confirmed cases of BSE in Northern Ireland is demonstrated in Fig 1. Weight loss and loss of condition were both recognised in 73% of cases. Reduced milk yield was recorded in 86% of affected, lactating cows. Fig 2 demonstrates the percentage frequency distribution of general clinical signs in 2,794 confirmed cases of BSE in Great Britain. Notably, the relative occurrence of the major presenting general clinical signs was similar in both countries.

Behaviour: The percentage frequency distribution of behavioural clinical signs in the 33 confirmed cases in Northern Ireland is shown in Fig 3. Comparative behavioural signs in 192 cases in Great Britain are shown in Fig 4. It is interesting to note that the frequency of behavioural signs is similar in cases in both countries with apprehension, temperament change and sensitivity to touch and sound being observed in over 80% of all cases. Kicking in parlour, panic stricken and nervousness of entrances would appear to be 3 behavioural signs in which differences in their respective recorded frequencies were noted. This may reflect the requirement to rely on individual herd owner's observations and the highly subjective nature of the particular categories of signs involved.

Locomotor/neurological: The percentage frequency distribution of locomotor/neurological signs in the 33 confirmed cases is shown in Fig 5. Corresponding clinical signs in 192 cases in Great Britain are presented in Fig 6. It is clearly apparent that both the frequency and distribution of such signs are almost identical in BSE cases in both countries, with ataxia and muscular tremors being the most commonly observed signs. The similarities in the observed locomotor/neurological signs may be a reflection of the objectivity of the various signs within this clinical category. Furthermore, it could be anticipated that these clinical signs should be readily demonstrable to the veterinary surgeon during a clinical examination.

Population distribution

Herd type: The specific incidence, between November 1986 and December 1989, in 44,767 dairy herds in Great Britain with at least one confirmed case of BSE was 10.4%. This compares with the specific incidence of 0.7% in 54,166 suckler herds and thus the probability of BSE occurring in a dairy herd is approximately 15 times greater. Too few cases have occurred in Northern Ireland to perform meaningful statistical analysis to compare with the findings from Great Britain. 28 of the cases in Northern Ireland have occurred in dairy herds, one in a suckler herd and 4 in mixed herds. Approximately twice as many suckler herds are present in Northern Ireland than dairy herds and suggests that the increased probability of BSE occurring in a dairy herd in Northern Ireland may be similar to Great Britain. It has been postulated that the major epidemiological determinant in BSE is scrapie infected ruminant protein in cattle feedingstuffs, giving rise to an extended common source epidemic (Wilesmith et al., 1988). The greater incidence in dairy herds may reflect higher levels of concentrate feeding in this type of herd. Certainly in all cases in Northern Ireland where records existed commercial concentrates were fed. This concurs with previous findings in Great Britain (Wilesmith et al., 1988).

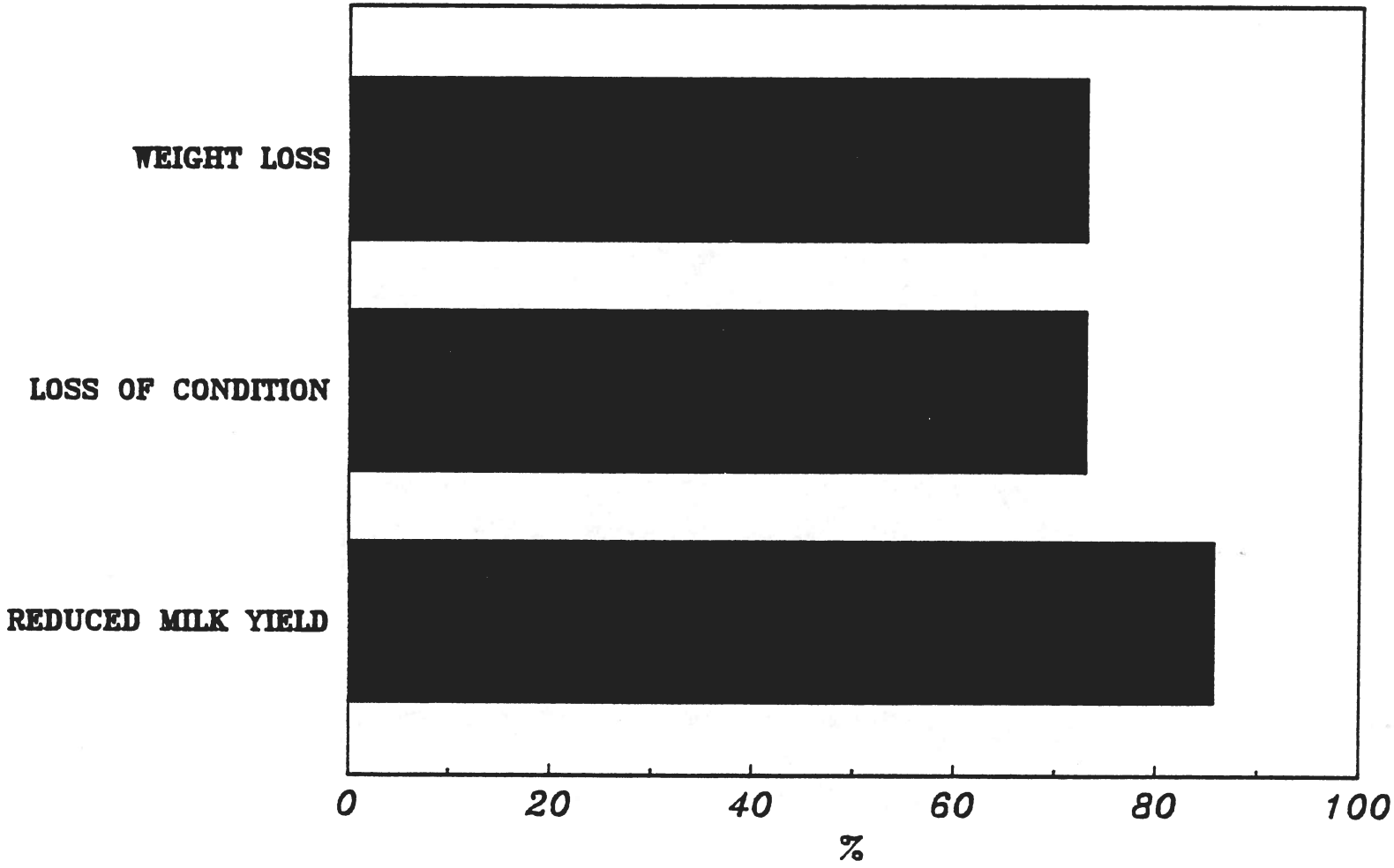

Fig 1. The percentage frequency distribution of general clinical signs in cases in Northern Ireland.

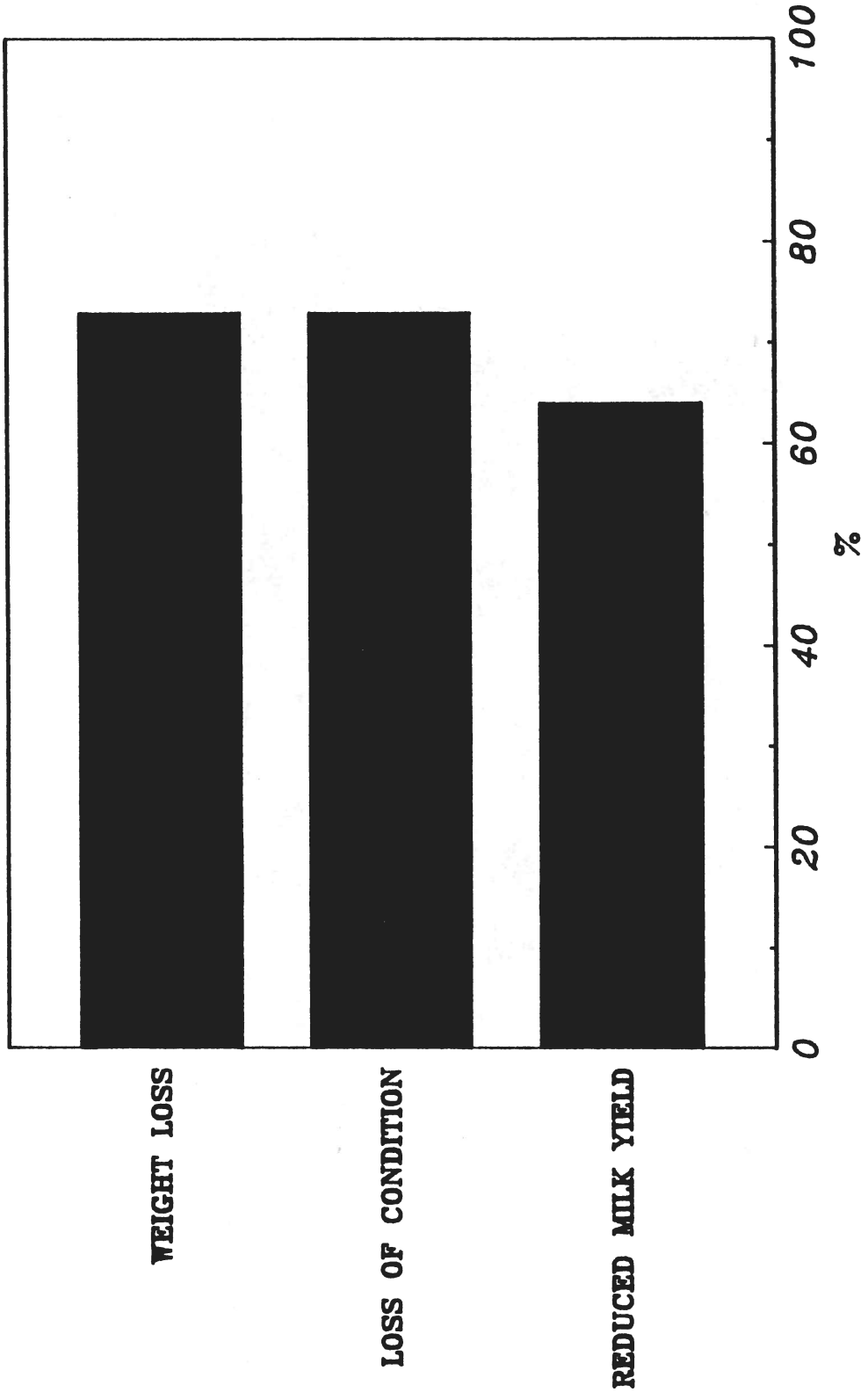

Fig 2. The percentage frequency distribution of general clinical signs in cases in Great Britain.

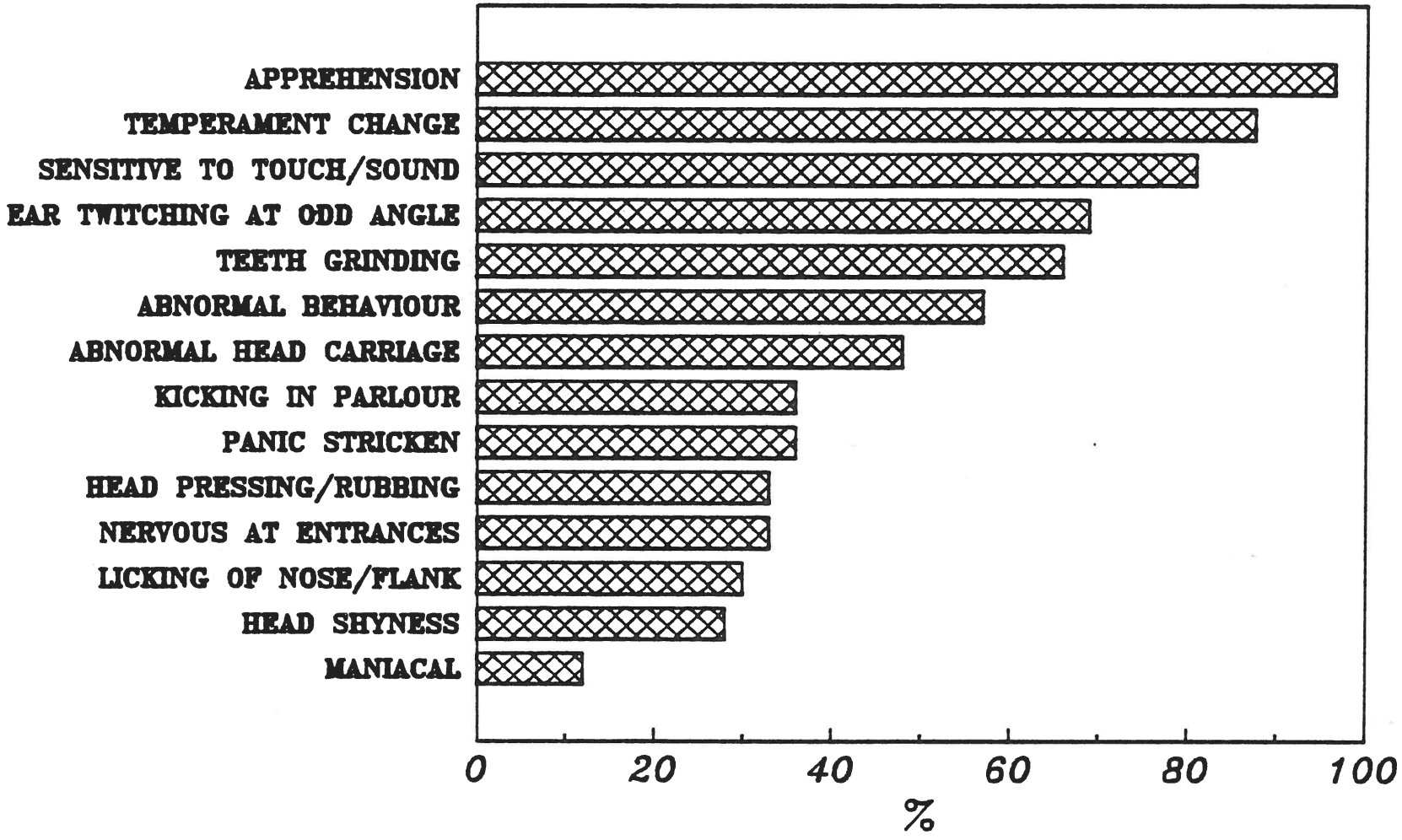

Fig 3. The percentage frequency distribution of behavioural clinical signs in cases in Northern Ireland.

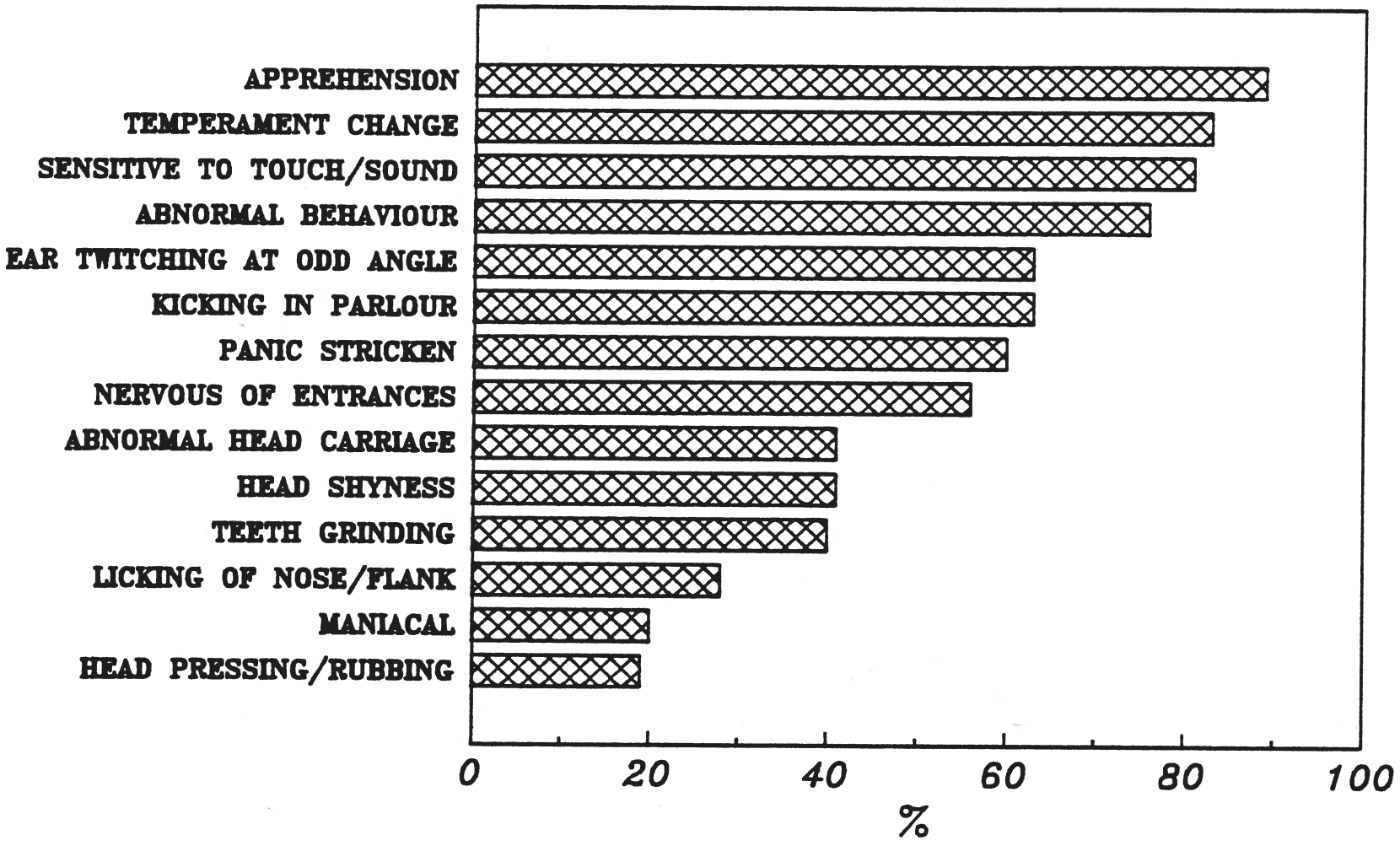

Fig 4. The percentage frequency distribution of behavioural clinical signs in cases in Great Britain.

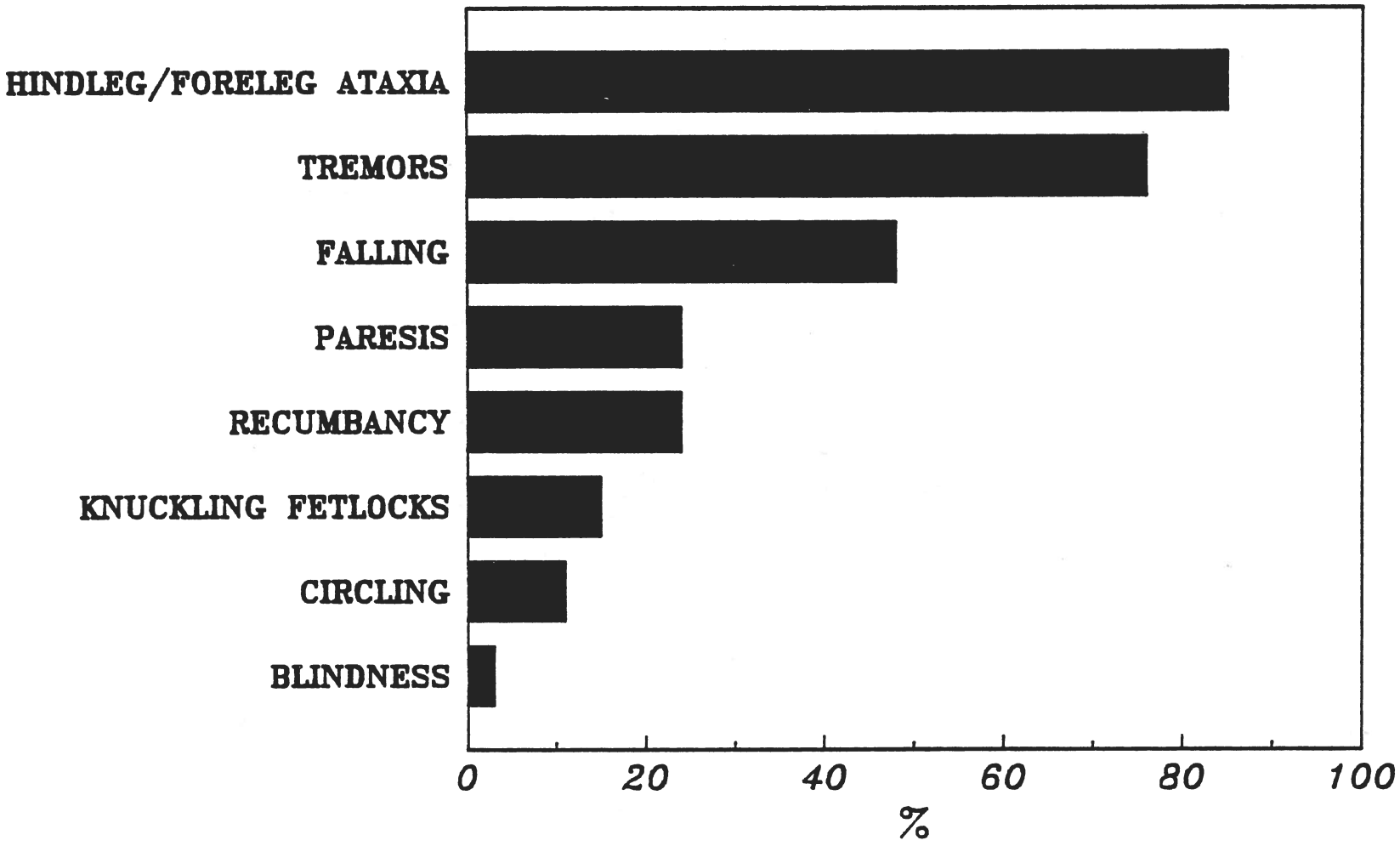

Fig 5. The percentage frequency distribution of locomotor-neurological signs in cases in Northern Ireland.

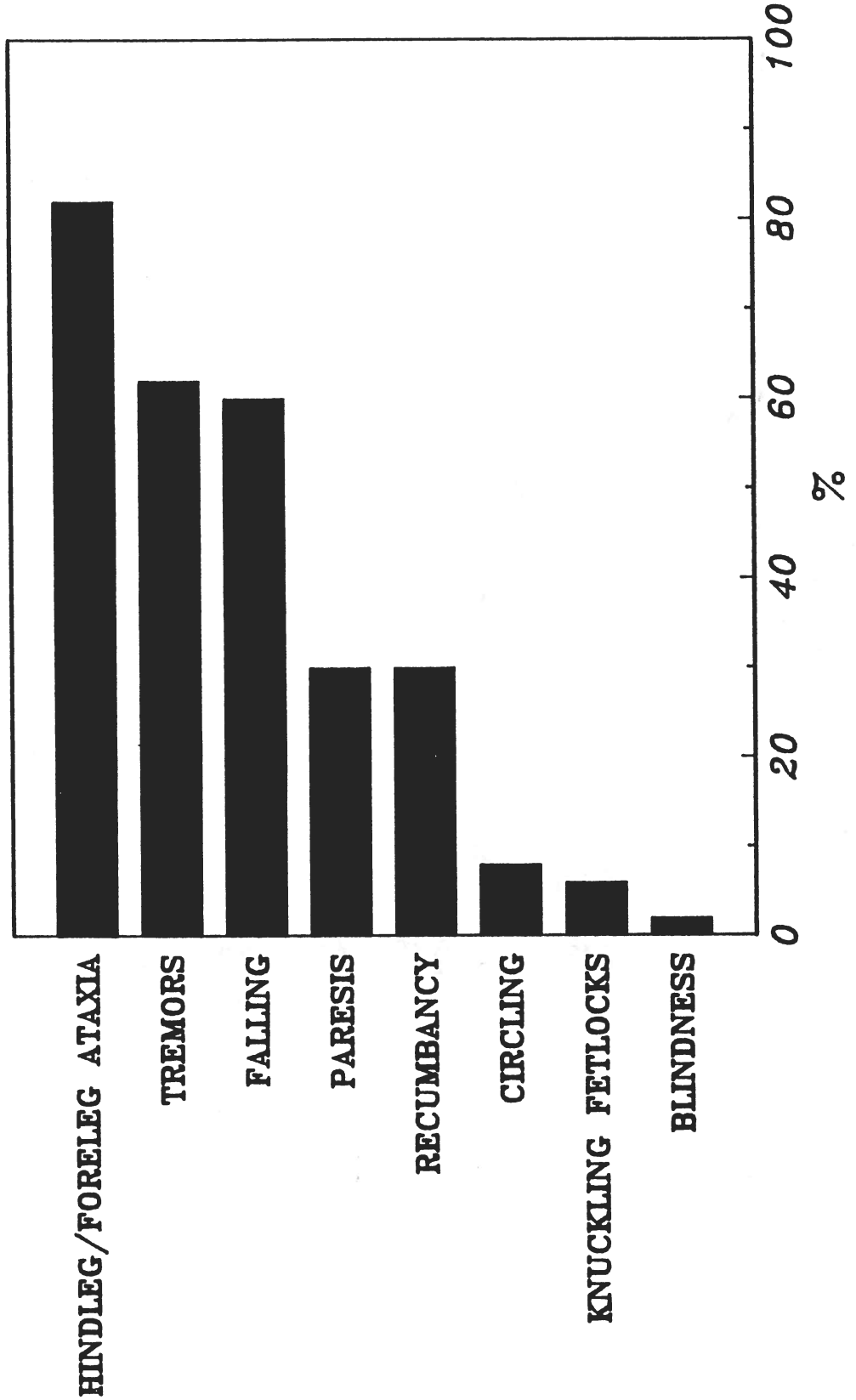

Fig 6. The percentage frequency distribution of locomotor-neurological signs in cases in Great Britain.

Herd size: The frequency distribution in Northern Ireland of dairy herds, with at least one confirmed case of BSE, by herd size is shown in Fig 7. The frequency distribution, by herd size of all dairy herds is also shown. Similar frequency distributions are shown for Great Britain in Fig 8. It is evident from both figures that the probability of BSE occurring in a dairy herd increases with the size of the herd. Thus in Great Britain, 52.2% of all BSE affected dairy herds have more than 99 cows. Such herds correspond to only 18% of all dairy herds in Great Britain. In Northern Ireland, 40% of BSE affected dairy herds have more than 99 cows. Such herds correspond to only 5.1% of all dairy herds in Northern Ireland. Wilesmith et al., (1988) suggest that the positive association between herd size and the risk of occurrence of a case of BSE is likely to be due to an increased probability of purchasing an infected batch of feedingstuffs with increasing herd size.

Breed of animal: Table 1 shows the distribution of confirmed cases of BSE in dairy herds by breed in both Great Britain and Northern Ireland. Statistical analysis using the Chi-squared test indicated that no statistically significant differences exist between the breed distribution in BSE cases and the breed distribution in the total dairy population in both Northern Ireland and Great Britain. These results failed to demonstrate any increased susceptibility to BSE associated with breed type.

Table 1. Distribution of confirmed BSE cases in dairy herds by breed, in Great Britain and Northern Ireland

Breed	% Confirmed Cases in GB (NI)	% distribution of breed in GB (NI)
Friesian	93.4 (97%)	89.7 (98%)
Ayrshire	1.7 (3%)	2.2 (2%)
Channel Isles	2.2	3.4
Others	2.7	4.7

Number of cases per herd: All of the 33 cases in Northern Ireland involve a single animal in each herd. Analysis of data in Great Britain up to December 1989 indicate that 64% of all BSE herds have only one affected animal and a further 18% have 2 affected animals. Whilst a small number of herds have had large numbers of affected animals (15 or more), to date there is no evidence of either lateral transmission within herds or vertical transmission from infected dams. However, as the postulated incubation period in cattle under natural conditions of exposure is 3-5 years, this is to be expected. Evidence for or against lateral and/or vertical transmission should become apparent within 1-2 years in Great Britain.

Age incidence: Fig 9 shows the percentage distribution of BSE cases in Great Britain in 1987 and 1988, by year of birth. Fig 10 shows the corresponding percentage distribution of BSE cases in Northern Ireland, between November 1988 and December 1989, by year of birth. Wilesmith et al., (1988) suggest that in 1982 cattle in Great Britain first became exposed to a transmissible agent in feed. They also suggest that infection is more likely to be acquired in calf hood. Thus, in Great Britain the greatest proportion of clinically affected cases which occurred in 1987 were

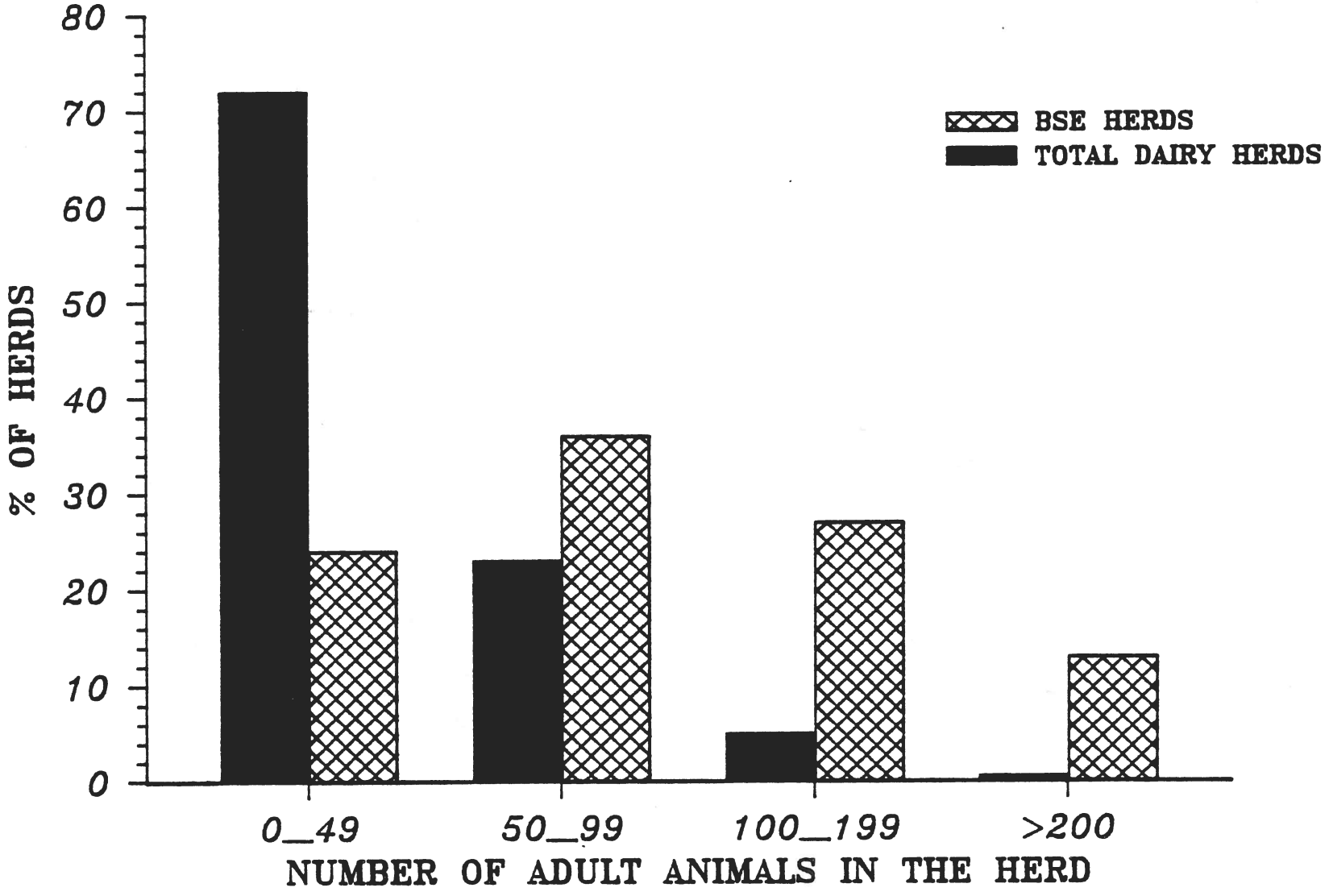

Fig 7. The frequency distribution, by herd size, of BSE dairy herds and all dairy herds in Northern Ireland.

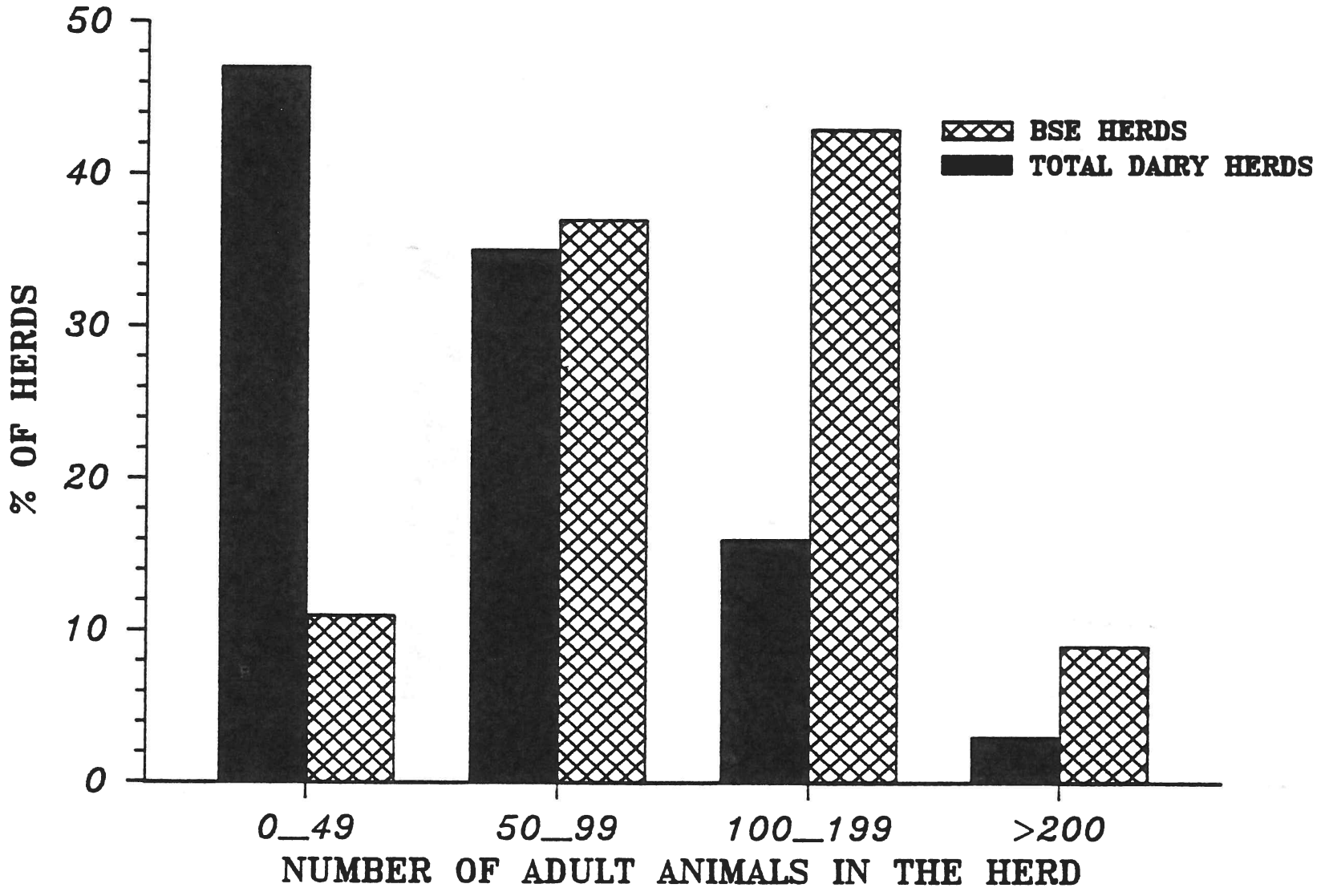

Fig 8. The frequency distribution, by herd size, of BSE dairy herds and all dairy herds in Great Britain.

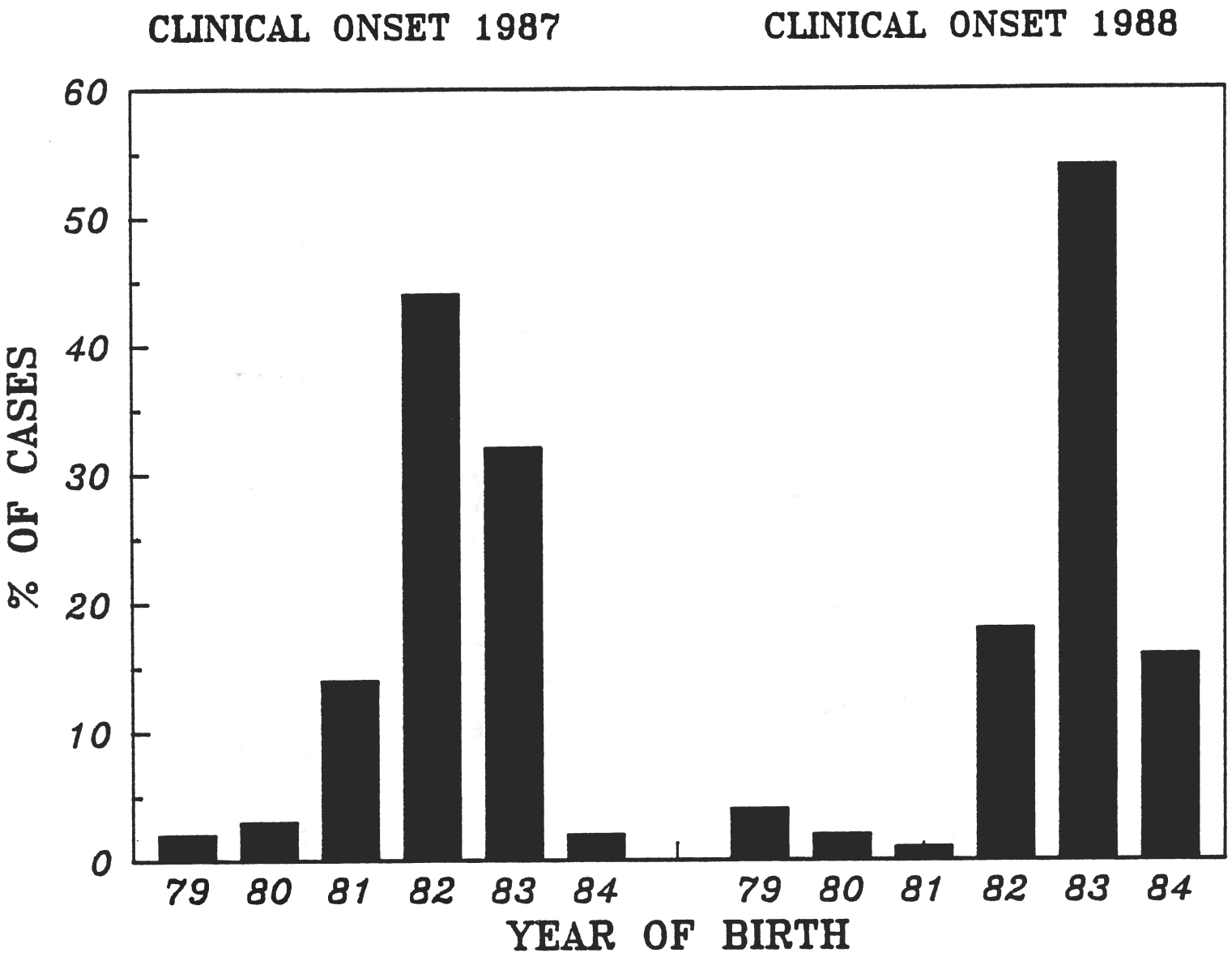

Fig 9. The percentage distribution of BSE cases in Great Britain in 1987 and 1988, by year of birth.

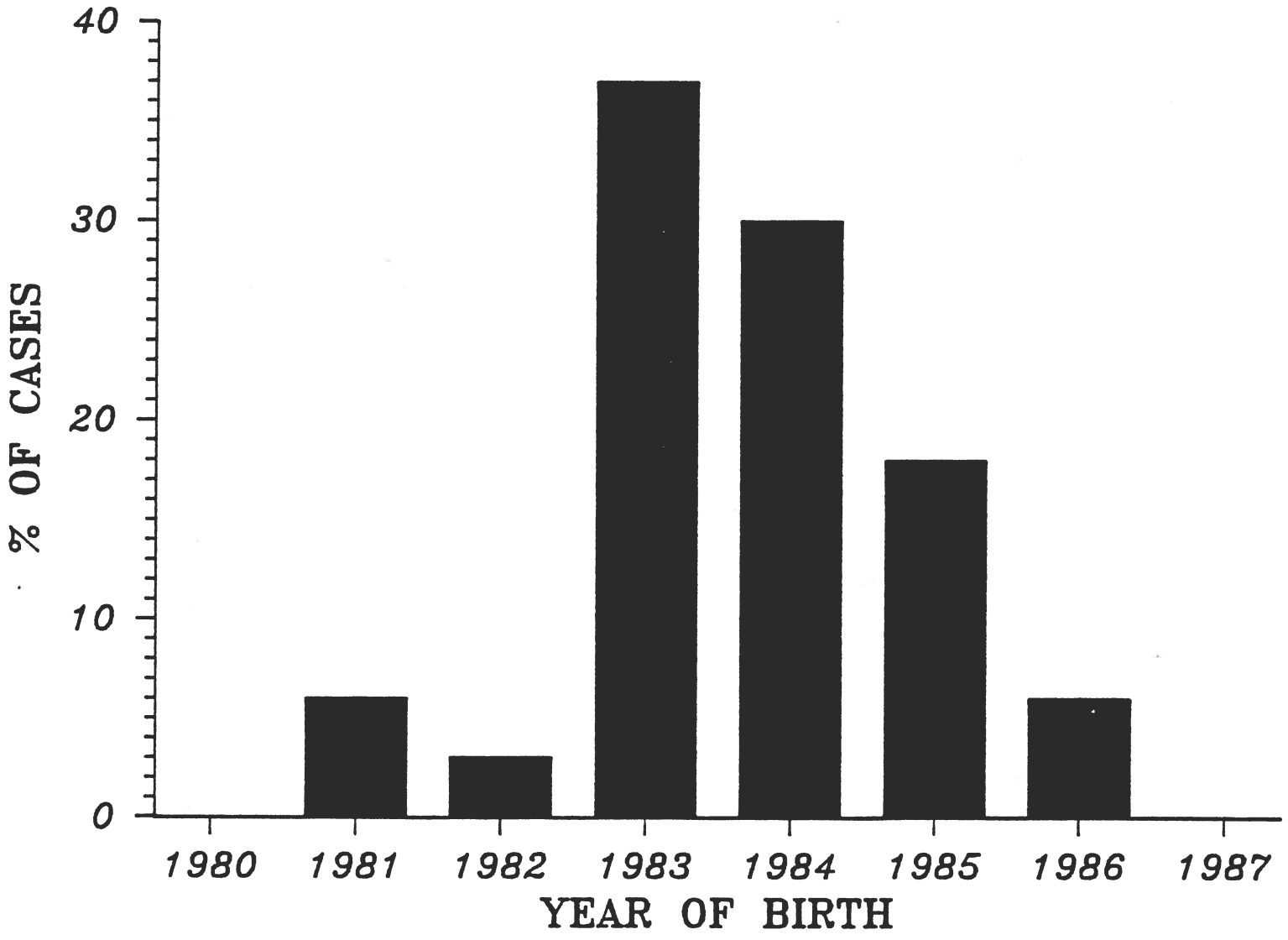

Fig 10. The percentage distribution of BSE cases in Northern Ireland, between November 1988 and December 1989, by year of birth.

calves in 1982, whereas the greatest proportion of clinically affected cases which occurred in 1988 were calves in 1983. Since the greatest proportion of BSE cases in Northern Ireland were calves in 1983 (only 3 cases born prior to 1983) it could be hypothesised that cattle in Northern Ireland first became exposed to the scrapie-like agent in feedingstuffs in 1983.

Prevalence: The monthly prevalence of BSE cases in Great Britain (by month of onset of clinical signs) between April 1985 and October 1989 is given in Fig 11. The first confirmed case was identified in November 1986 and a number of cases recorded per month gradually increased so that in December 1987 100 cases were observed. The first confirmed case in Northern Ireland was in November 1988 and in December 1989 3 were identified with an average of 2.4 cases per month during this period. The adult cattle population in Northern Ireland is approximately one-tenth of the Great Britain population. It could be hypothesised that if cattle in Northern Ireland are exposed to the same degree of risk to the BSE agent as cattle in Great Britain then the incidence of the disease in Northern Ireland should have reached 10 cases per month by December 1989.

CONCLUSIONS

The reasons for the chronological difference between the first confirmed case of BSE in Great Britain in November 1986 and the first confirmed case in Northern Ireland in November 1988 are not known. Wilesmith et al., (1988) suggest that a number of combined factors were significant in the occurrence of BSE in cattle in Great Britain. These include: a dramatic increase in the sheep population in Great Britain which commenced in 1980; a probable increase in the prevalence of scrapie-infected flocks; the greater inclusion of sheep heads in material for rendering; the greater inclusion of casualty and condemned sheep in material for rendering as a result of the reduction in the number of knackers yards; the introduction of continuous rendering processes during the 1970s and 1980s which may have resulted in the rendering of animal material at a lower temperature and, or, a shorter time than previously, and the decline in the practice of using hydrocarbon solvents and terminal heat treatment for fat extraction since the mid-1970s. Many of these factors were common to Northern Ireland over the same time period. However, one major difference is the low prevalence of scrapie in the indigenous sheep population of Northern Ireland. This would result in a reduced exposure to the scrapie agent and in a substantially lower incidence of BSE cases in cattle. This is authenticated by the finding that the observed prevalence of BSE in Northern Ireland is approximately one-third the predicted prevalence, based on comparable observed prevalence levels in Great Britain. Furthermore, a proportion of the BSE cases in Northern Ireland may have been the result of a known importation of meat and bonemeal from Great Britain in early 1983. Notably, this coincided with the apparent time period of first exposure of cattle in Northern Ireland to the scrapie agent, as determined by the age distribution of confirmed BSE cases. The importation of meat and bonemeal from Great Britain between 1983 and the suspension of the inclusion of ruminant-derived animal protein in ruminant feedstuffs in July 1988 (Order, 1988) may be a major aetiological determinant in the epidemiology of BSE in Northern Ireland.

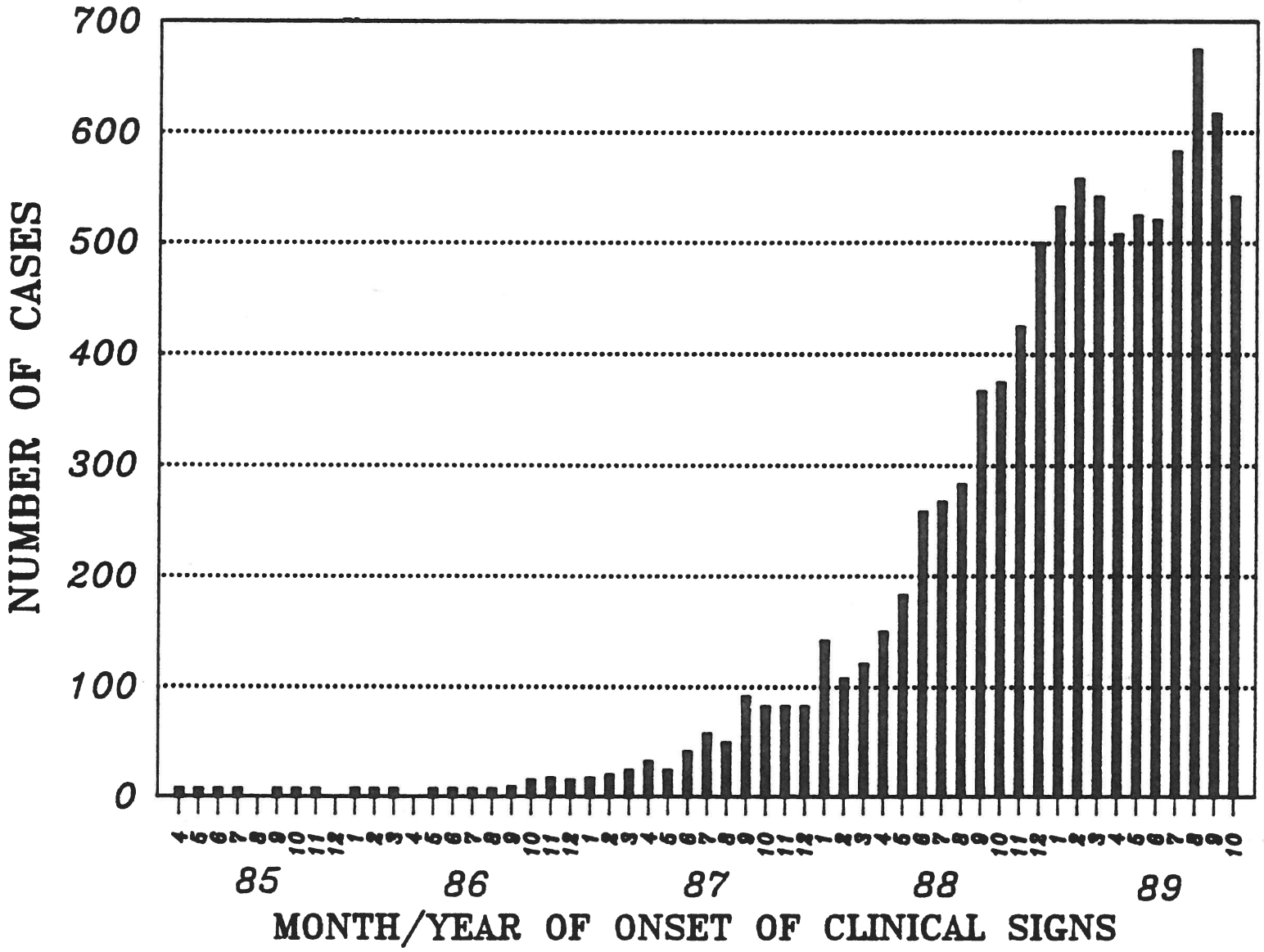

Fig 11. The monthly prevalence of BSE cases in Great Britain (by month of onset of clinical signs between April 1985 and October 1989.

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THE 1988 EUROPEAN SEAL MORBILLIVIRUS EPIZOOTIC

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First evidence of the 1988 European seal epizootic was seen during April 1988 when a large number of dead common seals and aborted seal pups were found on the Danish island of Anholt in the Kattegat. In the following months mortalities were also reported in many other seal colonies in the Kattegat and also in the Danish and Dutch Wadden Sea (Dietz et al., 1989a). By June the disease had spread to seal colonies in the Skagerrak and German Wadden Sea. Seal colonies along the eastern coast of England and the coast of Ireland became infected in early August. Evidence of infection in Orkney seals was also apparent in August but it appears that seals in other regions of Scotland were not affected until September. By the end of 1988, most seal colonies in Europe are believed to have become infected with morbillivirus.

Pollution was initially implicated by the media as a possible cause of the seal epizootic. Later a herpesvirus and a picornavirus were isolated from affected seals and considered as possible etiological agents (Osterhaus, 1988). Subsequently it was shown that the epizootic was unrelated to these viruses but serological examination of convalescent seals indicated rising antibody titres to canine distemper virus (CDV) or a closely related morbillivirus (Osterhaus and Vedder, 1988). We found lesions similar to canine distemper in many affected seals from the Irish Sea confirming CDV or a closely related morbillivirus as the cause of the epizootic (Kennedy et al., 1988a; Kennedy et al., 1989).

MORTALITY

It is estimated that approximately 18,000 common seals died in European waters during the epizootic. There is considerable variation in the reported mortality figures for different geographical locations. Up to 90% mortality has been reported in some individual seal colonies (Dietz et al., 1989a) but overall mortality in the Kattegat and Skagerrak has been estimated at 60%. A similar mortality figure has also been reported for seals along the eastern coast of England but the corresponding figure for Scotland is considerably lower (Harwood, 1989). Mortality in Strangford Lough, home of the largest common seal colony in Ireland, is estimated at 30-40% (R. Brown, personal communication). It is unclear whether such differences in apparent mortality rates are due to factors such as

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differences in "observer effect", the timing of infection in different areas or the presence of secondary infections. The virus reached seal colonies in Scotland later than those in England and at a time when congregations of breeding seals were beginning to disperse thus reducing the opportunities for lateral spread of the virus among seals. Such a difference of timing may have accounted for the apparently lower mortality rate in Scotland. Herpesviruses have been commonly isolated from affected seals in continental Europe (Osterhaus, 1988; Frey et al. 1989) but not from affected seals in N. Ireland (McCullough, unpublished observations). It is possible that such infection has increased the mortality rate in morbillivirus-infected seals. The mortality rate for grey seals (Halichoerus grypus) is considerably lower than for common seals. It is estimated that only a few hundred grey seals died in the North Sea (Harwood, 1989). The reason for this difference is unknown. Lesions of distemper have been found in this species (Kennedy, unpublished observations) indicating that it is susceptible to morbillivirus infection but the reason for the lower mortality is unknown.

DISTEMPER IN PORPOISES

During September-December 1988, 6 common porpoises (Phocoena phocoena) were found stranded on the coast of County Down in N.Ireland. We found lesions similar to canine distemper in all 6 of these animals and isolated a morbillivirus (Kennedy et al., 1988b). This is the only report of distemper in any species of marine mammal other than seals.

DISTEMPER IN LAKE BAIKAL SEALS

It has recently become apparent that several thousand Siberian seals (Phoca siberica) died as a result of morbillivirus infection in Lake Baikal from late 1987 until late 1988 (Grachev et al., 1989; Likhoshway et al., 1989; Osterhaus et al., 1989a). It is not yet known if there is an epizootiological relationship between this outbreak and the European epizootic.

PATHOLOGY OF SEAL DISTEMPER

We have necropsied approximately 140 common and 20 grey seals from the coast of N.Ireland during the period August 1988-February 1989. The main necropsy finding was pneumonia in approximately 75% of seals (Kennedy et al., 1989). Pneumonic lungs were frequently oedematous and failed to collapse. Dark red, sharply demarcated patches of consolidation were seen in all lung lobes. Interlobular and subpleural oedema and emphysema were common findings. Emphysema frequently extended into the mediastinum and subcutis of the neck; such seals had increased buoyancy and drifted with the tide. Congestion of the mucosae of the bronchi and upper respiratory tract were commonly seen.

Microscopic lung lesions were characteristic of bronchointerstitial pneumonia. There was marked congestion and oedema of alveolar walls and filling of alveolar lumina with serofibrinous exudate, leucocytes and macrophages. Proliferation of type II pneumocytes was a characteristic finding. Cytoplasmic acidophilic inclusions were common in bronchial epithelium, intraalveolar macrophages and multinucleated syncytia.

Lesions of non-suppurative demyelinating encephalitis were seen in the brain of many seals. Degeneration and necrosis of neurons in the cerebral cortex were frequent findings. There was marked focal gliosis and perivascular cuffing. Patchy demyelination was also present in many seals. Cytoplasmic and nuclear acidophilic inclusions typical of morbillivirus infection were frequently seen in neurons.

There was marked depletion and necrosis of lymphocytes in spleen and lymph nodes. Cytoplasmic acidophilic inclusions typical of morbillivirus infection were frequently seen in transitional epithelium of the renal pelvis and urinary bladder.

We used an immunoperoxidase technique to demonstrate morbillivirus antigen in tissues of affected seals. Antigen was detected in lung, trachea, brain, spleen, bronchial lymph nodes, pancreatic ductules, gastric mucosa and transitional epithelium of the urinary tract. This distribution of viral antigen is characteristic of canine distemper.

The gross and microscopic lesions in affected seals are identical to canine distemper of dogs and other terrestrial carnivores.

Lesions in affected porpoises from the Irish Sea were essentially similar to those in seals (Kennedy et al., 1988b).

MORBILLIVIRUSES

The Morbillivirus genus is a group of small antigenically related RNA viruses within the family Paramyxoviridae. Prior to the 1988 seal epizootic, this genus comprised measles virus (MV) of man, canine distemper virus (CDV) which affects a wide range of terrestrial carnivores including members of the Canidae, Procyonidae and Mustelidae families, rinderpest virus (RPV) in cattle and other large ruminants, and peste des petits ruminants virus of sheep, goats and other small ruminants. Evidence that the recent seal epizootic was caused by a morbillivirus is 1) the presence of lesions similar to canine distemper in affected seals (Kennedy et al., 1988a; Breuer et al., 1988; Kennedy et al., 1989); 2) the demonstration of morbillivirus antigen in tissues of affected seals by immunoperoxidase (Kennedy et al., 1988a and 1989) and immunofluorescence techniques (Breuer et al., 1988); 3) the isolation of morbillivirus from tissues of affected seals (Cosby et al., 1988; Kennedy et al., 1988a; Liess et al., 1989a); 4) the ability of infected seal sera to neutralise CDV (Osterhaus and Vedder, 1988; Liess et al., 1989b and 1989c) and 5) the experimental reproduction of distemper-like disease by inoculation of tissue homogenates from affected seals into CDV-naive dogs, mink and seals but not in CDV vaccinated animals (Osterhaus et al., 1988: Blixenkrone-Moller et al., 1989; Visser et al., 1989). While confirming the seal virus as a morbillivirus, these findings do not establish its exact relationship to the other known morbilliviruses. On the basis of studies described below, we believe that the seal virus is different from the other known morbilliviruses and have suggested the name phocine distemper virus (PDV) (Cosby et al., 1988). This name has now been proposed by the subcommittee of the International Committee for Taxonomy of Viruses. Morbillivirus infection commonly causes high mortality when introduced into susceptible populations of animals. It appears that the 1988 European seal epizootic resulted from introduction of PDV into an immunologically naive seal population.

Since morbillivirus infection had not been reported in any species of marine mammal prior to 1988, little is known about the epizootiology of these viruses in marine animal species. Knowledge of the exact relationship of PDV to the other morbilliviruses is central to an understanding of the origin and epizootiology of the virus. Several studies have been done in an attempt to determine the exact relationship of PDV to the other morbilliviruses.

CHARACTERISATION OF PHOCINE DISTEMPER VIRUS

Because of the close antigenic similarities among morbilliviruses, it is not possible to identify unequivocally a new morbillivirus such as PDV on the basis of antigenic characteristics alone. Other biochemical properties such as protein, messenger RNA and genome analyses and host range must also be considered. A variety of techniques has been employed to help elucidate the exact nature of PDV.

Immunoprecipitation studies

Cosby et al. (1988) assessed the relationship between PDV, CDV and MV by examining the ability of hyperimmune dog serum, serum from a human patient with subacute sclerosing panencephalitis and serum from an affected seal to immunoprecipitate PDV proteins from radiolabelled lysates. The mobilities of PDV proteins in sodium dodecyl sulphate-polyacrylamide gel electrophoresis indicate that the major virus structural protein (nucleocapsid; N protein) has an apparently higher relative molecular mass (65,000) than the N protein of MV, PPRV and CDV (60,000). It is similar in size to the N protein of many RPV strains. Although always greater than 60,000, the size of the N protein of RPV is not identical in all isolates (Diallo et al., 1987) and therefore the size of the N protein cannot be used for definitive identification of a new morbillivirus.

Rima et al. (1990) immunoprecipitated radiolabelled PDV antigens with monoclonal antibodies against the various MV and CDV proteins. Mixtures of CDV-specific monoclonal antibodies precipitated the nucleocapsid (N) and phosphoprotein (P) antigens strongly but the fusion (F) and haemagglutinin (H) proteins only weakly. This indicates that the respective F and H epitopes of PDV and CDV are probably similar but not identical. These tests confirmed the findings of Cosby et al. (1988) that the N protein of PDV migrates more slowly than those of MV and CDV but at a rate similar to that of RPV. The P protein of PDV migrates faster than that of CDV and at approximately the same rate as the P protein of MV whereas the H protein of CDV migrates more slowly than that of MV and PDV. The L proteins of CDV, PDV and MV migrate at the same rate. The FO protein of PDV appears to be larger than those of CDV and MV, but is only weakly immunoprecipitated. Precipitation of PDV and CDV antigens with infected seal serum and hyperimmune dog serum, respectively, show that the F1 protein of PDV and CDV co-migrate, suggesting that the F2 part of the F protein accounts for the difference in mobility of the total F protein. These results indicate that the apparent molecular weights of the various proteins of PDV, CDV, MV and RPV differ, and those of PDV are distinct from the other morbilliviruses.

Cosby et al. (1988) also examined Vero-cell cultures infected with PDV

and reference strains of MV, CDV and RPV by indirect immunofluorescence using a panel of monoclonal antibodies to CDV, MV and RPV. These studies indicated that PDV is more closely related antigenically to CDV than to either MV or RPV. However, PDV exhibited antigenic differences to the 4 test strains of CDV and also to the Convac strain of CDV used to prepare the monoclonal antibodies. Since all 5 strains of CDV have a high degree of antigenic conservation at the particular epitopes examined, the variation exhibited by PDV may indicate that it should be classified as a distinct virus. Similarly using a panel of monoclonal antibodies to a strain of RPV, there was a spectrum of cross-reactivity with RPV and the other morbilliviruses but differences between the cross-reactivity of CDV with RPV were demonstrated on several epitopes. There do not appear to be any natural isolates of CDV from host species other than dogs with which to compare the results of these studies. However, the degree of antigenic variation from standard strains of CDV isolated from dogs, characterises PDV as a new morbillivirus rather than a new strain of CDV.

Studies on RNA of phocine distemper virus

Hybridisation studies with RNA species extracted from PDV-infected cells indicate further distinctions between PDV and the other morbilliviruses (Rima et al., 1990). The only cross-hybridisations that we have observed in northern blots were 1) a spurious reaction at low stringency only between the F gene of CDV and a gene of PDV which corresponds in size to the N or P gene and 2) between the same RNA species and a RPV N gene cDNA clone. This clone contains a region conserved in morbillivirus genomes and the results do not support a close relationship between PDV and RPV.

The sizes of the viral messenger RNAs of PDV have also been studied. These appear to be similar to those of CDV (Rima, unpublished observations). The difference in the F protein message between CDV and MV has been used as a distinguishing characteristic between these two viruses. Since the size of the F message of PDV and CDV are the same, it appears that these two viruses form a subgroup within the Morbillivirus genus.

The technique of DNA-RNA hybridisation has also been used to examine RNA extracted from spleen of an affected seal (Mahy et al., 1988; Bostock et al., 1990). These workers applied short and long cDNA clones of RPV, PPRV, CDV and MV to seal virus RNA. The short cDNA probes cover regions of low homology between equivalent genes in different morbilliviruses and are specific for the virus from which they were prepared since they do not hybridise to non-homologous virus. Short MV, CDV and RPV probes did not hybridise with PDV indicating that the seal virus is not MV, CDV or RPV. The longer probes to CDV, RPV and PPRV used by these workers contain regions of high homology between equivalent genes of different morbilliviruses and cross-hybridise to non-homologous virus under conditions of moderate stringency. Some cross-hybridisation was obtained using these longer probes under conditions of moderate stringency supporting the findings of Cosby et al. (1988) and Rima et al. (1990) that PDV should be considered as a newly recognised morbillivirus.

HOST RANGE

Distemper-like disease has been induced in CDV-naive mink by experimental inoculation of lung homogenates from infected seals

(Blixenkrone-Moller et al., 1989). Mink protected by vaccination with a live CDV vaccine were not susceptible to PDV-induced disease. Mild distemper-like disease has also been induced in specific-pathogen-free dogs by inoculation of tissue homogenates from affected seals (Osterhaus et al., 1988). These species are also susceptible to CDV infection. There are no reports of experimental inoculation of PDV into other species of terrestrial carnivores.

We do not yet know whether the morbillivirus isolated from Irish Sea porpoises (Kennedy et al., 1988b) is more similar to PDV than to CDV or whether the neutralising antibodies found in US dolphins (Carman, 1989) were induced by infection with PDV or another CDV-like morbillivirus. Evidence of morbillivirus infection in cetaceans was unexpected since they are not members of the Order Carnivora. There are no data to indicate whether cetaceans are susceptible to CDV infection. However it seems reasonable to assume that most species of sea mammal including sealions, walruses, sea otters and whales are susceptible to PDV infection.

Because PDV and RPV are members of the same genus and the opportunity exists for farm animals grazing along the seashore to come into contact with infected seals, we considered that it was important to establish whether the virus could produce disease in domestic animals. We inoculated calves, sheep, pigs and chickens with cultured PDV and also with tissue homogenates from affected seals (McCullough, unpublished observations). Uninoculated sentinel animals were housed in contact with the inoculated animals in order to determine whether lateral spread of infection would occur. Evidence of seroconversion was detected in all inoculated animals but not in the sentinel animals indicating that they had not become infected from the inoculated animals. All animals remained clinically normal throughout the experiment. We found no evidence of virus replication in cells in nasal mucus samples of the calves and sheep or in cells collected by alveolar lavage from the calves. These results indicate that PDV, unlike RPV and PPRV, does not cause disease in these ruminant species.

PORPOISE MORBILLIVIRUS

We have isolated a morbillivirus from tissues of porpoises that had lesions similar to distemper in dogs and seals (Kennedy et al., 1988b). Examination of the porpoise morbillivirus by indirect immunofluorescence using a panel of monoclonal antibodies to CDV indicates that this virus differs antigenically from PDV in epitopes on the N, P, F and H proteins (McCullough, 1990). These results indicate that the porpoise virus is more closely related to CDV and PDV than to any of the other morbilliviruses. Biochemical studies are in progress to determine whether the porpoise virus is identical to PDV or CDV or is yet another newly recognised morbillivirus.

LAKE BAIKAL SEAL MORBILLIVIRUS

Grachev et al. (1989) applied synthetic oligonucleotide primers based on known sequences of CDV and MV RNA to spleen tissue from morbillivirus-infected seals. Primers made to sequences in the N and P genes common to MV and CDV reacted strongly with seal virus RNA while most of the primers made specifically to CDV sequences failed to react. These results suggest that Lake Baikal seals were infected with a morbillivirus closely related but not identical to CDV. On the other hand, recent work has shown that the Lake

Baikal virus is antigenically very similar to CDV (Osterhaus et al., 1989c). On the basis of known antigenic variation amongst CDV isolates, these workers suggest that it may be regarded as a strain of CDV. Further studies are required to determine the exact relationship between the Baikal morbillivirus, PDV and the other morbilliviruses.

SEROEPIZOOTIOLOGY

Several epizootiological studies have now been done in an effort to learn more about the incidence and geographical distribution of morbillivirus infections in marine mammals. Deitz et al. (1989b) found evidence of antibodies in 12 of 40 sera from harp seals (Pagophilus groenlandicus) and 4 of 90 sera from ringed seals (Phoca hispida) collected in Greenland during the years 1985 and 1986. Osterhaus et al. (1989d) reported serological evidence of morbillivirus infection in a small number of common seals admitted to a seal sanctuary in The Netherlands in the years 1984-88. Harwood et al. (1989) did not find evidence of morbillivirus infection in grey seals collected from the coast of Scotland between 1977 and 1987 although 71 of 73 grey seal sera collected in the autumn of 1988 from the Scottish coast had antibodies to CDV or a closely related morbillivirus. These findings indicate that morbillivirus infection has been extant in populations of Greenland harp and ringed seals and European common seals but not in North Sea grey seals for several years in advance of the 1988 common seal epizootic. It is not known if these populations have been infected with PDV, CDV or another morbillivirus. Osterhaus et al. (1989d) compared sera collected from common seals admitted to the Dutch seal sanctuary before 1988 with sera collected during the 1988 epizootic. They used CDV and RPV virus neutralisation assays and CDV and RPV enzyme linked immunosorbent assays (ELISAs). Sera collected in previous years were either (1) negative in all 4 tests or (2) positive in the CDV and RPV ELISAs but negative in CDV and RPV virus neutralisation test. Sera collected during the 1988 epizootic were usually positive in all 4 tests. These results may indicate that there are antigenic differences between the virus circulating in Dutch common seals before 1988 and PDV. Thus, it is possible that European common seals may have been infected with at least 2 morbilliviruses or strains of the same morbillivirus. If 2 strains/viruses exist, it appears that PDV is much more pathogenic in common seals. Although mortality in European grey seals was much lower than in common seals (Harwood, 1989; Dietz et al., 1989a) we have found lesions of distemper in 15 grey seals from the Irish Sea (Kennedy, unpublished observations). We do not yet know whether the virus in these animals is identical to PDV.

Serological evidence of morbillivirus infection has also been found in 2 of 5 sera from seals captured on the east coast of Canada (Bowen and Cornwell, 1989) indicating that such a virus is present in North American seal populations. However, high mortality has not been reported in these populations and it is not known whether this virus is PDV or another strain/virus of lower pathogenicity.

During an investigation of the 1987-88 mass mortality of bottlenose dolphins (Tursiops truncatus) along the central and south Atlantic coast of the United States, antibodies to CDV-like virus were found in 6 of 13 dolphins captured alive off Virginia Beach in October 1987 (Carman, 1989). This result together with the finding of morbillivirus infection in porpoises in the Irish Sea (Kennedy et al., 1988b) indicates that morbillivirus is present in cetaceans on both sides of the Atlantic Ocean. The exact

relationship of the dolphin and porpoise morbilliviruses to PDV and the other morbilliviruses is not yet known.

ORIGIN OF PHOCINE DISTEMPER VIRUS

The origin of PDV is unknown. Many epizootics of canine distemper have occurred in wild and captive canids in arctic and subarctic regions where there is opportunity for contact between such species and seals. Outbreaks of distemper in dogs and foxes in Canada and Greenland occurred in the winter of 1987/88 (Leighton et al., 1988; Dietz et al., 1989a). Since PDV differs antigenically and biochemically from laboratory strains of CDV we believe it is unlikely that European seals became infected from terrestrial canids.

There have been previous incidents of epizootic disease with high mortality in seals in several parts of the world. Many seals died along the New England coast of the United States from 1979 to 1981 as a result of influenza virus infection (Geraci et al., 1982). Herpesvirus infection killed seals in a seal orphanage in The Netherlands in 1984 (Borst et al., 1986). There was a mass die-off of crabeater seals (Lobodon carcinophagus) in Antarctica in 1955; many of these animals had pneumonia and although the cause of death was not determined, the epizootic was believed to have a viral etiology (Laws and Taylor, 1957). Pneumonia was also the main necropsy finding in an outbreak of disease in grey seal pups at St. Kilda (Gallacher and Water, 1964). Since PDV or another morbillivirus cannot be excluded as the primary etiological agent in many of these and other previous epizootics, the 1988 European outbreak may have resulted from infection of a local, naive seal population by a virus already established in seal colonies in other parts of the world.

Since our finding of distemper in porpoises from the Irish Sea (Kennedy et al., 1989b) is the first report of systemic viral disease in any cetacean species, there are no data to indicate the incidence of morbillivirus infection in these species. We do not yet know whether the porpoise virus is identical to PDV or whether porpoises played a role in the transmission of morbillivirus infection to European seals in 1988. Terrestrial mammals infected with morbillivirus excrete large amounts of virus and a similar situation probably pertains to infected porpoises. Furthermore, aerosol transmission of morbillivirus readily occurs in terrestrial mammals and probably also in porpoises. Since these viruses are highly infectious, it is possible that infected porpoises stranded near seal colonies could transit the virus to seals. Since porpoises normally avoid seals and do not spend part of their lives on land, it is more difficult to postulate interspecies transmission in the reverse direction. Regardless of virus origin, cetaceans especially whales and dolphins, migrate long distances and thus may possibly transmit infection to susceptible populations of marine mammals in other parts of the world.

The findings of Osterhaus et al. (1989d) that European seals may have been infected with a morbillivirus more closely related to CDV than to PDV during the years 1984-1988 and that the morbillivirus isolated from Lake Baikal seals is also more closely related to CDV than to PDV (Osterhaus et al. (1989c) indicate that seal populations may be infected with two or more different morbilliviruses or strains of the same virus. It is not known if the highly pathogenic PDV has arisen from a mutated pre-existing non-pathogenic morbillivirus already circulating in European seals.

Establishment of the relationships between PDV, and morbilliviruses from Lake Baikal seals and porpoises from the Irish Sea, requires gene sequencing of the respective viruses. Since all 3 viruses have now been isolated, such studies are feasible. Seroepizootiological studies of wild pinnipeds, cetaceans and other marine mammals are also necessary to determine the geographical extent of morbillivirus infection in these species.

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CHICKEN ANAEMIA AGENT: AN OVERVIEW

M.S. McNULTY*

Chicken anaemia agent (CAA) was first isolated in Japan. Initial characterisation of CAA demonstrated that it resisted treatment with chloroform or ether, and heating at 80°C for 15 mins. In addition, it passed through a membrane filter of 25 nm pore size (Yuasa et al., 1979). These findings indicated that CAA was a small, resistant, unenveloped virus. Consistent with these observations, Goryo et al. (1987a) demonstrated the presence of 19.1 $\pm$ 0.2 nm spherical, virus-like particles, which had a density in CsCl of 1.35 to 1.36 g/ml, in purified preparations of CAA. Thus CAA was within the size range for parvoviruses and possessed some of their physical characteristics, but the density was significantly lower than that described for parvoviruses.

CHARACTERISATION OF CAA

Further attempts to characterise CAA have been hampered by (1) its relatively poor growth in laboratory systems used routinely to propagate the virus, namely MDCC-MSB1 cell cultures and 1-day old, CAA antibody-negative chicks and (2) the fact that it takes between two and three weeks to accurately determine the infectivity of CAA preparations in MSB1 cells. In this laboratory, these problems have been overcome by using SDS-treatment to more efficiently release the virus from infected material, thereby improving the yields of virus from infected MSB1 cells. In addition an ELISA has been developed to detect CAA antigens. This has greatly facilitated the monitoring of purification procedures for the virus, allowing the development of an effective purification scheme based on sonication and SDS treatment of cell lysates, differential centrifugation, sucrose gradient and CsCl gradient centrifugation (Todd et al., 1990a).

Purified preparations contained large numbers of spherical or icosahedral CAA particles, which banded at a density of 1.33 to 1.34 g/ml in CsCl (Todd et al., 1990a). These were 22 nm in diameter when negatively stained with phosphotungstate and measured 26.5 nm when uranyl acetate was used as negative stain (McNulty et al., 1990a). Structural detail of the capsid was better revealed with uranyl acetate than phosphotungstate. The morphology of negatively stained CAA particles viewed down axes of 3- and 5-fold symmetry suggested that the capsid was composed of 32 structural units, consisting of 12 apical pentons and 20 face-centred hexons, arranged as in a class P = 3 icosahedron with a trianglulation number of 3 (McNulty et al., 1990a).

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The virus contained a single-stranded, circular DNA 2.3 kb in size. Only one major polypeptide (M_r 50,000) was identified in purified preparations of CAA (Todd et al., 1990a). This obviously does not account for the entire coding potential of the virus DNA.

Table 1. Comparison of CAA and other small DNA viruses

	CAA	Circovirus[a]	BFD virus[b]	Parvovirus
Size (nm)	22-26	17	14-16	18-26
Density (g/ml in CsCl)	1.33-1.34	1.37	1.378	1.39-1.42
Genome	SS-DNA circular	SS-DNA circular	SS-DNA circular	SS-DNA linear
DNA (kb)	2.3	1.76	1.7-2.0	c.5
Polypeptides (mol wt)	50	36	26.3 23.7 15.9	c.80 c.65 c.60

[a] Tischer et al., 1982
[b] Ritchie et al., 1989

In recent years, two other small DNA viruses with a single-stranded circular DNA genome have been described, namely porcine circovirus which was discovered as a contaminant of the PK(15) cell line (Tischer et al., 1982), and psittacine beak and feather disease (BFD) virus (Ritchie et al., 1989), which causes an unsightly, ultimately fatal disease in psittacine birds. Some of the major characteristics of CAA, circovirus, BFD virus and parvovirus are summarised in Table 1. The former three viruses obviously do not belong to the family Parvoviridae, which contains viruses with much larger, linear DNA genomes. The relationship of CAA, circovirus and BFD virus requires further investigation to determine whether they should be classified together in a new virus family.

DISEASE

Yuasa et al. (1979) first reported that 1-day old chicks inoculated intramuscularly with CAA had by 14 days after inoculation developed severe anaemia, haemorrhages throughout the body, atrophy of the thymus and bursa of Fabricius, yellowish bone marrow and liver changes. Mortality was about 50%. These observations were subsequently confirmed by other workers (Goryo et al., 1985; Bulow et al., 1986; McNulty et al., 1989a). A similar disease, known as blue wing or anaemia dermatitis syndrome, has been seen in the field, both in layer and broiler chicks in many countries including Japan (Goryo et al., 1987b), Sweden (Engstrom, 1988), West Germany (Vielitz and Landgraf, 1988), UK (Chettle et al., 1989) and USA (McNulty et al., 1989a).

By virtue of its effect on the lymphoid system, CAA is thought to be immunosuppressive, but there is only limited evidence to support this (Box et al., 1988; Otaki et al., 1988a,b). Nevertheless, flocks with clinical disease due to CAA often have a high prevalence of secondary bacterial infections.

EPIDEMIOLOGY

The key factor in the occurrence of the disease in the field is primary infection of in-lay breeder flocks. Non-immune breeders transmit the virus vertically to their progeny. Breeder flocks frequently appear to become infected early in lay or around peak egg production (Vielitz and Landgraf 1988; Chettle et al., 1989). This suggests that some birds may be latently infected and that the stress or hormonal changes which occur at the onset of lay cause reactivation of the virus. No clinical signs are seen in the breeders and there is no apparent effect on egg production, hatchability or fertility. However the progeny hatched from eggs laid over a period of 3 to 6 weeks begin to show signs of disease when aged 10-14 days (Vielitz and Landgraf, 1988). It appears that experimental parenteral inoculation of newly hatched chicks simulates the vertical transmission which triggers the field disease.

Vertically infected chicks excrete the virus and infect hatchmates by horizontal spread. It is known from experimental studies that even young chicks infected by contact do not develop clinical disease. Furthermore, an age resistance to experimental disease is rapidly acquired. Virtually all inoculated 1-day old chicks developed anaemia, whereas only two of sixteen chicks inoculated at 7 days of age became anaemic. Chicks inoculated at 2-4 weeks of age showed no disease, but the virus was recovered from their livers (Yuasa et al., 1979). The basis of age resistance is unknown, but it may be related to developing immune competence. Two week-old bursectomised chicks developed anaemia and persistent CAA infections, in contrast to intact chicks, which showed no anaemia, developed antibody and eliminated the virus (Yuasa et al., 1988). Chicks with maternally derived antibody are also resistant to experimental disease, even when inoculated parenterally with CAA at one day of age (Yuasa et al., 1980a; Bulow et al., 1986).

Immunosuppressive viruses such as IBD virus and Marek's disease virus act synergistically with CAA. One day-old chicks dually infected with CAA and immunosuppressive viruses showed higher mortality rates than chickens infected singly with either virus (Bulow et al., 1986). Thus CAA may contribute to apparent Marek's disease vaccine failures in the field (Otaki et al., 1988a,b). In addition, infection with immunosuppressive viruses early in life can overcome the protective effects of both age resistance and maternal antibody (Yuasa et al., 1980b; Bulow et al., 1986). Thus the epidemiology of CAA infection is very complex, and the clinical outcome is dependent on the interplay of several factors including route of infection, age, immune status, and presence of immunosuppressive agents.

Serological studies have indicated that antibody to CAA is widespread in commercial and SPF chickens worldwide (Yuasa et al., 1985; McNulty et al., 1988, 1989a, 1989b). It appears that in the UK most breeder flocks become infected during the growing period (McNulty et al., 1988). It is not clear whether this infection is acquired by lateral spread from a small number of vertically infected chicks or from residual virus in the house, or both. Because the majority of breeder flocks in the UK have antibody to CAA at

point of lay, outbreaks of clinical disease caused by CAA are rare in this country. However in other countries where a significant number of breeder flocks are not infected until they come into lay, clinical outbreaks have caused serious economic losses (Vielitz and Landgraf, 1988).

Although isolates of CAA from different geographical locations may show differences in their reactivity with mouse monoclonal antibodies to chicken anaemia agent (McNulty et al., 1990b), all isolates characterised so far belong to one serotype and one pathotype (Yuasa and Imai 1986; McNulty et al., 1989a, 1990c). So far no naturally occurring non-pathogenic isolates of the virus have been recognised.

Because CAA is present in many SPF flocks and because it is vertically transmitted (Yuasa and Yoshida, 1983), there is a possibility that CAA could contaminate avian vaccines and biologicals. There is therefore a need to eliminate CAA from SPF flocks. Any eradication procedure would be based on a programme of serological testing and culling. However the available serological diagnostic tests, namely serum neutralisation and indirect immunofluorescence are relatively cumbersome and expensive. To overcome these problems we have developed an antibody detecting ELISA for CAA, in which a monoclonal antibody is used to capture CAA antigen on the microtitre plate (Todd et al., 1990b).

Additional knowledge of the epidemiology of CAA infections is required before a logical programme for eliminating CAA from SPF flocks can be devised. The following questions are particularly important. What is the rate of vertical transmission from breeder flocks in which the majority of birds have antibody? Does vertical transmission occur at a higher frequency in such flocks at certain times eg point of lay and peak production? Does the virus establish a latent state and if so what are the triggers for reactivation? It has not been possible to answer these questions by conventional direct techniques such as virus isolation because of the inherent disadvantages of this method for CAA. However we have recently successfully applied the polymerase chain reaction technique to the detection of CAA-specific DNA sequences in chicken tissues (D. Todd, unpublished observations). The sensitivity and specificity of this technique may allow us to successfully investigate some of the above gaps in our knowledge.

SIGNIFICANCE

The economic significance of clinical outbreaks of disease following vertical transmission of CAA is well recognised, but has not been accurately quantified. These economic losses can be minimised by ensuring that breeder flocks have been exposed to CAA before they commence laying.

In a recent survey of clinically normal broiler flocks, the performance of broiler flocks with no antibody to CAA at slaughter and those in which 60% or more birds had antibody has been compared, using a computerised system for broiler production data (McIlroy et al., 1988). All broiler flocks were the progeny of breeder flocks with a high prevalence of antibody to CAA ie immune breeder flocks. Thus the purpose of the survey was to measure the effects of subclinical CAA infection. Serological testing was carried out by ELISA and the serological and production data were analysed as described by McIlroy et al. (1989). The results showed that flocks without antibody to CAA had a 15% ($p < 0.05$) higher net income per 1,000

birds and were 3.6% heavier (kg/bird) ($p < 0.05$) than flocks with a high prevalence of antibody. There was no effect on mortality, underlining the subclinical nature of the infection, or on feed conversion (M.S. McNulty, S.G. McIlroy, D. Bruce and D. Todd, unpublished observations). These results show that, contrary to what was formerly believed, subclinical CAA infection in broiler flocks derived from immune parent flocks has a very substantial, statistically significant economic impact. This finding has obvious implications for vaccination strategies designed to minimise the losses caused by CAA.

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OPEN SESSION

DATA REQUIREMENTS AND OBJECTIVES FOR ECONOMIC ANALYSES OF DISEASES IN FARM LIVESTOCK

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With production systems becoming more intensive, animal health has increasingly become a primary determinant of productivity of farm livestock. This has led to an increasing demand for control programmes and research into animal disease. However, the current tendency towards public expenditure restraint means that resources are becoming more scarce and hence there is an even greater need to take decisions which establish relative priorities. The need to base such decisions on sound economic criteria has consequently become more urgent (BVA, 1983, MAFF and AFRC, 1987).

Decisions about disease **control**, at the herd level as well at the national level, logically involve:
a) choice of disease(s) to be controlled;
b) for each disease, choice of control programme to be implemented.
A cost/benefit analysis of a control programme has often been used as the "economic" criterion in the decision process, i.e. if it has been estimated that benefits of the control programme are larger than its costs, and hence the benefit/cost ratio is greater than 1, it has been "economically" justified to implement the programme. However, cost/benefit analysis is not an adequate basis for the decision. To base decisions on sound economic criteria it is **the relationship between control expenditures and output losses** for a disease which must be established. In a cost/benefit analysis of a control programme only one combination of control expenditures and output losses is represented and hence only a single point estimate is calculated.

Decisions about **research** into animal disease in the public sector as well as in the private sector are generally concerned with allocation of available resources (capital, manpower etc.) to research on particular diseases. Estimates of the annual cost of diseases have been used to support these decisions, with the inference that the larger the annual cost due to a disease the greater the justification for more research (Howe et al., 1989). However, an estimate of the annual cost of a disease does not provide the appropriate information to base decisions on economic criteria. In this instance it is the information about **the expected benefits from additional research expenditures** that is required for the decision process.

This paper aims to identify the classes of data required to base decisions about animal disease control and research into animal disease on appropriate economic criteria. The availability of these data in the United Kingdom is explored. Some examples illustrate the extent to which data problems

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constrain further progress in the economic analysis of disease in farm livestock.

THE GENERAL ECONOMIC BASIS FOR DECISIONS

Resources are always scarce and hence choices have to be made from the several options available for allocating the available resources. Commonly, the aim is to allocate resources in such a way that the highest returns are obtained. The economic criterion for the decision to which option and how much resources should be allocated is **marginal returns**, i.e. additional returns to an increment in input. It implies that the returns to additional input should at least equal its costs, otherwise it is not economically worthwhile to increase the level of input. If the costs of additional input equals exactly the returns from additional output, the level of input is optimal (i.e. equimarginal principle).

Each control programme for a disease is a production process which uses particular inputs to produce a particular output. The inputs are control procedures, e.g. medicines, vaccines and labour,and the output is a reduction in the losses caused by disease. So each control programme for a disease is a resource-using process and therefore represents an option to allocate resources. The same principle applies to research into a disease. It uses capital, manpower etc. as input to achieve a reduction in the losses due to disease by, among other things, developing and improving control procedures. To take rational decisions about disease control and research on economic criteria, information about incremental returns to additional inputs (marginal returns) has to be available.

THE ECONOMIC FRAMEWORK FOR DECISIONS ABOUT DISEASE CONTROL

The economic costs of a disease exists out of two components, i.e. output losses (L) and control expenditures (E). Reduction of the production level and decrease of the quality of products are some typical elements of output losses. Costs of treatment, vaccination, hygiene procedures and extra labour are some examples of control expenditures.

Disease control activities aim to reduce output losses due to diseases as much as possible with the available resources. Therefore it is required to establish the **relationship between control expenditures and output losses** for each disease. For most diseases there are several options for controlling the disease involving varying levels of control expenditures and output losses.

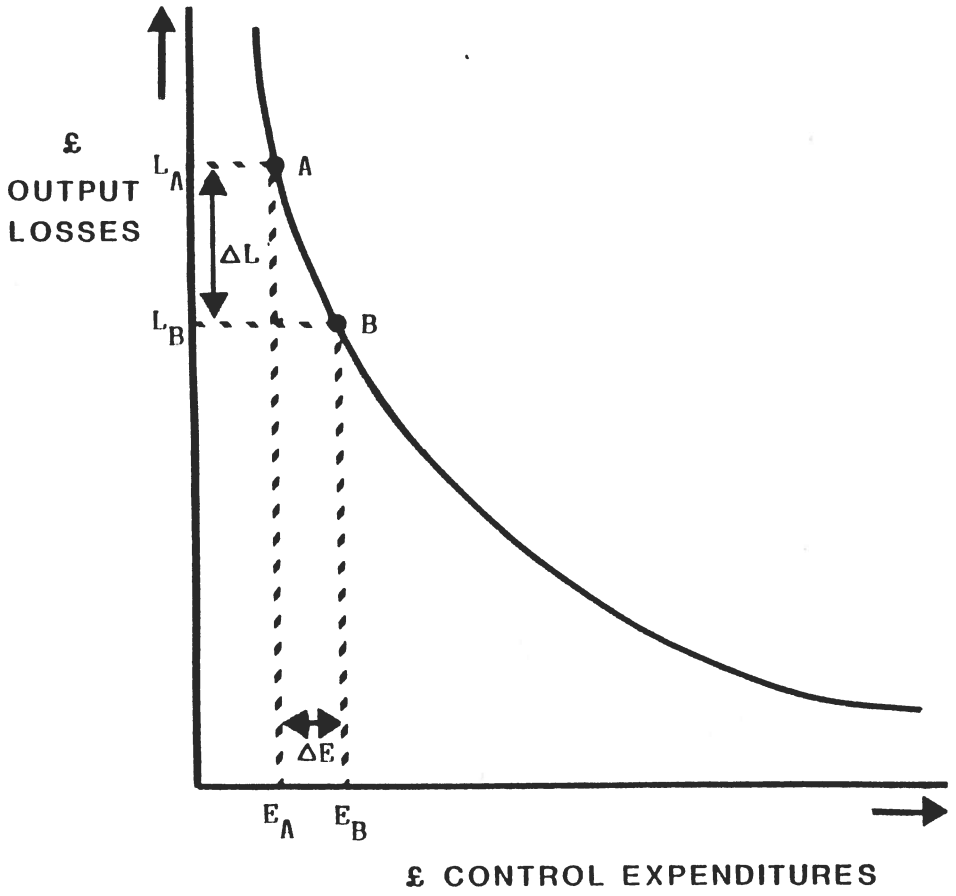

Fig. 1 The general relationship between output losses and control expenditures

Figure 1 shows the general relationship between output losses and control expenditures that is expected for most diseases*. Change from control programme A to B involves an increase in control expenditures of $E_B - E_A$ (ΔE) and a decrease in output losses of $L_A - L_B$ (ΔL). Because ΔL is greater than ΔE it is economically worthwhile at least to increase the level of control expenditures from E_A to E_B. The curve reflects that it gets increasingly more expensive to achieve successive incremental reductions in output losses; and hence returns to additional control expenditures diminish. Since both axes of the diagram have identical units it follows by simple mathematics that point C represents the economically optimal control programme (see Fig. 2). At this point the total losses T_C, i.e. sum of control expenditures (E_C) and output losses (L_C), are the lowest attainable. To the left of point C £1 of control expenditures reduces output losses by more than £1, to the right £1 on control achieves less than £1 reduction in output losses (see also McInerney and Turner, 1989). So the relationship provides information about the returns to an increment in control expenditures and the optimal level of control expenditures.

The shape of the curve representing the relationship between control expenditures and output losses may differ in detail for some diseases, although it has not yet been possible to confirm this by empirical work.

*Empirical research by the author has shown that this relationship exists for mastitis.

However, the general framework remains valid, i.e. the relationship between output losses and control expenditures, the criterion of marginal returns and optimal control policy.

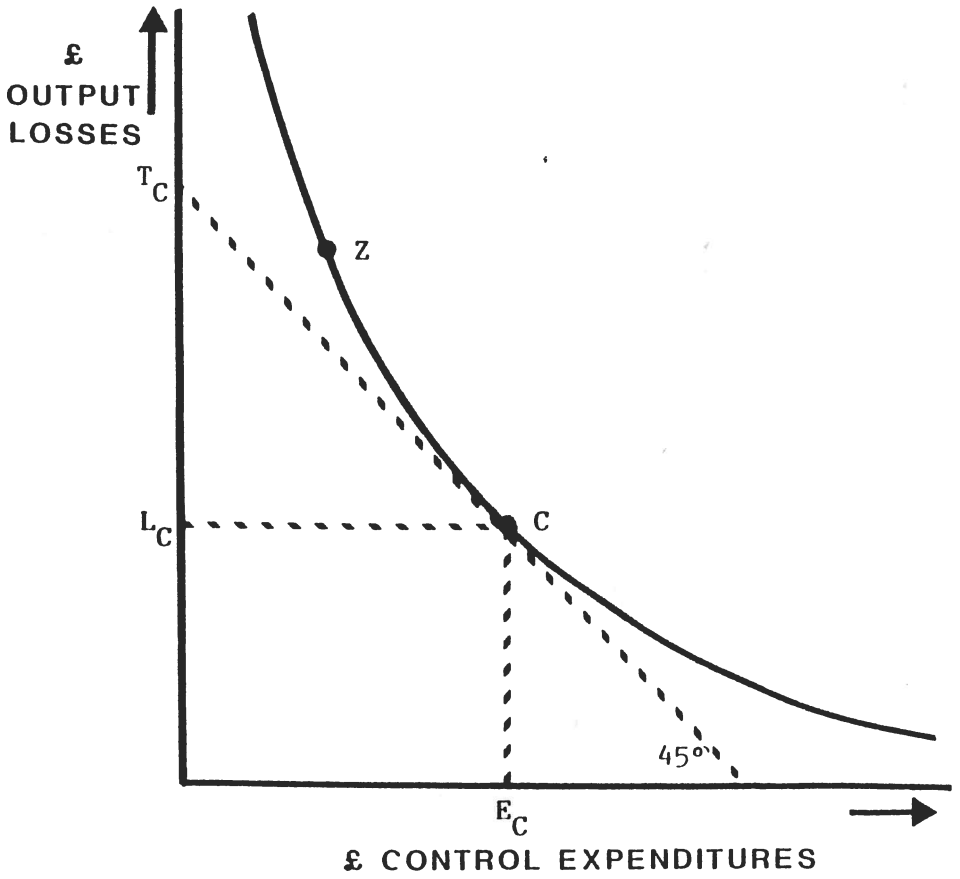

Fig. 2 Defining the economically optimal control program

A cost/benefit analysis of a control programme, which has often been used as the "economic" criterion in the decision process, represents only one combination of control expenditures and output losses. For example, point Z in Fig. 2 represents a control programme with a benefit/cost ratio > 1, i.e. the reduction in output losses exceeds the control expenditures incurred. However, it is only a single point estimate and does not tell anything about the relationship between control expenditures and output losses for the disease. In addition, a programme which satisfies the benefit/cost ratio criterion certainly does not have to represent the economically optimal control programme to adapt.

The establishment of the relationship provides the full information required to take decisions on an economic basis. It can imply that it is economically more justified, i.e. that higher returns are obtained, to allocate available resources to controlling several minor diseases than to controlling a few major diseases and, of course, vice versa.

CLASSES OF DATA REQUIRED

The classes of data required to establish the relationship between output losses and control expenditures are listed in Table 1.

Table 1. Classes of data required to establish the relationship between output losses and control expenditures

Fundamental knowledge of disease
Occurrence of disease
Effects of disease on production process
Effects of disease outside production process
Identification of control options
Benefits of control programmes
Costs of control programmes

A major condition before economic data collection can be started is that, first of all, a fundamental knowledge of the disease has been built up, e.g. cause of disease and type of animals affected have to be known. Basic veterinary research is mainly concerned with the gathering of this sort of information. As long as essential **technical aspects** of a disease are not fully understood it is impossible to know where and which data need to be collected as a basis for economic analysis. Sometimes decisions have to be taken even before a sufficient understanding of a disease has been obtained, e.g. Bovine Spongiform Encephalopathy, however this type of situation is exceptional. Most diseases are present for some time before decisions on control programmes are taken and hence it is possible to gather information about technical aspects of a disease.

Data about **occurrence** of disease, especially under different control programmes, are indispensable for an economic analysis. Occurrence of disease is a major factor that influences the magnitude of economic output losses due to disease. Economic analyses usually cover a particular period of time, e.g. a year and hence information about occurrence of disease during the relevant time period is required. Incidence data provide this information. Point prevalence values are commonly easier to collect than incidence rates and have been often used in economic analyses. However, these only provide information about disease occurrence at a specific point in time and hence can only be useful for an economic analysis if information about duration of disease is also available.

The **production process**, i.e. technical transformation of resources into products by the animal, can be affected in a number of different ways by a disease. The level of **input** used can decrease or increase, e.g. reduced feed intake or increased labour input. Commonly, a disease reduces the capability of an animal to be productive, either completely (e.g. by death) or only partly (e.g. by a parasite infection). The length of time period for which the level of **output** is decreased can vary from a temporary to permanent reduction. Another aspect is the **quality of products**. Some diseases reduce quality of output and hence cause extra losses.

There are also possible **effects outside the production process** which may affect other populations and are of immediate concern. Production of other livestock may be affected by disease, and wildlife can be affected also (e.g. the effect of tuberculosis on badgers). Processing industries may have to take action because of presence of the disease. Human health can be threatened when disease is transmissible to people, i.e. is a zoonosis. Any or all of these elements may have to be included in an economic analysis depending on the unit of interest, e.g. herd or national level.

For most diseases, there is not a single method available for control. Different techniques are available and the frequency or the length of time period of application are generally variable. These **options, and their costs and benefits**, need to be identified to establish the relationship between control expenditures and output losses still occurring. Some costs, for example material costs, are not hard to estimate but others may be more difficult to include, e.g. labour input or disruption of trade. Benefits of control programmes arise from a decrease in output losses due to disease. Reduction in output losses can be obtained in two ways:
- reducing the presence of disease;
- reducing the effects of disease.

Control programmes often consist of two elements: treatment and prevention. Treatment aims mainly at reducing effects of disease by making disease less severe and accelerating recovery. Prevention procedures usually aim at reducing disease occurrence. Reductions in incidence are generally more easily perceived than decrease in output losses per case. The latter may be overlooked therefore and real benefits may be underestimated.

These general classes of data are required to establish the relationship between control expenditures and output losses which is necessary to take rational decisions about disease control on an economic basis.

THE AVAILABILITY OF THE DATA REQUIRED

Much basic research has been done to disentangle the technical aspects of diseases,and results have been published in the literature. For many diseases a comprehensive knowledge has been established which has led to the introduction of technically effective control programmes. Although for some diseases understanding of essential technical aspects may still be lacking because of their complexity or their recent existence, in general data concerning technical aspects are available for most diseases for which control programmes are considered.

A distinction between diseases controlled at national level and those controlled at herd level has to be made in considering the availability of incidence data. Incidences of diseases controlled at national level (notifiable diseases) are systematically reported and published in the annual Animal Health Reports (MAFF, 1988a). However, this concerns only a small number of diseases and is only related to the single control programme implemented. The situation concerning incidence data for diseases controlled at herd level is in complete contrast. At present there is no national data collection system to provide information about incidence of such diseases in the United Kingdom. However, there are some miscellaneous sources available. Data are collected by the Health Schemes, the Veterinary Investigation Centres, VIDA II (Kirby, 1989) and in the abattoirs; however they concern a small group of diseases and can only give an indication of the average national situation. As far as is known, the National Mastitis Surveys of 1977 and 1981-1983 (Wilson et al., 1982, Wilesmith et al., 1986) are the only recent surveys on a national level which collected data both about incidence and applied control programmes,and hence can provide information about their relationship. Overall, the conclusion is that availability of data about incidence is poor in the United Kingdom, especially for diseases controlled at herd level.

In the literature, experiments have been described which aim to estimate the effects of diseases on the production process (Gavora et al., 1980, Hawkins, 1979, Morris 1973 and Muirhead, 1983). Commonly, this involves only

direct effects on the level of output while results are dependent on specific conditions of the experiments which therefore are often rendered inconclusive. Effects on the quality of output are usually not taken into account, nor are the effects on inputs used, nor outside the production process. So data about effects on and outside production process are typically scarce and those which are available have to be interpreted with care.

With regard to the availability of data about control options for notifiable diseases and the costs and benefits involved, commonly only one of the available control options is applied for a notifiable disease and hence data available relate only to this specific situation. Costs of current control programmes are monitored in MINIM, a management information system to support developing process of Ministry of Agriculture, Fisheries and Food (MAFF, 1988b). Benefits of current control programmes are hard to estimate because no information is available about what the disease situation would have been in the absence of a control programme. Information about costs and benefits of other options are not available and can only be obtained from those other countries where these options are applied. However, conditions will probably differ from those in the United Kingdom and so simulations will be necessary to estimate costs and benefits of these control programmes should they be implemented in the United Kingdom.

For diseases with no compulsory control programme a wide variety of control procedures is applied in the herds. Data about costs and benefits involved in these options are only available from published projects. These usually cover only a few of the options and the results are often inconclusive through variation. Although much of the information required is available on farms it is not collected at present and therefore is unavailable for economic analyses.

EXAMPLES

Three diseases are used to illustrate specific instances of data problems.

Aujeszky's Disease

The UK eradication programme for Aujeszky's Disease implemented in 1983 is well known and has been widely discussed, not least because the costs of the programme turned out to be much higher than originally estimated. Before implementation of the programme it was estimated that 200 to 300 herds would have to be slaughtered and the programme would cost at least £6 million (Anon, 1985). It became very clear soon after the start that this had been a gross underestimate. By the end of 1987 513 herds and more than 430,000 pigs had been slaughtered under the eradication programme (MAFF, 1988a) and the costs were significantly higher than expected.

There are two points to make about Aujeszky's Disease in the context of this paper. First, much discussion has been held about the explanation for the unexpected rise in the costs of the control programme. It can be assumed that it has been generally accepted that lack of accurate data about the number of animals and herds infected was a major reason for the large increase in costs. Secondly, as far as is known, before the start of the programme an attempt was made to estimate only the costs of the programme. No attempt was made to estimate the benefits or to analyse the other options available, e.g. vaccination, so that a decision could be supported by a full economic analysis. Done (1987) tried to make an analysis of the costs and benefits of the implemented slaughter policy and a hypothetical elimination programme.

The results of the analyses were very sensitive to the assumptions concerning the benefits, i.e. the situation of Aujeskzy's Disease without the interference of a control programme.

Sheep Scab

In 1988 a review of the options available for controlling sheep scab was made. A Working Party of MAFF analysed the expected costs and benefits of six different possible control strategies. In principle, this makes it possible to establish the relationship between varying levels of control expenditures and related output losses and to determine the economically optimal control programme. However, the analysis was severely hindered by lack of data, especially data concerning residual incidence of sheep scab under the different control policies. Therefore the results had to be presented under "best" and "worst" assumptions. For example, the total losses (i.e. the sum of output losses and control expenditures) of a control programme varied from £93 million for the "best" situation to £224 million for the "worst" situation (MAFF, 1988c). The great variation in results associated with lack of data limited the value of the information becoming available from the economic analysis.

Bovine Virus Diarrhoea/Mucosal Disease

Bovine Virus Diarrhoea/Mucosal Disease (BVD/MD) is one of the diseases which has received much attention in recent years. It was the subject of a CEC-seminar in 1985 (Harkness, 1987) and of a SVEPM workshop in 1987 (Thrusfield, 1987). Much research has been done and is still being done to understand the complex mechanism of the disease. Analyses of possible control strategies for BVD/MD have been made (Spedding et al., 1987, Bennet & Done 1986) and the results are summarised in Table 2.

Table 2. Summary of results of cost/benefit analyses (£ million) of control programmes for BVD/MD (+ = net benefit, - = net cost)

Author	Spedding et al., (1987)	Bennet & Done (1986)
Do nothing	- 120	- 47
Test and eliminate	- 557[a]	- 8[a]
Vaccination	+ 8	- 37

[a]On a 5-year basis, the others on a 1-year basis.

The variation in results of these analyses was mainly associated with differences in data for (a) the population with MD, (b) the number of animals found to be virus - positive and (c) the efficiency of a test - eliminate programme to obtain a disease free herd. This demonstrates very clearly the effect of a lack of accurate data on the results of an economic analysis and how this may lead to wrong decisions. There is no point in making an economic analysis as long as necessary and reliable data are not available. At present several essential data are unavailable, e.g. technical aspects of the disease complex, effects of disease on and outside the production processes and the effects of control procedures, and so an economic analysis for BVD/MD is severely hampered.

SOME ASPECTS OF THE ECONOMIC FRAMEWORK FOR DECISIONS ABOUT RESEARCH

First of all, it is important to distinguish between two types of research, namely basic and applied research. Basic research, sometimes called pure or interest-oriented research, covers research that aims to develop a fundamental knowledge of a disease, e.g. cause, mechanism of transmission and effects caused by disease. This knowlege is required before applied research can be done. The benefits of basic research are hard to identify and even harder to value. Therefore it is very difficult to base decisions about basic research on conventional economic criteria, and this has to be done more as an "act of faith", although it is recognised that someone still has to decide about what resources to allocate to this type of research. Basic research is not considered further in this paper. Applied research, also called task-oriented research, includes the development of new control procedures and the improvement of existing control programmes. Hence benefits of this type of research are less hard to identify and to estimate, in principle at least. Benefits arise from the reduction in total losses due to disease to be achieved by implementation of new or improved control programmes. It is important to notice that it is the reduction in total losses due to disease which needs to be considered and not just the reduction in output losses. The latter is often only taken into account but generally a newly developed control procedure does not only cause a further reduction in output losses, but also involves an increase in control expenditures.

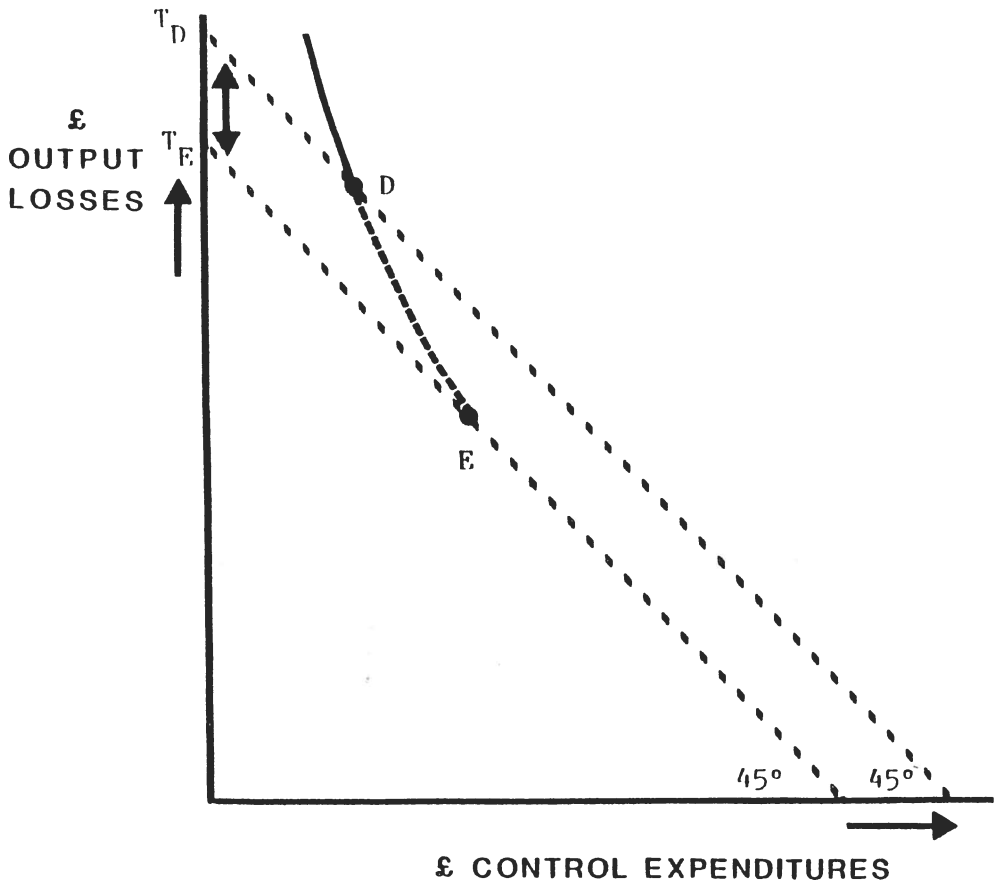

Fig. 3 Reducing the total losses by development of a new technique

The effects of applied research on the relationship between control expenditures and output losses due to a disease are shown in Figs. 3 and 4. There are several types of effects to be distinguished. First, research can lead to development of a new **technique** which extends the options available for control (see Fig. 3, movement from D to E). The newly developed control programme E has lower total losses (T_E) than the existing control programme D (T_D). The reduction in total losses ($T_D - T_E$) represents the potential benefits of research. Secondly, the efficiency of existing **technology** (i.e. collection of techniques to control a disease) can be increased by improving existing control procedures (e.g. improving the efficiency of dry period therapy for mastitis). This moves the curve inward, i.e. for the same level of control expenditures the level of output losses has decreased (see Fig. 4, movement from F to G). The reduction in total losses ($T_F - T_G$) represents the potential benefits of research.

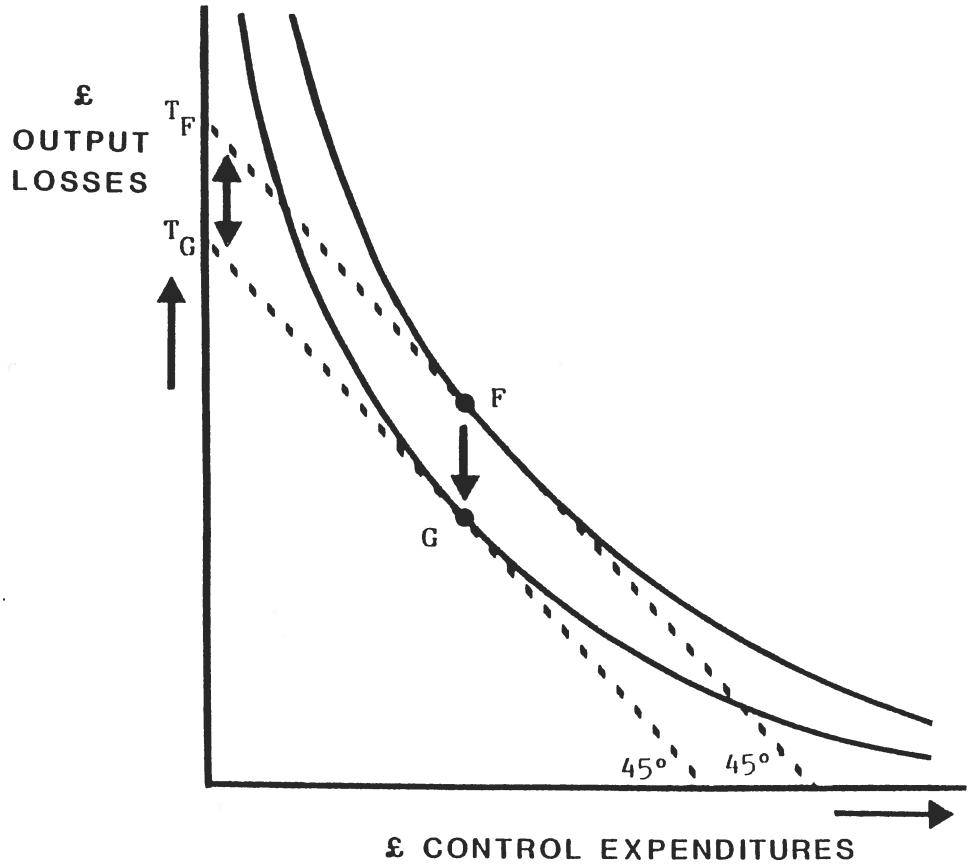

Fig. 4 Reducing the total losses by improving the efficiency of existing technology

One factor that influences the benefits of research is the degree of acceptance of the developed control programme by livestock owners in the situation of a non-compulsory programme. Benefits of research can be considerably decreased if a developed control programme is not accepted for a certain reason. So the potential benefits weighted for degree of acceptance represent the real benefits of research.

The expected benefits to additional expenditures on research, i.e. **marginal returns**, is the information required for the decision process. Only if the benefits are expected to equal at least the expenditures involved it is economically worthwhile to allocate resources to the research project.

An estimate of the annual costs of a disease does not provide any of the information required. It does not tell anything about how much it will cost to achieve a reduction in the total losses due to a disease by applying more research and therefore can never be used legitimately as a justification for more research.

CLASSES OF DATA REQUIRED

Some classes of data additional to those mentioned in Table 1 are necessary to provide the information required for the economic assessment of research. Research is done in the public sector as well as in the private sector and hence **expenditures** made in both sectors need to be included. **Benefits** of research originate from the reduction in total losses due to improved control programmes. The classes of data required to estimate benefits of research correspond with data required for decisions on control policies, i.e. **costs and benefits involved with control programmes** and have been discussed previously in this paper.

THE AVAILABILITY OF THE DATA REQUIRED

Data required to estimate benefits of research in the UK are scarce. Costs of research are borne in several sectors. MAFF is not able to give any accurate data about its expenditures on research and development into animal disease. On a request to Chief Scientist's group, information was given that probably about 10% of annual expenditures on R&D was for animal disease (i.e. £11.6 million for 1988/1989), but any breakdown into individual diseases was certainly not possible. Expenditures by the Agriculture and Food Research Council are monitored by ARICS, a research information system. This system does provide information about research expenditures specified for diseases. The National Office of Animal Health collects some information about the expenditures on research by the pharmaceutical industry. The six veterinary schools are able to provide information about their departmental research expenditures.

EXAMPLE

In the Corporate Plan of the Agricultural and Food Research Council (AFRC, 1988) a table with the approximate annual cost of research into several diseases and its estimated potential annual benefit is given. It has been estimated that the research projects for 13 different diseases cost £3.92 million a year and the potential benefits are £372 million a year. For example, the benefits of the development of calf respiratory vaccine are estimated at £60 million a year for an annual outlay of £0.55 million on research. No information about methodology and data used has been provided. The awareness of the lack of appropriate data necessary for such estimates questions the accuracy of this type of exercise. In addition, expenditures involved in the implementation of new control procedures have probably been excluded, nor has the degree of acceptance of possible new control programmes been included.

CONCLUSION

To take rational decisions about animal disease control on economic criteria the relationship between control expenditures and output losses needs to be established. Decisions about research into animal disease requires information about the expected benefits to additional expenditures on research. The dataset necessary to provide this information has been identified in broad terms. Unfortunately, these data are scarce at present in the United Kingdom and this constrains further progress in the economic analysis of disease in farm livestock. Collection of data is a major requirement to fulfil the need to base decisions on economic criteria. There is no use in making economic analyses when results have no meaning because of lack of data. This leads to the conclusion that there is an urgent need for a data collection system. The National Animal Health Monitoring System in the United States is an example of such a system. The data collected by this type of system are useful not only for economic analyses, but also for e.g. epidemiological studies and food safety control.

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PHYSICAL INJURIES IN DAIRY COWS: ASSOCIATIONS WITH SEASON, COW CHARACTERISTICS, DISEASE, AND PRODUCTION

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Physical injury to the body and limbs of dairy cows varies from hardly visible hair loss and thickening of the skin covering single protruding points of the body or limbs to extensive necrosis and inflammation of several muscle groups or even bones. Such conditions are, however, rare as primary veterinary treatments. Incidence rates for veterinary treatment of periarthritis of the hock (tarsal joint) up to 13 cases per 100 cow-years are, however, reported in some herds (Blom, 1981).

The prevalence of physical injuries observed during herd visits of herd health advisors ought to be important indicators of the herd health status. The occurrence of injuries observed by the meat inspection staff at the slaughter houses could, similarly, be important indicators of product quality. Systematic measurement of such injuries and the equivalent signs of "ill health" have been suggested as potentially the most objective measures of animal welfare (Ewbank, 1986). Experiments to investigate dairy cow housing types, design, flooring, and equipment often use the occurrence of physical injuries to the animals as a main dependent variable although insufficient standardization of injury recording is claimed to be a major deficiency of many such studies (Webb & Nilsson, 1983). The need for valid estimates of the prevalence of injuries that reveal "true" changes over time in any kind of dairy cow population is thus evident. To provide such estimates, knowledge about the effects of individual cow and herd characteristics such as age, stage of lactation, season, other health disorders, milk yield, and body weight is important because such risk factors are important for other disease entities. Consequently they can also be expected to affect the risk of acquiring injuries. Despite this, relatively little interest has been paid to these factors in the veterinary literature.

The purpose of this study was to quantify the effect of these risk factors on the risk of dairy cows acquiring physical injury on body and limbs. The results are expected to support the creation of variables for monitoring cow populations to provide valid estimates of the prevalence of injuries for herd health, animal welfare, and experimental purposes. This paper describes the applied statistical technique in detail, presents a summary of some important results, and discusses the potential inferential problems of the analysis.

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MATERIALS AND METHODS

Data

A subset of data from loose housed dairy herds collected from 1978 until early 1982 to estimate the effect of dairy cow housing systems on health, reproduction, production, and economy (Oestergaard, 1985) were used for this study. Included were cows from 15 herds with Danish Black and White cows which can be compared with Holstein Friesians, and 3 herds with Red Danish cows which are dual purpose cows with approximately the same size and milk yield as the Danish Black and White breed (average herd size $\pm$ sd = 85 $\pm$ 21). The loose housing systems were equipped with either solid floor and scraper or slated floor. Four of the 18 herds were grazing during one or more summer season or had access to an exercise pen in the study period. Technicians made weekly visits to all herds and were responsible for recording milk yield, body weight at calving (actual weight at first calving and heart girth measurement at the subsequent calvings), body weight in spring and fall and at culling, and dates of calvings and cullings. In addition, technicians made strong recommendation to achieve a feed ration of 5 kg dry matter of fodder beets, molasses or beet pulp, approximately 7 kg DM of silage or hay fed ad libitum, and 5 kg DM of concentrate. The flat rate feeding principle (Oestergaard, 1979) was applied in all herds. All treatments requiring injections and/or use of antibiotics were performed by the local practicing veterinarians who recorded cow identification number, date of treatment, and diagnosis. Recordings were verified by the technicians.

The claw health of each cow was recorded at least twice in each lactation at the time of claw trimming. Heel erosion (erosio ungulae) was recorded in 5 degrees from 0 representing no erosion to 4 representing exposure of the pododerm, and sole ulcers were recorded in 6 degrees from 0 representing no sign of sole ulcer to 5 representing an exposed pododerm (Smedegaard, 1964a&b). Trimming took place four times per year in each herd and each cow was trimmed at least twice per lactation with six months interval. Therefore most cows where trimmed during the first 3 to 4 months in milk and again approximately 6 months later. Some cows required claw trimming more frequently. Detailed descriptions and analyses of the recordings and findings at these claw trimmings are presented by Thysen (1987) (herd level analysis) and Enevoldsen et al., (1990a&b) (cow level analysis).

The conditions under study, physical injuries to body and limb(s), were obtained as follows: In February-March, April-June, and October-December all cows in all herds were examined by one of three members of the scientific staff at the Institute of Internal Medicine at The Royal Veterinary and Agricultural University in Copenhagen. Prior to each visit the three observers examined a number of cows together to minimize observer differences. Presence or absence of the symptoms 1) contusion and/or 2) wound with or without exudation on 5 locations on the cows were recorded. Every symptom in the two categories was scored from 1 (least severe) to 3 (most severe). Only the highest score of a given symptom on a given location was recorded. The locations examined were: 1) tarsal joint, 2) thigh, 3) costal arch/hip-/ischial area, 4) neck and shoulder, and 5) carpal Joint. All data were stored in the database at the Danish Institute of Animal Science after correction of recording and coding errors revealed by built-in logical checks in the computer programmes.

Data edition

Based upon the observations of each cow described above and recordings

of injuries three dependent variables were created according to location:
HOCK: Clinical condition of the tarsal joint area.
KNEE: Clinical condition of the carpal joint area.
BODY: Clinical condition of the costal arch, thigh, hip and ischial areas.
The variables HOCK and BODY could each have one of four values (degrees) as estimates of the clinical condition of the location. These values were defined by one or more symptom as follows:
Degree 1: Alopecia. Minor hyperkeratosis.
Degree 2: Major hyperkeratosis. Edema. Bursa formation.
Degree 3: Wound(s) and/or inflammation with exudation.
The variable KNEE differed by including bursa formation into degree 3. For the analysis, the last observation made no later than 300 days in milk (DIM) in a lactation was selected.

All variables were coded as indicator (dummy) variables. Continuous variables (e.g., Body Weight) were initially ranked from lowest to highest and then grouped into 7 categories of approximately equal size (i.e. 13-16 % of observations in each group). The variable Disease included veterinary treatments only. The following "subvariables" were created: 1) Limb (L); disorders of limbs (primarily periarthritis of hock, interdigital phlegmon and sole ulcer). 2) Severe (S); disorders expected to be severe enough to impair foot and leg health or increase lying time, e.g., severe metritis, left displaced abomasum and rumen acidosis, summer mastitis and peritonitis. 3) Other (Ot); all other disorders, e.g., other mastitis, retained placenta, and non-severe metritis. A cow was categorized as having experienced one of these "diseases" (L, S, or Ot) if at least one treatment was performed between 21 days before latest calving and the day of examination for physical injury.

The cows were categorized according to maximum degree of heel erosion in front (HEF) and hind (HEH) digits. The cows status with respect to sole ulcer (SU) was determined by the highest degree recorded (0-5). Only cows with recordings from claw trimmings performed between calving and 60 days after the day when the cows were examined for presence of injuries were included in the data set. When several observations of claw health were available for a cow in one lactation the most severe degree was used for the analysis. The variables, Herd and Year of Calving were combined into one variable (Herd-Year of Calving) which indicated the calving year and the herd identity. Calving year was from November 1 to October 31. The data set contained only 215 observations from lactations 4-9 which consequently were combined leaving the variable Parity with four classes.

Statistical analysis techniques

The interrelationships between the dependent variables were analyzed with a log-linear modelling procedure (Proc Catmod, SAS Institute Inc., 1985). A Likelihood Ratio (LR-) test was used as a summary measure of the Goodness of Fit of the models. The statistical significance of terms eliminated from models was also assessed by the LR-test. The odds ratio (OR) was selected as the epidemiological measure of interest to describe the association between independent variables (risk factors) and the three injury-variables. OR's, variance estimates, and -2log (Likelihood)-values (for model reductions) were obtained by means of the SAS-procedure Proc Logist (Harrell, 1986) which allows logistic regression analysis of ordinal dependent variables. For a variable to be ordinal, what caused the degree of injury to increase from, 2 to 3, must have been an extension of what caused it to increase from 0 to 1 and from 1 to 2. This assumption was checked

prior to the regression analysis by means of logit cumulative probability (LCP-) curves (Harrell, 1985). LCP-curves also were useful to screen the data set for categories with empty cells which might cause numerical problems in the logistic regression analysis (Hosmer & Lemeshow, 1989) and to reveal whether a variable could be included in the logistic regression model as an untransformed continuous variable.

Modelling strategies

For log-linear modelling of the relationship between the dependent variables, the modelling strategy suggested by Andersen (1988) and used by Mousing (1988) was applied. The first step was to set up the possible combinations of variables. For three variables A, B, and C, three models were possible: 1) A given B and C given B (denoted A-B-C), 2) C-A-B, and B-C-A. Of these three models the "best model(s)" which was defined as the model with the best fit measured by the Likelihood Ratio test (i.e., the model with the highest p-value) was selected for further analysis. In the second step, the statistical significance of terms in the selected best model was assessed also by means of the LR-test. Terms were removed if the p-value of the difference in LR-chi-square values was larger than 0.05.

The logistic regression models to estimate the relationships between the hypothesized risk factors and the dependent variables were built according to the following strategy: First, lactation 1 and lactations 2-9 were analyzed separately in all phases of the analysis because these two groups were expected to represent two different populations due to the markedly different body weight, production level, disease patterns, and conditions prior to and around calving (interaction assumed a priori). Second, logistic regression modelling was performed in three stages according to the principles outlined by Rothman (1986) and Hosmer & Lemeshow (1989): 1) Data reduction, 2) model selection, and 3) estimation of effect.

Data reduction: The purpose of this stage was to create as few variables with as few categories as possible while maintaining a valid description of the variation in the data. Prior assumptions of a linear relationship between the logarithm of the odds (logits) and values of the independent variables were avoided by categorizing all independent variables and including them in the models as indicator variables. The other variables were grouped to select as biologically reasonable categories as possible and to avoid categories with fewer than 5 % of the observations. The reference indicator variable (the control group) was as far as possible chosen as the group with the highest number of observations (to increase stability of parameter estimates) and the lowest occurrence of physical injury, e.g., cows not treated for a particular disease. It was attempted to form enough categories of each variable to enable a visual inspection of plots of regression coefficients obtained against the categories of the independent variables. E.g., the regression coefficients estimating the effect of each Body Weight category compared with the seventh category (coefficient equal to zero) were examined. Such graphical analysis gave detailed information about the statistical relationship between the regression coefficients conditional on all other variables in the model. Ordinal (e.g., three coefficients representing four degrees of sole ulcers) and nominal (e.g., five coefficients representing six bimonthly calving periods) independent variables were examined similarly.

The information obtained from this examination of the regression coefficients were used to create a set of new variables with only two or three categories. E.g., if the coefficients for the bimonthly calving

periods May-June, July-August, and September-October were markedly larger than the remaining periods, the variable Month of Calving was recoded into a binary variable with the value 1 for calving May-October and 0 otherwise. For ordinal variables, "non-significant" indicator variables were only excluded from the model if they were "adjacent" to the reference indicator variable. E.g., if the coefficients for sole ulcer Degrees 1-3 were similar to Degree 0, but markedly smaller than Degrees 4-5, then the variable Sole Ulcer was given the value 1 for Degrees 4-5 and 0 otherwise; if the coefficients of Degrees 1, 2, and 5 were larger than degrees 0, 3, and 4, then Sole Ulcer was assigned the value 1 for degrees 1-5 and 0 otherwise. If no obvious trends or biologically reasonable patterns were revealed, a binary variable with approximately equal numbers of observations representing reasonable divisions like high and low BW, and Winter and Summer calving season was formed in the case of continuous or nominal variables, while ordinal variables like Veterinary Treatment for disease and Sole Ulcer was recoded as treated/not treated and sole ulcer/no sole ulcer. No variables were removed from the analysis at this data reduction stage.

Model selection: The purpose of this stage was (as formulated by Hosmer & Lemeshow, 1989): to find the best fitting and most parsimonious, yet biologically reasonable model to describe the relationship between the dependent and the set of independent (predictor) variables. The recoded set of variables were analyzed with all possible two-way product terms except terms including the variable Herd-Year of Calving. Through an Analysis of Deviance, product and lower order terms were removed from the models until the difference in values of -2 log(likelihood) of the models at the corresponding degrees of freedom was statistically significant at the 5-10 percent level (the LR-test).

Estimation of effect: To provide epidemiologically interpretable effect estimates for the product terms, combined variables were created by forming indicator variables as follows: if the final model included the terms, A, B, and A*B (A and B both dichotomized into High and Low, and "*" indicating interaction between A and B), a model with the terms HighA+HighB, HighA+LowB, and LowA+HighB (coded as indicator terms) provides estimates of the effects of these terms relative to LowA+LowB. If the data reduction stage had revealed a clear trend in the categories of a variable, or the variable was ordinal, effects were estimated for all these categories to provide a maximum amount of information about the relationship and making adjustments for the effects of other variables and interactions. In all stages of the regression analysis Herd-Year of Calving was forced into the models.

RESULTS

Table 1 gives the distribution by severity and parity group of the injuries observed on HOCK, BODY, and KNEE. In Table 2 the results of the log-linear analysis of the interrelationship between HOCK, BODY, and KNEE are presented. The analysis revealed that only the models BODY-KNEE-HOCK ($p=0.1963$) and KNEE-HOCK-BODY ($p=0.2766$) could be accepted in lactation 1. None of these could be reduced further. The latter model implies that KNEE and BODY occurred independently of each other within levels of HOCK (or conditional on HOCK). KNEE and HOCK were strongly associated as were HOCK and BODY. In lactations 2-9, KNEE-HOCK-BODY was the "best" model ($p=0.1677$) and this model could be reduced to the model KNEE|HOCK BODY. This model implies that BODY occurred independently of KNEE given HOCK (or HOCK given KNEE) and that KNEE and HOCK were strongly associated.

Table 1. Distribution of physical injuries by location[a], lactation groups, and degree[b] of severity.

	HOCK	BODY	KNEE
Lactation 1 (N=1793)			
Degree 3	2.1	2.1	2.3
Degree 2	8.6	1.1	15.2
Degree 1	65.0	4.5	66.6
Lactation 2-9 (N=832)			
Degree 3	2.8	4.1	8.4
Degree 2	8.8	3.2	25.3
Degree 1	60.9	6.1	52.6

[a]HOCK: Area around the tarsal joint. BODY: Costal arch, hip, ischial, and thigh area. KNEE: Area around the carpal joint.
[b]Defined in the text.

Table 2. Relationships between physical injuries observed on tarsal joint (HOCK), body (BODY) and carpal joint (KNEE) measured by likelihood ratio test obtained by log-linear modelling.

	Lactation 1			Lactations 2-9		
Model[a]	df	chi-sq	P	df	chi-sq	P
1. BODY-KNEE-HOCK	14	18.2	0.1963	20	27.1	0.1330
2. HOCK-BODY-KNEE	15	81.6	0.0001	21	87.1	0.0001
3. KNEE-HOCK-BODY	14	16.6	0.2766	20	25.9	0.1677
4. KNEE|HOCK BODY	22	40.3	0.0099	28	40.6	0.0581
5. KNEE HOCK|BODY	23	97.9	0.0001	29	101.6	0.0001
6. BODY|KNEE HOCK	23	100.0	0.0001	29	103.0	0.0001
Effects of model reductions						
Diff.: 4-3	8	23.7	0.005	8	14.7	0.08
Diff.: 5-3	9	81.3	0.0001	8	75.7	0.0001
Diff.: 6-1	9	81.8	0.0001	9	75.9	0.0001

[a]E.g., model 1 is read: BODY given KNEE and HOCK given KNEE, i.e., the model assumes independence of BODY and HOCK given KNEE. Model 6 is reduced from model 1 and assumes independence of BODY given KNEE and HOCK. The reduction cannot be accepted (Diff.: 6-1 is highly significant).

Detailed descriptions of the data used for forming the independent variables subjected to the logistic regression analysis are given elsewhere (Blom, 1983; Enevoldsen et al., 1990a,b&c; Hindhede & Thysen, 1985; Thysen et al., 1985; Thysen, 1987). Examination of the LCP-curves revealed that only the relations BODY vs date of examination in lactation 1 and 2-9, KNEE vs date of examination in lactations 2-9, and KNEE vs Body Weight in lactations 2-9 could be modelled on an untransformed continuous scale. All independent variables were, however, analyzed as categorical variables for interpretational reasons (Rothman, 1986). Assumptions of ordinality of the dependent variables were violated with respect to HOCK (lactation 1) and KNEE vs date of examination. This could imply that the recording procedure changed during the study period. The ordinality assumptions were also somewhat violated for the claw health variables (sole ulcer and heel erosion). This problem appeared to decrease when the variables were dichotomized.

Tables 3-4 provide summaries of the selected variables comprising the final logistic regression models, the estimated odds ratios (OR's), and the interactions considered. The following is an example of how to interpret the very condensed representation of results in tables 4-5: for the dependent variable HOCK in lactation 1 (table 3), the following OR's for the independent variable Observation Season (OS) are presented: OR=1.8 for observations made in Spring compared to Winter given the cows have calved in July-December and given they have not been treated for either Limb (L) nor Severe (S) combined with Other (S|Ot). OR=3.9 for OS=Spring compared to Winter when the cows have calved in January-June and no treatment for L or S|Ot are performed, i.e., V=0. For OS=Fall compared to OS=Winter, the OR's are 2.1-2.3 given V=0. The complexity of the associations is a reflection of the two significant interactions between Observation Season and Calving Month, and between Observation Season and Disease Treatment.

DISCUSSION

The log-linear modelling strategy applied initially (table 2) appeared to be a very convenient method for exploring the data prior to a more rigorous analysis of more specific hypotheses. The method is an attractive ("objective") alternative to a tedious examination ("subjective") of numerous two-way frequency tables. In the planning stage of experiments or observational studies, historical data could be analyzed in this way to aid in selecting recording procedures that would have a greater chance of producing uncorrelated variables. The method can also be expanded to include explanatory variables in the models.

The current results indicate that if a summary measure - an "injury score" - was created by disregarding location, potentially important information would have been lost since BODY occurred independently of KNEE and HOCK in lactations 2-9. In other words, it is very likely that the etiology of BODY differed from the etiology of KNEE and HOCK. E.g., occurrence of BODY is known to be strongly associated with construction of stall dividers and stall dimensions. Type and amount (if any) of bedding is, of course, an important factor for KNEE and HOCK.

The most important part of an epidemiological analysis, a thorough biological discussion of the findings obtained, is beyond the scope of this paper and is given elsewhere. The discussion is instead restricted to the potential inferential problems associated with the presently applied analytical strategy to estimate risk ratios for factors with an expected effect on the risk of physical injury.

Table 3. Summary of effect estimates from final[a] logistic regression models of injury occurrence on HOCK, BODY, and KNEE in parity 1. N=1793.

Independent variable	Contrasts b) and Odds Ratios by model and dependent variables HOCK	BODY	KNEE
Obs. Season OS=S(Apr-Jun), F(Oct-Dec), W(Jan-Mar)	OS=S vs OS=W 1.8(CM=4-6;V=0); 3.9(CM=1-3;V=0) OS=F vs OS=W 2.1-2.3(V=0)	OS=S vs OS=W 4.4(SU=0;HEF=0); 1.6-1.9(SU=1) OS=F vs OS=W 2.2-2.8	OS=F vs OS=W 1.3
Calving Month CM=1(Jan-Feb), 2(Mar-...., 6(Nov-Dec)	CM=4-6 vs CM=1-3 1.4-1.6(OS=F,W;V=0) 0.7(OS=S;V=0); 0.6(OS=F;V=1); 1.5(OS=W;V=1)	CM=2-3 vs CM=1,4-6 3.3(BW=0)	Not included
Days in Milk DIM[c]=1,2,..,7	DIM=2-5 vs other 1.4	Not included	DIM=3-7 vs other 2.0-2.6(BW=1;HEF=0); 1.4(BW=0;HEF=0;FCM=0) 0.6(BW=0;HEF=0;FCM=1)
Milk Yield FCM[c]=1,2,..,7	FCM=5-7 vs other 1.3(SU=0); 0.8-0.9(SU=1)	FCM=5-7 vs other 1.8	FCM=5-7 vs other 0.7(DIM=1:HEF=0); 2.4(BW=1;DIM=1;HEF=0) 1.6(BW=0;DIM=0;HEF=0)
Body Weight BW[c]=1,2,..,7	BW=4-7 vs other 0.5(HEH=1)	BW=1-2,5-6 vs other 2.2(CM=1); 0.7(CM=0)	BW=3-7 vs other 2.0(OS=F,W); 2.0(OS=S;DIM=1)
Vet. Treatm. V=L(Limb), S(Severe), Ot(Other)	V=L,S|Ot vs other 1.6(OS=S;CM=1); 1.3-1.4(OS=W); 0.7(OS=F;CM=0)	V=S vs other 1.9	Not included
Sole Ulcer SU=degree 0-5	SU=1-5 vs other 1.5-1.7(FCM=0)	SU=1-5 vs other 1.3-1.6(OS=F,W; HEF=0); 0.6(OS=S; HEF=0); 1.3-6.2 (OS=S;SU=1)	Not included
Heel Erosion Front digits HEF=degree 0-4	Not included	HEF=1-4 vs other 0.2-0.3(OS=S,F; SU=0); 0.7(OS=S;SU=1)	HEF=2-4 vs other 2.9(BW=1;DIM=1; FCM=1); 0.4(BW=1; DIM=1;FCM=)
Heel Erosion Hind digits HEH=degree 0-4	HEH=2-4 vs other 1.7(BW=0)	Not included	Not included
Interactions	OS(S)*CM,OS(F)*V, FCM*SU,BW*HEH	OS(S)*SU,SU*HEF	OS(S)*BW,CM*BW, DIM*BW,FCM*HEF, BW*HEF

[a]In addition, Herd-Year of Calving is included in all models

[b]The values mentioned first are contrasted versus (vs) the remai ning (other) and assigned the value one (1) in the table. "Other" are assigned the value zero (0).

[c]Values are ranked and equally sized groups 1-7 formed (1=lowest)

The prevalence OR (POR) calculated in this study is a valid estimate of the disease risk ratio between an "exposed" and an "unexposed" population if the disease or the exposure does not alter survival in the study population (Kleinbaum et al., 1982). It is difficult to know to what extent such bias occurs, but since incidence data with respect to chronic diseases with an indistinct time of onset and period of duration are very difficult and expensive to obtain, POR's are the best available risk ratio estimates.

Table 4. Summary of effect estimates from final[a] logistic regression models of injury occurrence on hock, body, and knee. Parities 2-9. N=832.

Independent variable	Contrasts b) and Odds Ratios by model and dependent variables		
	HOCK	BODY	KNEE
Parity A=2,3,4+(=4-9)	A=4-9 vs a=2: 1.5	A=4-9 vs A=2: 2.9 A=3 vs A=2: 1.8	A=4-9 vs A=2: 2.0 A=3 vs A=2: 1.2
Obs. Season OS=S(Apr-Jun), F(Oct-Dec), W(Jan-Mar)	OS=S vs OS=W 4.1(A=2);2.5(A=3-9) OS=F vs OS=W 3.6(A=2);2.2(A=3-9)	OS=S vs OS=W 3.2(SU=0);2.9(SU=1) OS=F vs OS=W 1.4(SU=0);3.3(SU=1)	OS=F vs other 1.6(V=1) 0.8(v=0)
Calving Month CM=1(Jan-Feb), 2(Mar-...., 6(Nov-Dec)	Not included	CM=3-5 vs other 11.3(V=1) 2.1(V=0)	CM=3-5 vs other 1.5
Days in Milk DIMc)=1,2,..,7	DIM=4-7 vs other 1.7(A=2);1.6(A=3-9)	DIM=1-3,7 vs other 2.4	Not included
Milk Yield FCMc)=1,2,..,7	Not included	FCM=1-2,5-7 vs oth 3.2	Not included
Body Weight BWc)=1,2,..,7	Not included	BW=1-2,4-6 vs other 3.6	BW=5-7 vs other 1.4
Vet. Treatm. V=L(Limb), S(Severe), Ot(Other)	V=L,S vs other 0.8(SU=0;A=3-9); 2.0(SU=1;A=3-9)	V=L vs other 3.2(CM=1) 0.6(CM=0)	V=L,S,Ot vs other 3.0(OS=F) 1.5(OS=W,S)
Sole Ulcer SU=degree 0-5	SU=1-5 vs other 2.0-2.4(V=1;A=3-9); 0.8-0.9(V=0;A=3-9)	SU=1-5 vs other 3.3(OS=F); 1.4(OS=W);1.3(OS=S)	SU=4-5 vs other 2.0(HEH=1); 0.7(HEH=0)
Heel Erosion Front digits HEF=degree 0-4	Not included	Not included	Not included
Heel Erosion Hind digits HEH=degree 0-4	Not included	Not included	HEH=2-4 vs other 2.6(SU=1)
Interactions	A*DIM,DIM*BW, DIM*V,BW*HEH,V*SU	CM*V,OS*SU	OS*V,HEH*SU

[a]In addition, Herd-Year of Calving is included in all models

[b]The values mentioned first are contrasted versus (vs) the remaining (other) and assigned the value one (1) in other cells of the table. "Other" are assigned the value zero (0).

[c]Values are ranked and equally sized groups 1-7 formed (1=lowest)

Many of the potential biases are introduced by the "uncontrolled" conditions inherent in an observational study. This disadvantage is, however, somewhat outweighed by the relatively high external validity of this study due to the diversity of management conditions represented in the data set. The most important potential bias in these results is probably culling. On average 40% of cullings occurred before 24 weeks post partum and the median herd life was approximately 2 years (Thysen, 1985). DIM was included in the analyses and if the effect of a factor differed during lactation because of culling, interactions with DIM ought to be significant. This was the case in several models. Other analytical methods are necessary to get an unbiased estimate of the effect of DIM per se.

Visual inspection LCP-curves and of plots of regression coefficients obtained against the categories of the independent variables played a central role in the creation of the independent variables in this analysis. Such an

approach is definitely subjective and could be called data-dredging but, in principle, the approach does not differ from polynomial regression, other non-parametric regression methods or fitting of e.g., sinus functions. Such "objective" methods also attempt to find the best fitting function to describe the relations in the data. In all cases the inherent danger is to overinterpret the associations revealed.

Despite the attempt to create variables with a reasonable dispersion the inclusion of all possible two-way interaction terms inevitably introduced "empty cells" which might have caused numerical problems in the regression procedure (Hosmer & Lemeshow, 1989). The control of such problems was attempted by rigorous examination of the s.e.'s of the regression coefficients which tend to increase when numerical problems arise (Hosmer & Lemeshow, 1989). Estimates with large s.e.'s relative to the coefficients were also given less weight in the evaluation of the results. The terms causing problems, were, however, kept in the models to maintain validity of the coefficients which could be estimated with sufficient precision. In this analysis, the modelling strategy primarily served to find the best model of the dependent variable. The variance estimates obtained from the logistic regression procedure are probably not valid for the OR's calculated from the smallest samples. In such cases it might be better to use Fisher's Exact test for testing the unadjusted OR. The estimated CI's must be liberally interpreted.

The final evaluation and discussion of the effect estimates obtained with different models, although part of a modelling strategy, introduced further "subjectivity" in the analysis. An alternative approach would have been to calculate expected probabilities of injuries with a final model for different combinations of independent variables. With the latter approach, the weak parts of the data (the variable combinations with few observations) would not have been discovered and unjustified conclusions might have been the result. With the applied method some feel for the data is maintained.

The rough categorization of the variables made it possible to examine numerous interactions. The drawback of this procedure is the lack of specificity. E.g., several diseases with rather different etiology are pooled into one category - these diseases may have had completely different effects on the dependent variable. In this analysis examination of possible interactions were given higher priority than specificity of categorization.

The variable milk yield illustrates very well the most important problem in any observational study. It is extremely difficult to determine whether high milk yield truly is a risk factor for a disease because treatment and culling strategy invariably differ according to yield. Milk yield must, however, be considered when evaluating non-experimental data like these. This variable also illustrates the importance of considering interaction terms in the statistical analysis of health data since several of the effects revealed would not have been detected without considering interactions.

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PREVALENCE OF TRYPANOSOMA CONGOLENSE IN EAST AFRICAN ZEBU CATTLE UNDER HIGH TSETSE CHALLENGE

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Studies began in 1986 in the Ghibe River Valley in South West Ethiopia to obtain information on the health and productivity of East African Zebu cattle, managed in village herds and under levels of comparatively high tsetse challenge. Ghibe is one of a number of sites which are part of the African Trypanotolerant Livestock Network coordinated by the International Livestock Centre for Africa. The Network was established in 1983 to achieve, among other things, a better understanding of genetic and acquired resistance to trypanosomiasis and of the environmental factors that affect susceptibility. The East African Zebu are susceptible to trypanosomiasis and one of the objectives of the study was to compare aspects of their health and productivity at this site with those of trypanotolerant breeds at network sites in other countries in Africa. Preliminary results for the first 18 months have been presented by Mulatu et al. (1988). However, as the project continued it became apparent that many individuals were repeatedly being detected parasitaemic despite treatment. These observations together with the fact that overall trypanosome prevalence was increasing and appeared to be higher than might be predicted from the level of tsetse challenge suggested that trypanocidal treatment was becoming less effective. Indeed, there were more infections detected in animals that had been treated in the previous month than in animals that had not been treated (Woudyalew Mulatu et al., 1990).

This analysis of the data has been undertaken in an attempt to distinguish between new and recurrent infections. The paper summarises these findings and also describes the results of a trial designed to compare the efficacy of two levels of the trypanocidal drug used in these herds.

MATERIALS AND METHODS

Baseline Survey

East African Zebu cattle from 7 traditionally managed village herds in the Ghibe Valley in South West Ethiopia are being sampled and weighed monthly. Blood collected from the ear is used to determine the packed cell volume (PCV) by micro-haematocrit centrifugation and to detect the presence of trypanosome species Trypanosoma congolense, Trypanosoma vivax or Trypanosoma brucei by the dark ground/phase contrast buffy coat method (Murray et al., 1977). When parasitaemia is detected in a blood sample and PCV is less than 26%, or when clinical signs of

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trypanosomiasis are apparent without parasitaemia being detected, the animal is treated with an intramuscular injection of 3.5 mg/kg diminazene aceturate*. When parasitaemia is detected but PCV is 26% or above the animal is not treated.

Data from samples collected between January 1988 and June 1989, the period when trypanosome prevalence was highest, are reported in this paper. An average of about 600 cattle were sampled each month.

Field trial

Two of the 7 herds, containing 39 and 60 animals respectively, were selected for more frequent sampling in June 1989 to compare the efficacy of the 3.5 mg/kg dose of diminazene aceturate (Berenil) with that of a 7 mg/kg dose. All animals were sampled on 13th June 1989 and then at 13 approximately 10-day intervals until 22nd October 1989. On each occasion blood samples were analysed for PCV and presence of trypanosomes using the methods already described. At the 1st sampling animals found to be parasitaemic and to have a PCV of 20% or greater were given one of 3 treatments: no Berenil, 3.5 mg/kg or 7 mg/kg Berenil. Animals found to be parasitaemic and with a PCV less than 20% were treated with either 3.5 or 7 mg/kg Berenil. The same treatment protocol was used at 20 day intervals, i.e. at samplings 3, 5, 7, 9, 11 and 13, irrespective of any treatment given at a previous sampling. At the 1st sampling the first animal entering the crush, tested and found positive was given 7 mg/kg, the next 3.5 mg/kg, the next left untreated and so on. The order in which treatments were given was randomised on each subsequent occasion. Thus, assignment of a treatment to an animal was as far as possible at random. Treatment with 7 mg/kg was also permitted if clinical signs of trypanosomiasis were apparent without parasitaemia being detected, but these cases were not considered in the statistical analysis. On intermediate sampling occasions 2, 4, 6, 8, 10, 12 and 14 blood samples were analysed for PCV and tested for presence of trypanosomes, but no treatments were given.

For statistical analysis, cases of parasitaemia which occurred in samples 1, 3, 5, 7, 9 and 11 (defined as day 0) were assembled together, and a subset of records defined for which samples were available on both the following 2 sampling occasions (days 10 and 20). Berenil is reputed to provide protection against reinfection for up to a maximum of about 22 days (Rogers, 1985), but this varies both with the level of dose and level of tsetse challenge. A new infection results in a detectable parasitaemia from about 7 days. Theoretically, therefore, any parasitaemia detected at 10 or 20 days after treatment is likely only to be a consequence of an uncured infection.

Proportions of cases detected parasitaemic again on days 10 and 20 were compared between treatment by the Chi-square test. Mean PCVs on days 10 and 20 were compared by analysis of variance which also allowed for effects of sampling occasion and age.

In order to evaluate any possible bias due to variations in animal susceptibility to trypanosomiasis, a subset of cases was also formed so that only the first case recorded was used for each animal. These were subjected to the same statistical analyses.

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*Berenil (Hoechst)

RESULTS

Baseline survey

The mean prevalences of T. congolense, T. vivax and T. brucei observed in monthly samples from cattle in 7 herds between January 1988 and June 1989 are shown in Table 1. T. congolense was the predominant species and its prevalence gradually increased with age up to about 32 months of age (Table 2), remaining constant thereafter. T. vivax, on the other hand, showed no increase in prevalence after 8 months of age and its incidence was much lower than that of T. congolense. The prevalence of T. brucei was lower still. Ninety four per cent of detected parasitaemias were associated with a PCV less than 26% and were treated. These represented 91% of all treatments, the remainder being for cases where clinical signs were apparent, but where no trypanosomes were detected.

Table 1. Mean monthly trypanosome prevalence in 7 herds of cattle sampled between January 1988 and June 1989.

	Age (months)		
	< 9 (1369)	9 - 32 (3714)	> 32 (6370)*
T. congolense	3.0	14.1	30.7
T. vivax	2.1	5.6	4.2
T. brucei	0.3	0.9	1.2
Mixed infections	0.1	0.2	0.4

*Number of samples.

Table 2. Mean monthly prevalence of T. congolense in 7 herds of cattle sampled between January 1988 and June 1989 and classified by age.

Age (months)	No. of samples	Prevalence of T. congolense
0 - 4	659	1.8
5 - 8	710	4.2
9 - 12	781	10.8
13 - 16	821	11.1
17 - 20	622	12.5
21 - 24	523	16.2
25 - 28	538	21.4
29 - 32	429	18.4
33 - 36	404	30.7
37 - 40	392	26.8
41 - 44	290	30.7
45 - 48	214	32.2

Since T. congolense was the predominant species and the one giving most repeated infections (Woudyalew Mulatu et al., 1990), subsequent statistical analysis was confined to this species alone and samples containing either T. vivax or T. brucei were ignored. In order to devise a method for distinguishing between possible new and recurrent infections, the distribution of parasitaemic blood samples with varying levels of PCV was first examined for the two age groups : 9-32 and > 32 months (Table 3). Two thirds of samples from animals over 32 months of age with PCV less than 21%, but less than 5% of samples with PCV greater than 26%, were found to contain T. congolense. Less than 2% of samples with PCV greater than 26% were detected with T. congolense in the younger age group. It was decided, therefore, that a blood sample with a PCV greater than 26%, and without T. congolense detected, would be considered 'negative'; it was felt that the occurrence of a T. congolense infection following a series of 'negative months' defined in this way would be more likely to indicate a new infection rather than a recurrent one.

Table 3. Distribution of PCV and percentages of samples detected with T. congolense in cattle between 9 and 32 months and greater than 32 months of age.

	Age (months)			
	9 - 32		> 32	
PCV (%)	No. of samples*	Proportion parasitaemic	No. of samples*	Proportion parasitaemic
< 17	129	0.65	389	0.84
17 - 18	137	0.55	420	0.73
19 - 20	274	0.44	865	0.58
21 - 22	202	0.34	535	0.49
23 - 24	228	0.23	556	0.35
25 - 26	950	0.10	1596	0.19
27 - 28	678	0.02	821	0.05
29 - 30	448	0.01	482	0.04
31 - 32	184	0.00	146	0.02
> 32	235	0.01	.194	0.00

*Samples included only if either no trypanosome detected or T. congolense detected alone.

The data were re-analysed taking into account for each animal and each sample in turn the numbers of consecutive months found to be negative prior to the month in question. Table 4 shows values for prevalence of T. congolense derived when samples were restricted to those preceded by zero, one, two, three and four consecutive negative months respectively. T. congolense prevalence decreased from 32.8 to 16.6% and from 15.3 to 7.5% respectively in the two age groups as the number of preceding negative months increased from 0 to 2. When 3 negative months were used the prevalence in the older age group was reduced yet further to 14.1%, but thereafter a small reduction was at the expense of a corresponding reduction in sample size. A 'new infection' was consequently defined as one occurring in an animal which had had 3 consecutive samples with PCV > 26% and parasitaemia not detected. From Table 4 these values were 7.8 and 14.1% in the younger and older age groups respectively.

Table 4 also shows the prevalence of T. congolense one month following treatment, itself preceded by one or two negative months. Similar figures were obtained for the older age group when either one or two negative months preceding a treatment were considered. There were too few samples to consider 3 negative months and the values in the table are, therefore, the best possible estimates of the response to treatment of a 'new infection'. Table 4 shows that approximately 29% of 'new infections' in cattle over 32 months of age were detected parasitaemic again a month later. A small proportion of these parasitaemias may have arisen from new infections which occurred once the period of Berenil protection had elapsed. This would have been considerably lower than the monthly prevalence figure of 14% estimated above, and so the majority are likely to have been due to recurring infections which failed to respond to treatment. Similarly, a high incidence of recurrent infections are also indicated in the younger group.

Table 4. Mean prevalence of T. congolense from monthly samples collected between January 1988 and June 1989 in cattle over 8 months of age classified according to their PCV and parasitaemic status in previous months.

Previous months*				Age (months)			
				9 - 32		> 32	
4	3	2	1	No. of samples	Prevalence	No. of samples	Prevalence
.	.	.	.	3472	15.3	6028	32.8
.	.	.	-	1416	9.0	1530	20.2
.	.	-	-	790	7.5	662	16.6
.	-	-	-	461	7.8	340	14.1
-	-	-	-	304	6.3	217	13.4
.	.	-	+	89	20.2	233	28.8
.	-	-	+	42	16.7	89	29.2

* . all samples included irrespective of level of PCV or presence or absence of trypanosomes.
- no trypanosome detected and PCV > 26%.
+ T. congolense detected and treated with 3.5 mg/kg Berenil.

These observations led to the design of a trial to investigate in more detail the rates of relapse to the 3.5 mg/kg treatment and to compare its efficacy to that of a 7 mg/kg treatment.

Field trial

Six of the 99 animals were less than 7 months of age and these have been omitted from the statistical analysis. The remaining 93 were at least 15 months of age at the start of the trial. The average 10-day prevalence of T. congolense among these animals was 30%. Only 17 cases of T. vivax and 5 cases of T. brucei were recorded throughout the 14 sampling occasions.

Case records were restricted to the 151 involving T. congolense alone. These are shown in Table 5 classified by treatment group and with the numbers and proportions detected parasitaemic again over the next 20 days. There was no effect of age group on proportions detected parasitaemic when animals older and younger than 32 months were compared. Only 53% of controls (parasitaemic animals not treated) were detected parasitaemic again 10 days later, but by day 20, 81% had been detected parasitaemic on at least one of the two occasions. Cases treated with either dose of Berenil and with PCV $\geq$ 20% resulted in significant reductions in the numbers of detected parasitaemias at day 10 (3.5 mg/kg $P < 0.01$; 7 mg/kg $P < 0.001$). At day 20, however, the proportion of cases treated with 3.5 mg/kg and detected parasitaemic (.59) was not significantly different from the controls (.72) but the proportion of cases treated with 7 mg/kg and detected parasitaemic (.18) remained significantly lower than the controls ($P < 0.001$). Treatment of cases with PCV < 20% resulted in no significant differences between the effects of the two doses but numbers of cases were small. Table 5 also includes results based on one case per animal. It was possible to use 78 of the 93 animals which met the criteria for inclusion of a case record. These results were similar to those for all 151 cases suggesting that there was no apparent bias in the analysis of the data due to possible variations in animal susceptibility.

Table 5. Numbers of cases of T. congolense detected with T. congolense again over next 2 sampling occasions, classified by levels of PCV and levels of treatment of Berenil on day 0.

	PCV (%) at day 0	Dose of Berenil at day 0 (mg/kg)	No. of cases	No detected parasitaemic again at day 10	at day 20	by day 20*
All cases	$\geq$ 20	0	43	23 (.53)	31 (.72)	35 (.81)
		3.5	41	8 (.20)	24 (.59)	25 (.61)
		7	40	2 (.05)	7 (.18)	9 (.21)
	< 20	3.5	12	1 (.08)	3 (.25)	4 (.33)
		7	15	1 (.07)	4 (.27)	5 (.33)
	Total	0	43	23 (.53)	31 (.72)	35 (.81)
		3.5	53	9 (.17)	27 (.51)	29 (.55)
		7	55	3 (.05)	11 (.20)	14 (.25)
One case per animal+	Total	0	17	9 (.53)	12 (.71)	13 (.76)
		3.5	30	6 (.20)	14 (.47)	15 (.50)
		7	31	3 (.10)	6 (.19)	9 (.29)

* Parasitaemic at day 10 or day 20 or both.
\+ Only the first case recorded used for each animal.

Mean PCVs at days 0, 10 and 20 for the 151 cases adjusted for age and month of sampling are shown in Table 6. Packed cell volume for cases treated with PCV $\geq$ 20% on the day of treatment showed an increasing trend at day 10 with increasing dose ($P < 0.001$) and this became even more marked at day 20. Whereas PCV in controls gradually decreased from 24.2 to 22.9% over the 20 day period, the 7 mg/kg dose resulted in an increase from 24.3 to 27.1%. Treatment of cases with PCV <

20% resulted in a rapid increase in PCV from 17.4% on day 0 to 23.2% on day 10. Cases treated with 7 mg/kg showed a further slight but non-significant increase in PCV on day 20.

Table 6. Mean values of PCV at days 0, 10 and 20 following cases of T. congolense, classified by level of PCV and level of treatment on day 0.

PCV (%) at day 0	Dose of Berenil at day 0 (mg/kg)	No. of cases	Mean PCV (%)		
			day 0	day 10	day 20
≥ 20	0	43	24.2	23.4	22.9
	3.5	41	24.0	25.1	24.0
	7	40	24.3	25.7	27.1
Average s.e. of difference between 2 means			0.69	0.65	0.83
< 20	3.5	12	17.4	22.8	23.0
	7	15	17.5	23.6	24.9
s.e. of difference between means			0.56	1.15	1.53

It was possible to extend 138 of the 151 records to 30 days, and when PCVs were compared at day 30 the above trends were maintained. By this day the 7 mg/kg dose had resulted in a significantly higher mean PCV being achieved (26.6%) than either the control or the 3.5 mg/kg treatment in both PCV groups (≥ and < 20%). The control and 3.5 mg/kg treatment groups had similar PCVs averaging 23.0%. As at day 20, proportions detected parasitaemic at least once over the 30 day period were similar between controls and 3.5 mg/kg treated cases and the proportion of the 7 mg/kg treated cases that were detected parasitaemic during the 30 day period (0.46) remained lower than the controls (0.86).

DISCUSSION

This paper has attempted to develop methods for distinguishing new and recurrent trypanosome infections in situations where only one monthly sampling is possible and has used 3 negative months prior to the sample in question as the criterion for the definition of a new infection. This method can only be approximate for it depends on a fixed threshhold for PCV for determining negative and positive samples and ignores individual animal variations in susceptibility which may change with age (Table 3). There is also the problem that parasitaemia may be detected in as little as 50% of infected cases (Table 5) due to the periodicity of waves of parasitaemia which frequently fall to indetectable levels. Nevertheless the results obtained suggest that a substantial proportion of the observed prevalence of T. congolense was due to recurrent infections.

The paper has also described the design and analysis of a simple field trial which, over the comparatively short period of 14 weeks, provided evidence of the relative efficacies of 2 doses of Berenil. The trial was conducted under conditions of high tsetse challenge and high trypanosome prevalence which allowed numbers of cases to be accumulated quickly. There is often debate over the most appropriate dosage of Berenil to be used in the treatment of animal trypanosomiasis in different situations, and, provided sufficient numbers of animals are tested, the approach

could also be used in situations where tsetse challenge is lower.

The high levels of recurring infections at the site coincided with lower levels of animal productivity. Mulatu et al. (1988) reported mean calf weight at 8 months of 62 kg and mean cow weight post-partum of 204 kg over the first 18 months of the study. An analysis of body weight from January 1988 onwards suggested reductions from these values of about 15% and 10% in calf and cow body weight respectively. Further statistical analyses are needed, however, to determine to what extent these reductions in productivity may be due to trypanosomiasis. An increase from 3.5 to 7 mg/kg of Berenil would likely increase average PCV and reduce the numbers of detected parasitaemias, and this in turn may result in some improvement in productivity. However, even at this dose relapses occurred (Table 5) suggesting the possible existence of drug resistance among certain stocks of T. congolense. Resistance by T. congolense to chemotherapeutic drugs has been reported previously in Ethiopia (Scott & Pegram, 1974). Consequently a number of field isolates have been obtained for testing for this. At the same time it is clear that additional measures are needed to reduce overall levels of trypanosomiasis. A tsetse control campaign is therefore being mounted using insecticide impregnated screens. Subsequent monitoring of trypanosome prevalence and animal health and productivity will allow the benefits of this campaign to be assessed alongside alternative chemotherapy regimes.

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THE APPLICATION OF SOME MULTIVARIATE STATISTICAL METHODS IN EPIDEMIOLOGY - AN EXAMPLE: A COHORT STUDY OF RESPIRATORY DISEASE IN PIGS

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Observational field studies usually provide epidemiologists with large amounts of data, including consecutive, explanatory and confounding variables, with many interrelations. To analyse such data, multivariate modelling methods, such as generalized linear multiple regression, loglinear regression and logistic regression, can be used. However, their use is limited to certain sorts of variable and/or relationships, and they are difficult to process with numerous variables. For multidimensional analysis of large tables of data, multivariate descriptive methods that do not suppose any hypotheses on the distributions of, or relationships between, the variables to be analysed also have been developed (Benzecri et al., 1979; Fenelon, 1981; Escofier and Pages, 1988). These methods allow the identification of the main structures and relationships that exist in large amounts of data.

The objective of this paper is to present an application of one of these methods - factorial analysis of correspondence - in a cohort study of respiratory disease in pigs.

MATERIALS AND METHODS

Data collection

The cohort comprised three whole batches of pigs raised in three commercial herds in north Brittany. Each piglet born alive was ear-notched and individually followed from birth to slaughter or natural death. Of the 679 piglets born alive that entered the cohort, 542 lived to slaughter.

Each pig was visited 16 times on the farm for clinical examination as follows: at birth; once a week to the farrowing unit (4 visits); 5 visits to the weaning unit; and 6 visits to the fattening unit. Respiratory lesions were scored at slaughter. For each pig, an individual questionnaire was completed, including information about: sow health status and conditions of farrowing; clinical signs and management conditions recorded at each visit to the farm; weight at birth, 4 weeks (weaning), 8 weeks, 12 weeks (entering the fattening unit), 16 weeks, 23 weeks and slaughter; respiratory lesions at slaughter; and serological status against Mycoplasma hyopneumoniae (according to an ELISA test) of sows and pigs at 5 days, 4 weeks, 12 weeks, 16 weeks, 20 weeks, 23 weeks and slaughter. The final data table contained 575 raw variables.

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Data analysis

For statistical analysis, BIDOUI programs were used. The analysis was performed in three steps. First, each variable was analysed descriptively in the whole cohort and on each farm. Synthetic variables were constructed for respiratory lesions and the pathology of the sow at farrowing. Respiratory lesions were described as follows: pneumonia scoring (0 [no lesions] to 28) is the sum of the score of each lobe of the lung considering the relative surface affected; rhinitis scoring (0 [no lesions] to 10) is the sum of the degree of atrophy of each snout and the deviation of the median septum. A score for pathology of the sow at farrowing (0 [no clinical signs] to 30) was calculated considering rectal temperature, length of farrowing, anorexia, vulval discharge, mastitis and agalactia. In addition, average daily gain was calculated for the intermediate periods between weighing and from birth to slaughter.

The second step consisted of an analysis of the relationships between pairs of variables, using either a Chi-square or correlation test.

For the third step, factorial analysis of correspondence was used. In order to have homogeneous variables, quantitative data were transformed into ordered qualitative variables. Data with a Normal distribution were transformed in 5 clusters whose limits were defined around the mean. The clusters, with m the arithmetic mean and s the standard deviation, were: (1) from the minimum to m-0.75s; (2) m-0.75s to m-0.25s; (3) m-0.25s to m+0.25s; (4) m+0.25s to m+0.75s; and (5) m+0.75s to the maximum. For the other data, the clusters were defined either considering biological limits (e.g. absence of lesions or a serological result below the positive threshold), or in order to separate the population into clusters of the same size. The successive analyses are presented with the results.

RESULTS AND DISCUSSION

Size of the samples for the analysis

The multivariate analysis was performed on files including only pigs with no missing data: File A of 482 pigs with no missing consecutive data; the same file separated into three files of 184, 161, 137 pigs for each farm; File B of 373 pigs with no missing consecutive, explanatory or confounding data.

Consecutive variables : univariate analysis

The consecutive variables concern pig performance (growth and health). The mean values of growth and respiratory lesions are presented in Table 1. There is no significant difference between Files A and B.

Consecutive variables : multivariate analysis

The first analysis was performed on File A (482 pigs). The active variables were: average daily gain (ADG) from birth to weaning (FAR); ADG from weaning to 8 weeks (PWB); ADG from 8 weeks to entering the fattening unit (PWE); ADG from entering the fattening unit to 16 weeks (FTB); ADG from 16 to 23 weeks (FTM); ADG from 23 weeks to slaughter (FTE); pneumonia (PNE); rhinitis (RHI); pleuritis (PLE); lung abscesses (ABC). These ten variables were transformed into 45 binary active variables (one variable per cluster).

The average daily gain from birth to slaughter (GRO) was introduced as a supplementary variable. With 45 active variables, percentages of inertia of the three first factors were 7.0, 5.9 and 4.7%, respectively.

Table 1. Consecutive variables: mean growth and respiratory lesions

Population	File A	File B	Farm 1	Farm 2	Farm 3
Size	482	373	184	161	137
Average daily gain[a] (birth to slaughter)	544	543	527	559	549
Average score for pneumonia	5.6	5.5	6.9	6.2	3.2
Average score for rhinitis	2.3	2.3	3.0	1.0	2.9

[a] grams per day

The second analysis was performed on File B (373 pigs). The active variables were the same, except for pleuritis and abscesses which were introduced into the analysis as supplementary variables. With 40 active binary variables, the percentages of inertia of the three first factors were 7.3, 5.4 and 5.2%, respectively.

The results of these two analyses were not different. Thus, the graphical results are presented only for the analysis of File B.

On the projection maps defined by factorial axes 1, 2 and 3, the distribution of performance and the partition of pigs can be successively analysed. Each axis obtained by factorial analysis of correspondence is a graphical representation of a synthetic factor calculated with all the variables introduced into the analysis. The first, second and third axes represent the first, second and third factors, respectively. The interpretation is usually made on the graphs, but not on the numeric values of the factors.

On the first plan (factorial axes 1 and 2), growth per day is a result of both axes 1 and 2, following a straight line from the lower right to the upper left of the map (Fig. 1). The level of pneumonia is related to the second axis. The level of rhinitis is poorly related to the first axis, along which animals with no lesions are on the left side of the map, and animals having lesions with different degrees of severity are on the right.

The analysis of growth rate at different ages shows that two main periods of life can be distinguished (factorial axes 1 and 2 in Fig. 2). The first factor is related to growth during the first period of life of the pigs. In the farrowing unit, growth rates increase along the first axis, as in the weaning unit, with a particular position of pigs with very low growth after

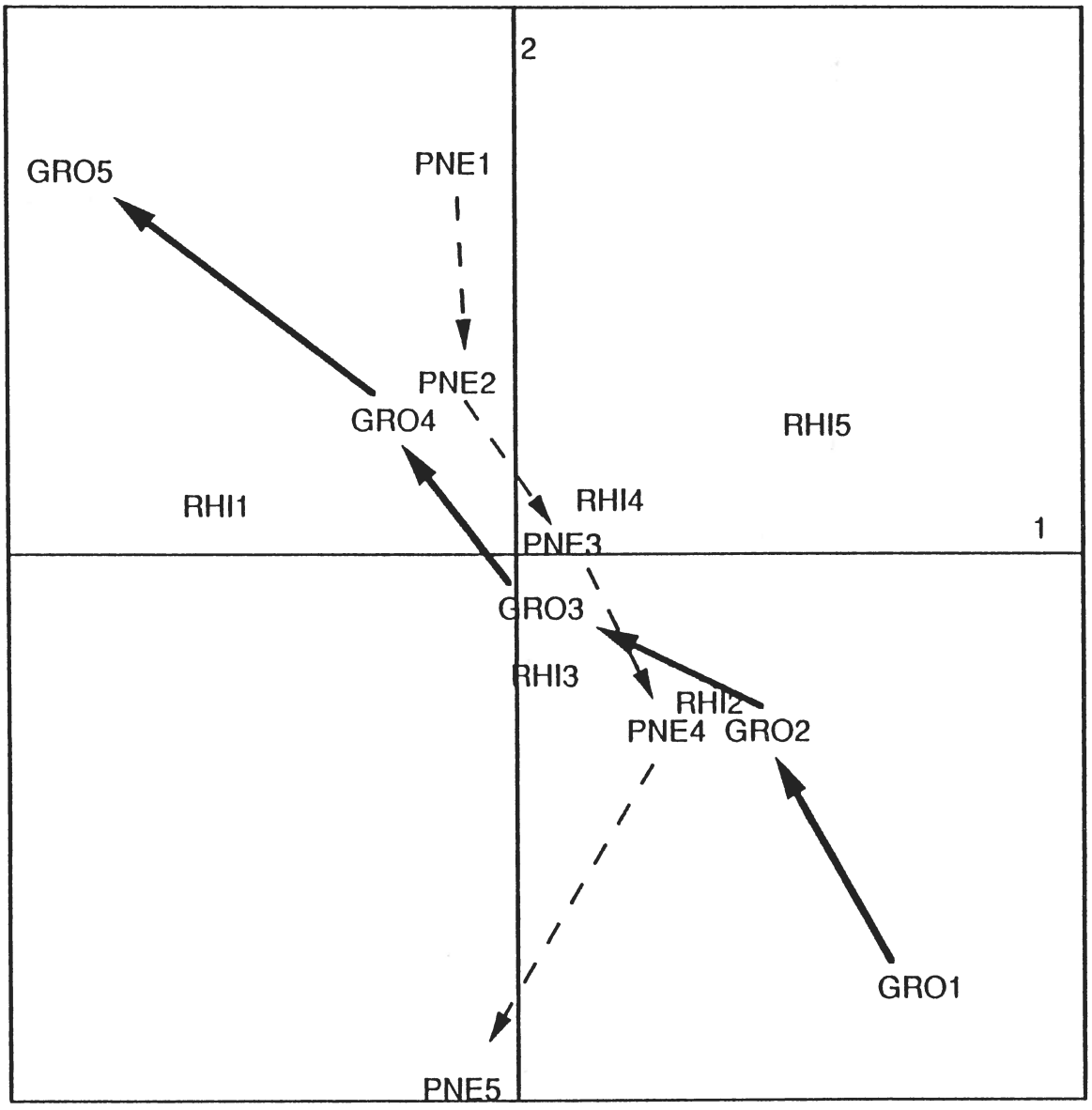

Fig. 1 Plan 1*2 : growth per day and respiratory lesions

GRO : average daily gain from birth to slaughter (1:very poor to 5 : very high)

PNE RHI : pneumonia and rhinitis at slaughter (1 :no lesion to 5 : severe lesions)

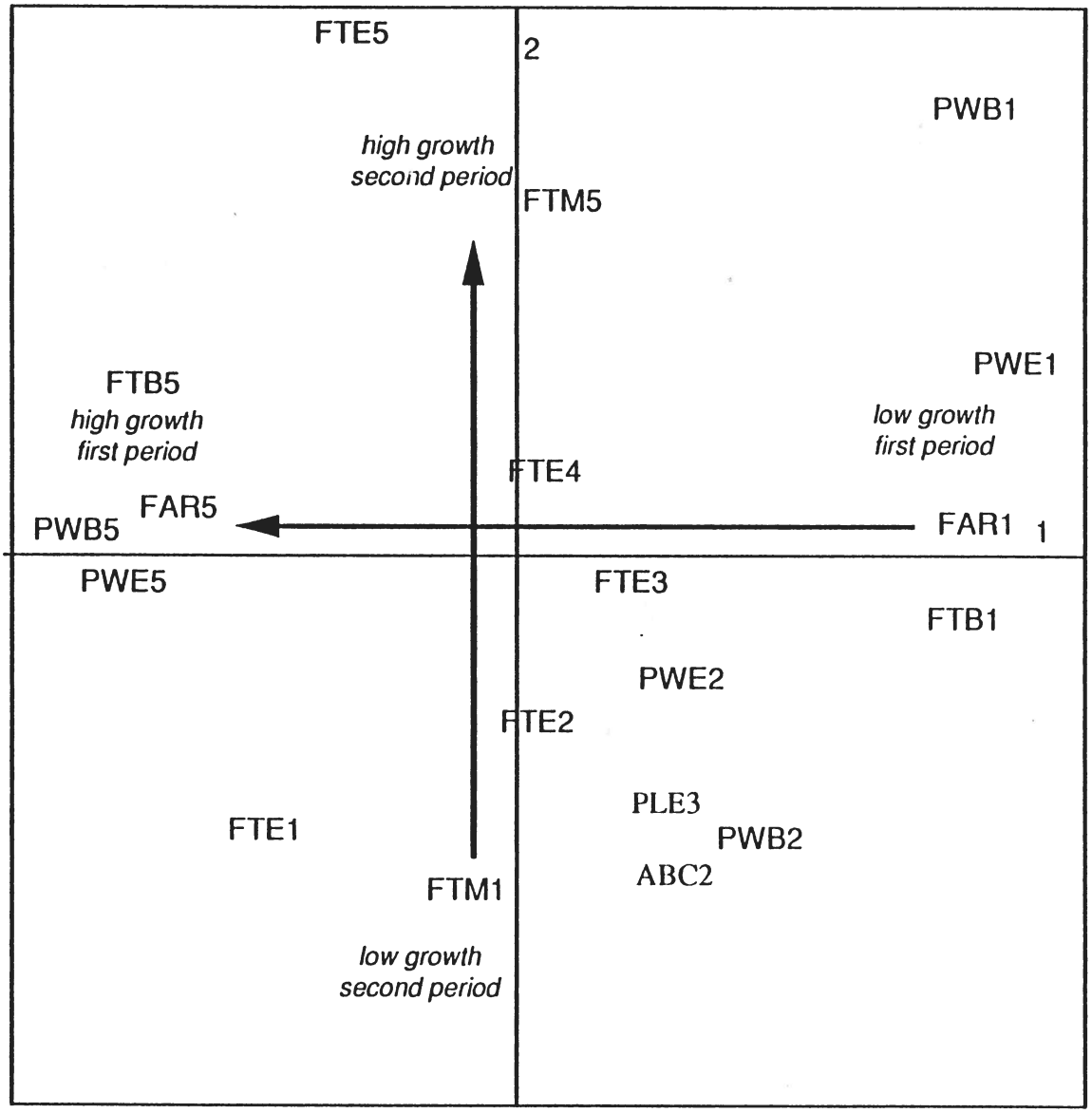

Fig.2 Plan 1*2 : growth rates at different ages

FAR : average daily gain in farrowing unit (1:very poor to 5:very high)
PWB : average daily gain in first period of post-weaning (1 to 5 : id)
PWE : average daily gain in second period of post-weaning (1 to 5)
FTB : average daily gain in first period of fattening (1 to 5)
FTM : average daily gain in second period of fattening (1 to 5)
FTE : average daily gain in the end of fattening (1 to 5)

PLE :pleuritis (1 : no lesion to 3 : severe lesions)
ABC : lung abcesses (2: presence)

weaning. The second axis expresses growth during the second part of the fattening period: from 16 to 23 weeks, and from 23 weeks to slaughter. The two factors are independent and so growth in the first and second part of life seem to be independent. Growth in the first period of fattening follows an intermediate gradient between axes 1 and 2. The second factor is also expressing the degree of severity of pneumonia. Therefore severe lesions of pneumonia are related to low growth in the second period of fattening, but not with growth at other periods. Absence of rhinitis lesions is associated with high growth in the first part of life, but no influence of lesions of rhinitis on late growth can be seen. Severe lesions of pleuritis and lung abscesses, introduced as supplementary variables, are associated with low growth in the second part of life.

On plan 1*3 (not represented here), the third axis distinguishes pigs with very low growth rate at the end of the fattening period from the others, and severe lesions of pneumonia from absent or moderate lesions (clusters 1 to 3).

This first analysis shows different profiles of performance: low or high growth in the first part of life, low or high growth in the second part of life, with a particular profile of pigs with low growth at the end of life. Pneumonia lesions are associated with low, late growth; absence of rhinitis is associated with high early growth.

Relationship between clinical signs of respiratory disease and lesions

The clinical signs of respiratory disease (coughing and sneezing) recorded at each visit were introduced into the analysis as supplementary variables (Fig. 3). Coughing does not appear to be associated with either severity of lesions or growth rate, neither on axis 2 nor 3. In contrast, sneezing in the farrowing unit and weaning unit are associated with lower growth and presence of rhinitis at slaughter.

Influence of serological status of sows and pigs

The relationship between maternal serological status and performance is difficult to interpret (Fig. 4). Pigs produced by sows with lower serological titers against Mycoplasma hyopneumoniae 5 days after farrowing are located on an average position on the first axis (early growth) and on the upper side of second axis, characterizing few pneumonia lesions and high late growth. This would suggest that colostral antibody levels do not influence protection of piglets. On the other hand, higher serological titers of piglets at 5 days are slightly related to fewer lesions.

At slaughter, pigs with high antibody levels have more severe lesions and lower late growth, but average early growth.

The serological status at intermediate ages is slightly associated with severity of lesions, but no simple relationship can be established.

Factors related to the farm and sow

Factors related to the farm and sow can be considered either as explanatory variables or as confounding variables. A few of them are presented in Figs. 5 and 6.

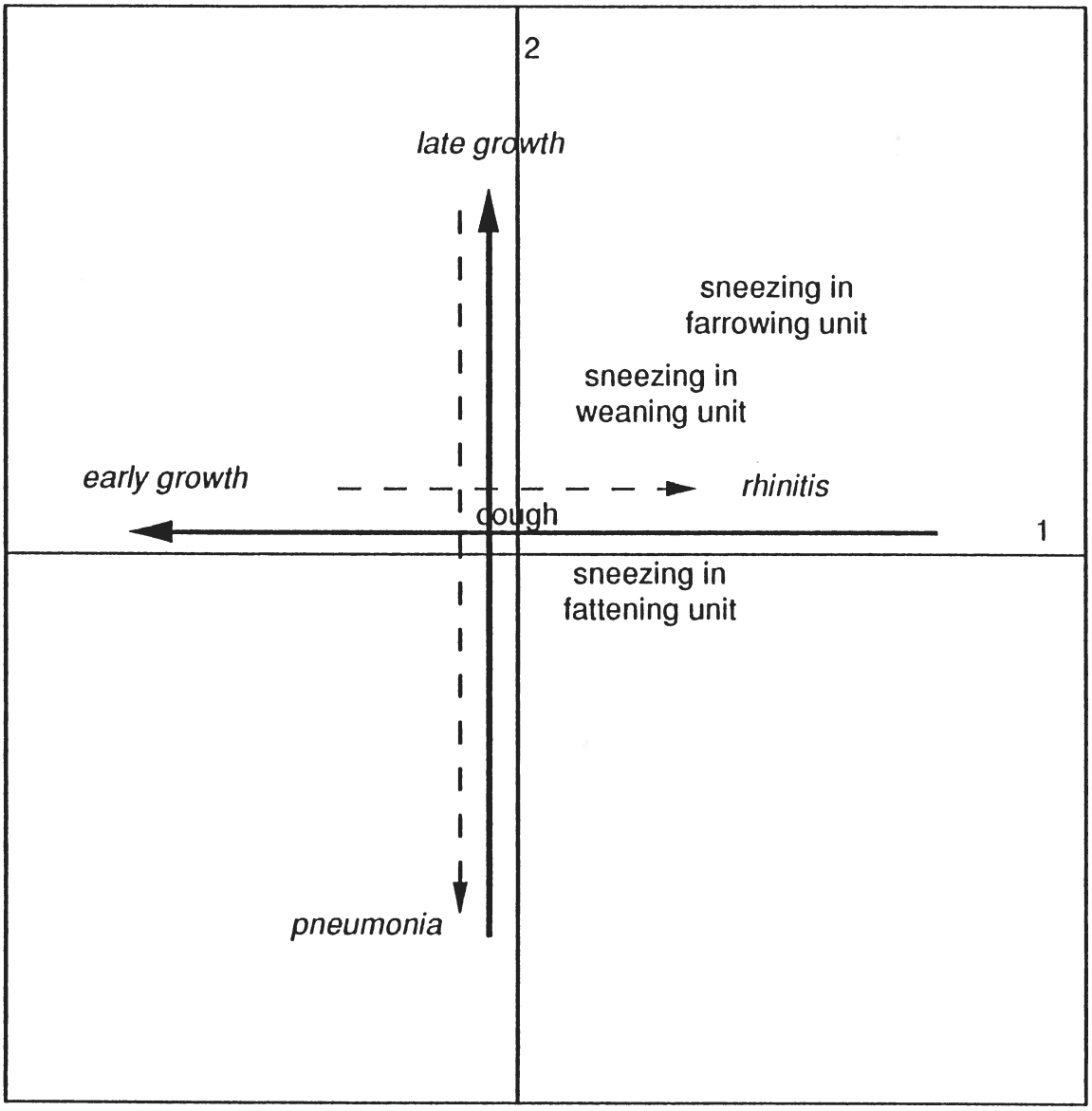

Fig.3 : plan 1*2 : clinical signs of respiratory disease

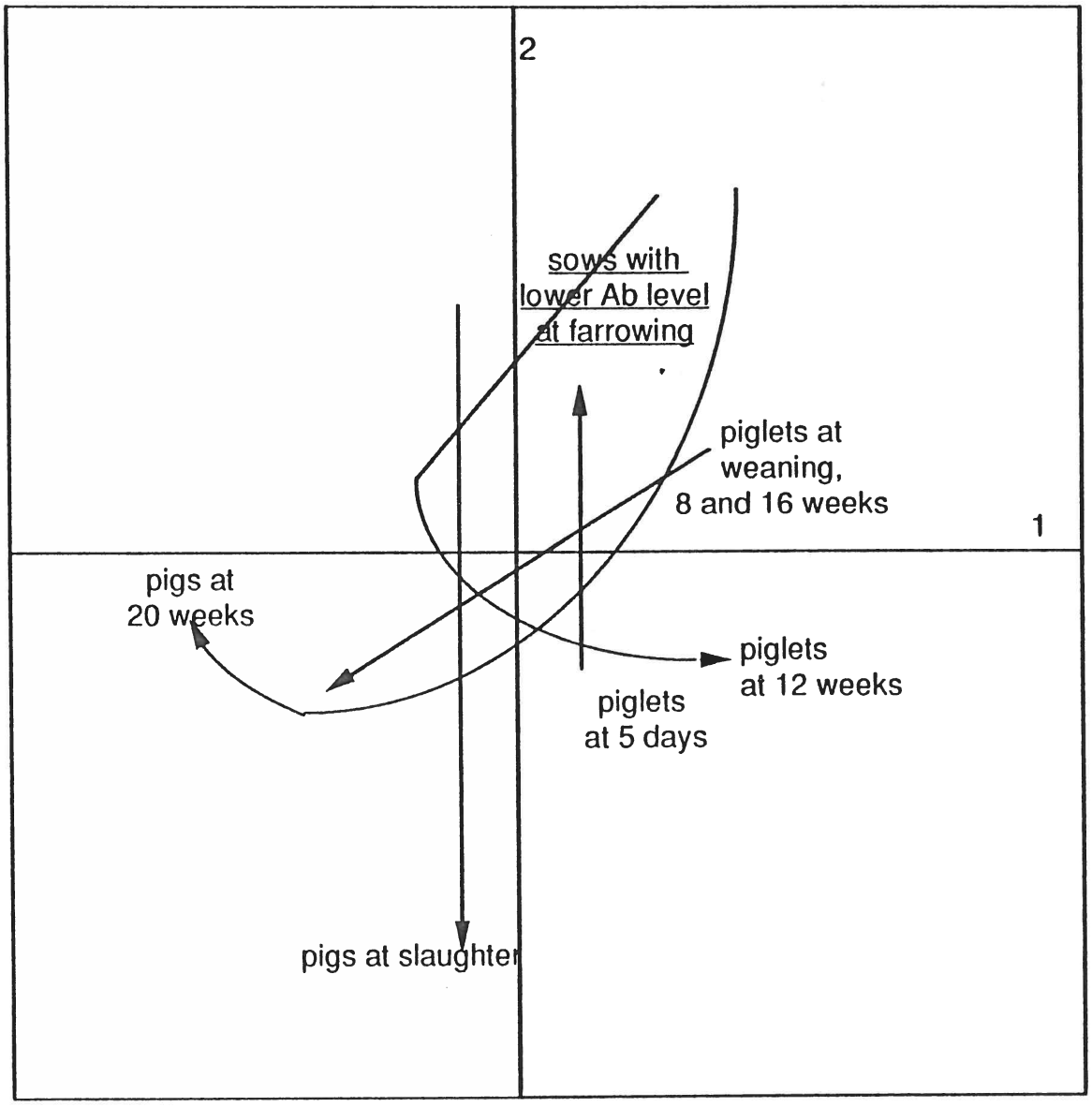

Fig.4 : Serological status of sows and piglets

The average position of pigs born from sows with lower serological titers at farrowing is indicated

The arrows indicate growing levels of antibodies of pigs at different ages

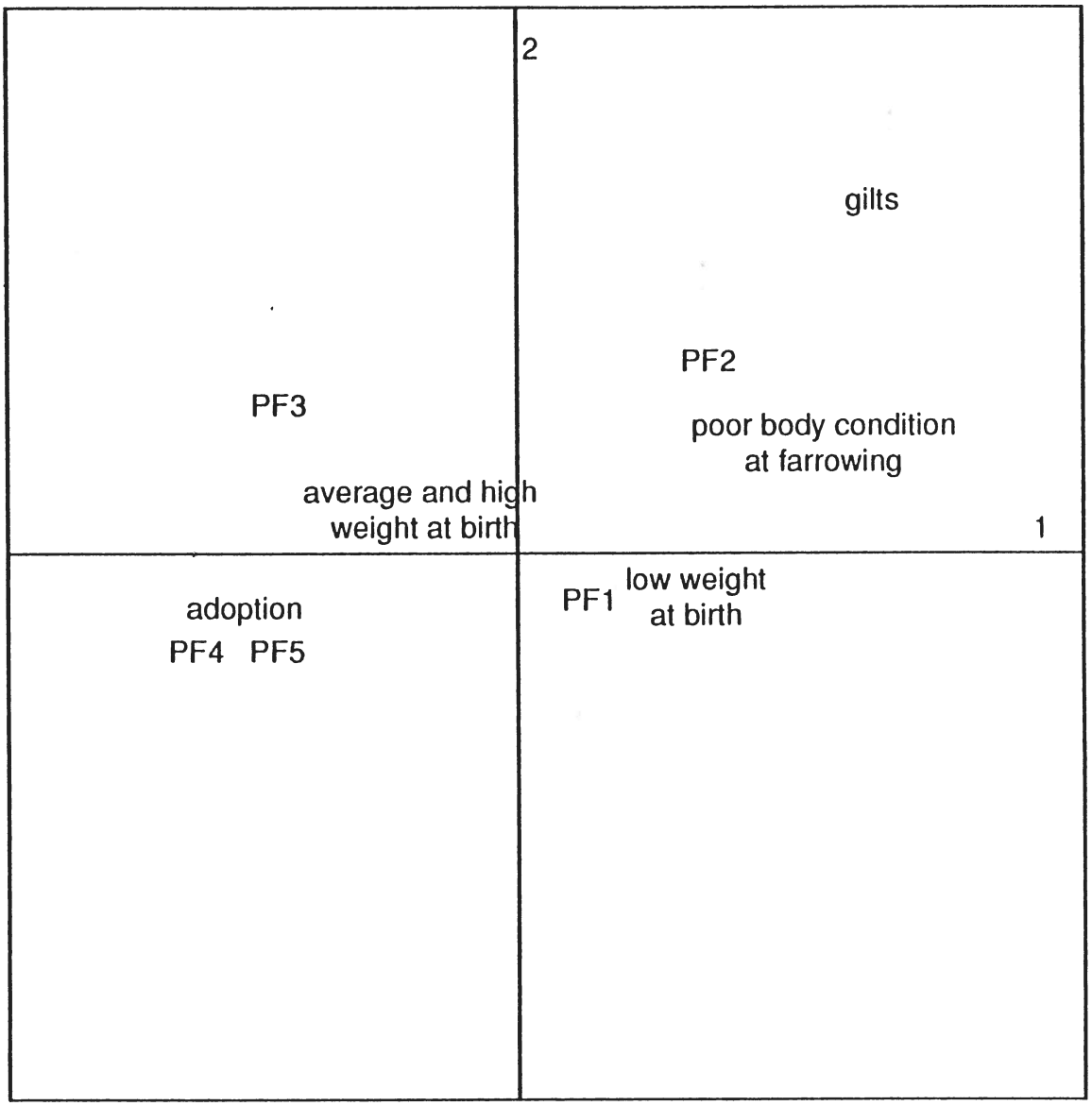

Fig.5 : Factors related to sow and farrowing

PF : pathology of the sow at farrowing (1 : absence to 5 : severe)

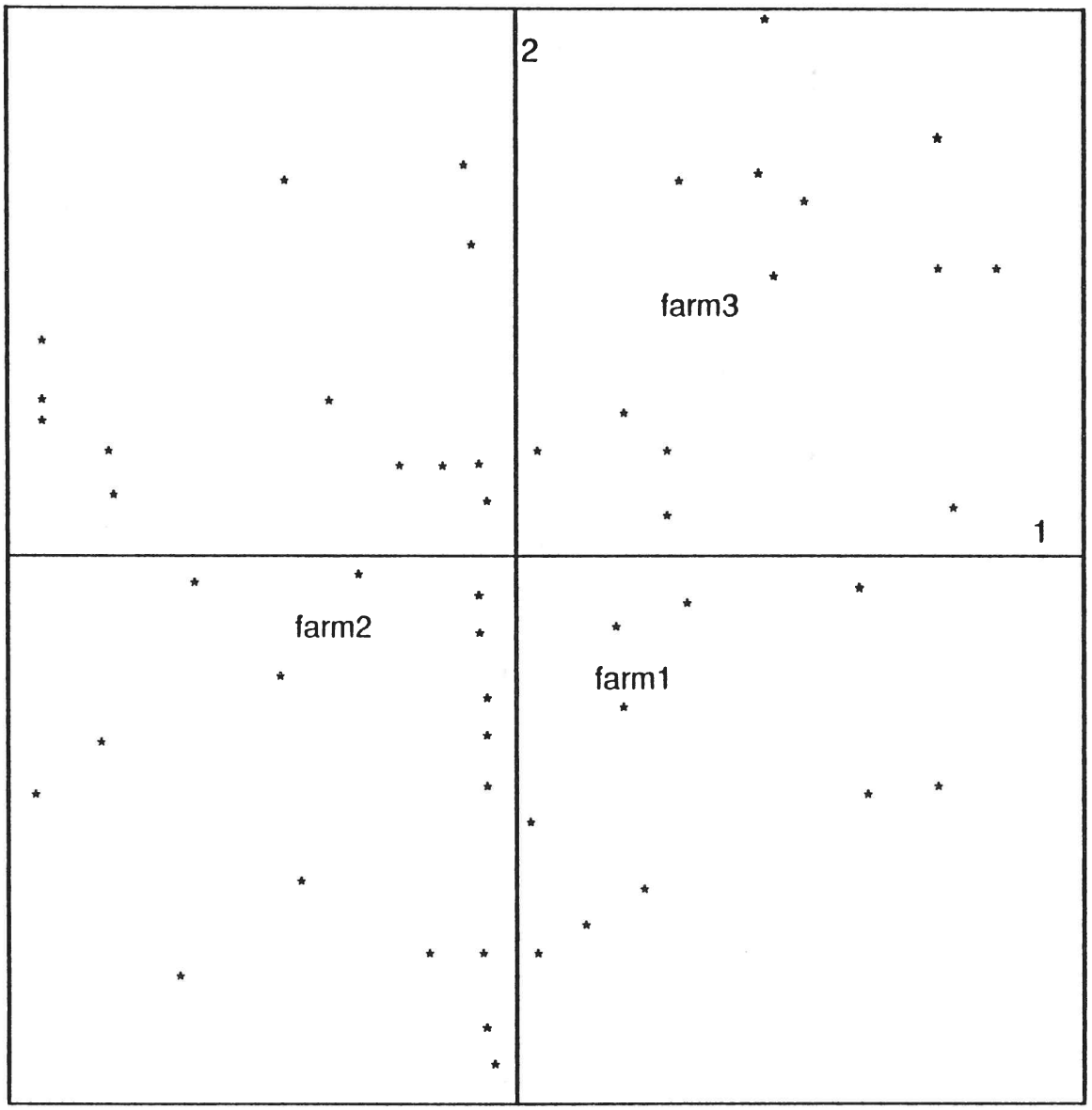

Fig.6 : Influence of the farm and the sow

Mean position of the pigs from each farm
Mean position of the litter of each sow (*)

Poor body condition of the sow at farrowing is related to lower early growth. The presence of pathology of the sow at farrowing appears independent of growth or health status of the pigs. Adoption of piglets is not related to lower performance. Piglets with lower weights at birth grow a little more slowly in the first part of their lives, but birth weight is not related to later growth. Parity of sows divides the population into two groups: pigs produced by gilts have lower early growth, but higher late growth and fewer pneumonia lesions. It must be taken into account that, in the sample, all the gilts belong to the same farm. The average positions of the three farms show differences in the profiles of their whole performance. Farm 1 has poorer results with lower average growth rates, both in the early and late periods. Farms 2 and 3 have similar global average daily gains, but this result is obtained in two different ways: Farm 2 is characterized by quick early growth and poor late growth, accompanied by more severe lesions of pneumonia. Farm 3 is characterized by slow early growth, especially after weaning, quick late growth and few lung lesions. Eventually, the differences observed in the performances of each litter are very important. As they induce a clustering effect of the results obtained by the pigs from the same litter, the quantitative methods that could be used in further analyses to compare results from groups of pigs must be adapted.

In this descriptive analysis, the relationships can be visualised without formulating a hypothesis about the nature of the relationship.

Multivariate analysis at the farm level

The average positions of the farms show their differences considering their performance profiles. In a further step, factorial analysis of correspondence is performed independently for each farm.

For Farms 1 and 2, the interpretation can be done on the two first factors (Figs. 7 and 8). The growth is the result, as in the global analysis, of early and late growth that are almost independent. Lung lesions are inversely related to late growth, and rhinitis to early growth.

For the third farm, the distribution of the pigs on plan 1*2 has a characteristic shape, typical of the high strength of the first factor (Fig. 9). Along the second axis, extreme clusters are located on the upper side of the map, and on the other hand, average clusters are situated on the lower side. Therefore, an interpretation cannot be made on these axes. On the plan defined by the first and third axes, it is possible to identify the same structures as in other farms and the whole sample, except for rhinitis which is associated with quick late growth (Fig. 10).

CONCLUSIONS

With the help of multivariate descriptive methods, the main relationships between health and growth performance of this cohort of pigs have been identified. These associations exist in the whole cohort and, if one particular farm is studied, in all the pigs on that farm. On the plans defined by the first factors, two main axes can be identified and used as a scale for performance. The first axis expresses early growth of piglets (from birth to 16 weeks). The second axis expresses late growth and lung lesions at slaughter, which are inversely related. The description of explanatory or confounding variables underlines the influence of clustering effects due to the origin of pigs (farm and sow). The predictive value of clinical signs

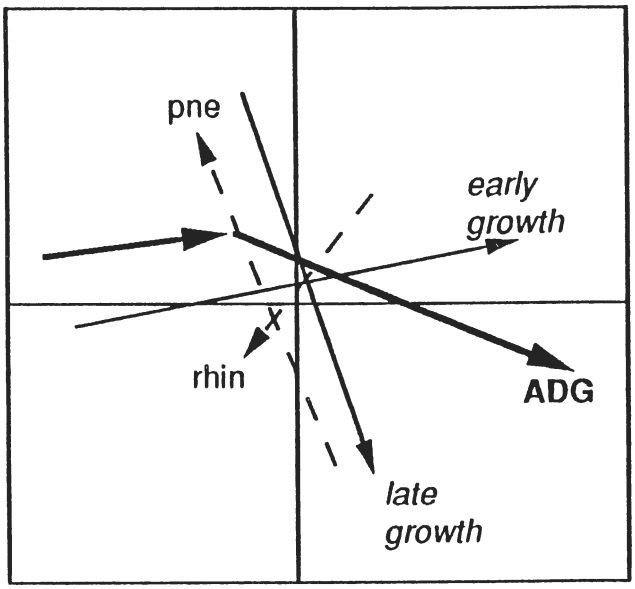

Fig.7 : Analysis in farm 1 : plan 1*2

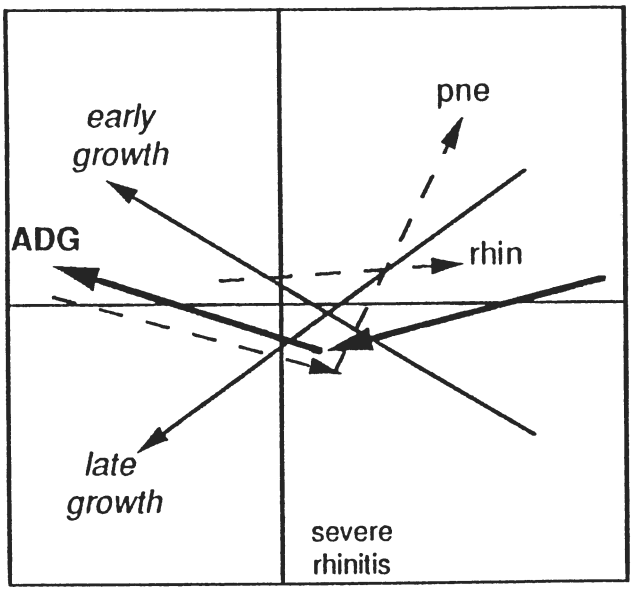

Fig.8 : Analysis in farm 2 : plan 1*2

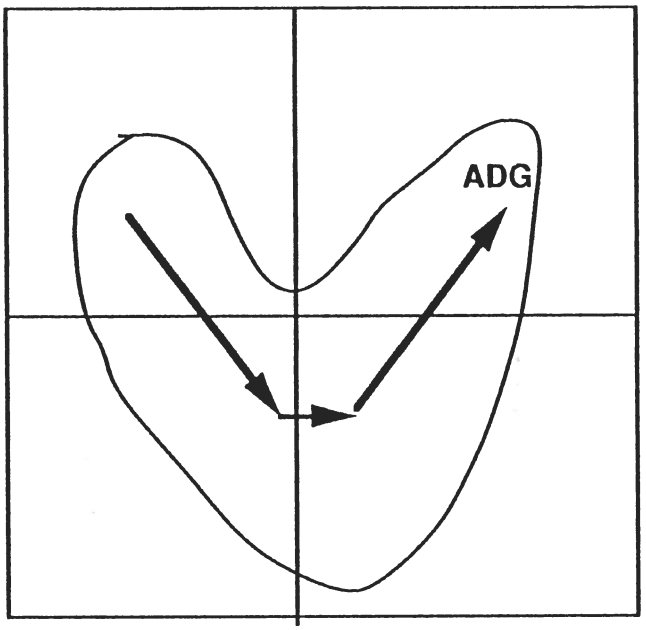

Fig. 9 : Analysis in farm 3 : plan 1*2

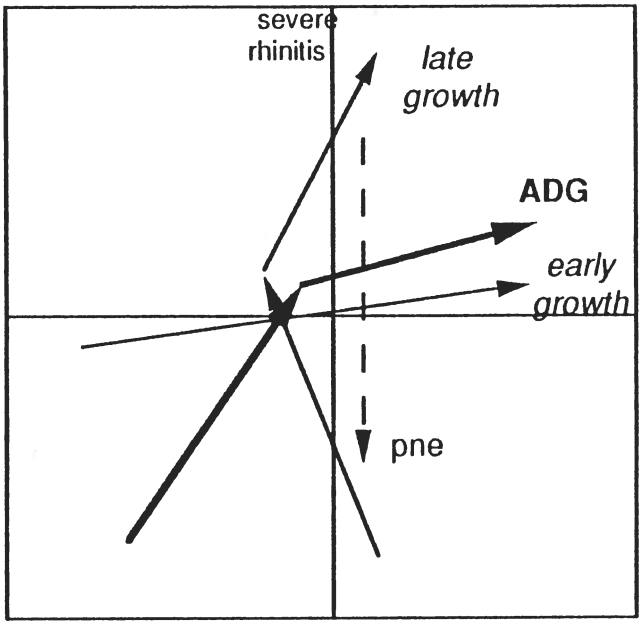

Fig.10 : Analysis in farm 3 : plan 1*3

of respiratory disease is difficult to assess in this sample. The relationships between serological status against Mycoplasma hyopneumoniae and respiratory lesions appear to be complex. The influence of both management conditions and other determinants must therefore be considered.

The graphic presentation of the results of such an analysis allows the rapid identification of complex interrelations that exist in numerous data, but it is not possible to quantify these relations. Therefore, they are adapted to initial analysis of large tables of interelated data.

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A REVIEW OF STATISTICAL TECHNIQUES TO EVALUATE SPACE-TIME CLUSTERING IN DISEASE OCCURRENCE

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Disease clustering has long been intriguing, since clusters bring with them prospects of clues to causal explanations (Rothman 1987). Space-time clustering of disease outbreaks is defined as the occurrence of a pattern of cases of disease that are closer together in time and distance than could be expected if the only underlying mechanism governing the distribution of the disease were random allocation (Grimson 1979). Decision rules are necessary to determine whether perceived clusters of disease cases constitute actual clusters, or merely clumps in an overall pattern consistent with random distribution of cases. Hence, statistical techniques are used to distinguish the presence of clusters of cases from random allocation (Klauber 1974).

The purpose of this paper is to review and illustrate some statistical techniques used for space-time clustering. The review will be restricted to techniques suitable for space-time clustering, therefore techniques for detecting clustering in either space or time alone were not considered.

GENERAL APPROACH

The null hypothesis states that the dates of onset are distributed at random among the places of onset of the cases. Generally, the interest is in a one-sided alternative hypothesis; the information that cases are further apart in space and time than expected is of virtually no value.

The terminology used throughout this paper is as follows. The number of cases will be denoted by n. For each i, $i = 1,2,.....n$, case i has a time coordinate T_i, and two space coordinates X_i and Y_i. We assume that the cases are listed in chronological order, so $T_1 \leq T_2 \leq ....\leq T_n$. Some of the statistical methods require the computation of a distance between each possible pair of cases. The number of possible pairs of cases will be denoted by N; it can be shown with basic combinatorial principles that $N = n(n-1)/2$. Let thee distance in time and space between the k^{th} pair of cases, $k = 1,2,...N$, be denoted by E_k and D_k, respectively. if the k^{th} pair consists of cases i and j, then $E_k = |T_j - T_i|$,and

$$D_k = [(Y_i - Y_j)^2 + (X_i - X_j)^2]^{.5}.$$

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Some methods require the user to specify threshold values in space and/or in time. Ederer, Myers & Mantel (1964) stressed the importance of choosing suitable values for these thresholds noting that 'Clusters may remain undetected if the unit of study is too large or too small'. A clustering within a year is missed if the period of study is a month, and vice versa. There are several ways of choosing a sensible threshold value. It can be the average value of the distances, it may be some percentile chosen from a histogram of distances, or it may be dictated by biologic knowledge on incubation period, generation time, ways of transmission etc. If there is some uncertainty about the appropriate value of the threshold, several different values may be used in applying a method. In general the necessity to choose threshold values requires care on the part of the user.

STATISTICAL TECHNIQUES

I. Ridit method

The ridit method was proposed by Bross (1958). It was used by Pinkel et al. (1963) to test for space time clustering of leukemia in children. This approach begins by measuring the time and spaces distance between cases for all possible pairs. A threshold value d^* must be chosen for the distance in space, pairs whose distance in space exceeds d^* are called unrelated pairs. The time distribution of the pairs below the threshold d^* is compared to the distribution of the pairs whose distance in space is above d^*. The temporal distribution of all the pairs further apart than the space threshold is calculated first. This gives the value of the empirical cumulative distribution function (CDF) of the unrelated pairs for each time coordinate E_k. The value of the CDF is called the 'ridit' for a pair. Each pair with a space distance smaller than the threshold is assigned a ridit according to the time coordinate E. The spatial distance below the threshold is divided into several intervals. In each of these intervals the average ridit is calculated. Under the null hypothesis of no space-time clustering, the expected average ridit for each interval is .5. If there is a space-time clustering a small average ridit (< .5) is likely to be observed for some of the intervals.

To decide about a significant difference from .5, or to test the difference between two ridits, an approximation to the standard normal distribution is used. The variance of a ridit is calculated as $1/(12 * n)$. The test statistic S is calculated as:

$$S = \frac{r_a - .5}{[1/(12\ n) + 1/(12\ N_a)]^{.5}} \qquad S \sim \text{Approximately Normal}(0,1)$$

where r_a = average ridit in interval a
n = # cases in the identified population
n_a = # cases in interval a
a = 1,2.... (= # of space intervals defined in the area below the threshold d^*)
.5 = average ridit under the null hypothesis.

The variance of the difference between two ridits is given by:

$$Var(r_a - r_b) = [1/(12 \cdot n_a)] + [1/(12 \cdot n_b)].$$

so :

$$S = \frac{r_a - r_b}{[1/(12\ n_a) + 1/(12\ n_b)]^{.5}} \qquad S \sim AN(0,1)$$

$a,b = 1,2,\ldots\ldots$ and $a \neq b$

A 95% confidence interval for a ridit is given by :

$$r_a \pm 1.96 * (1/(12\ n))^{.5}.$$

An important assumption of this technique is the Uniform distribution in the identified population and a uniform distribution in each of the pieces below the cutoff. This assumption is necessary in order to estimate the variance of the ridits. If the assumption is violated, the real variance can be either larger or smaller (Mantel 1979). Violating the assumption will have a large impact on significance tests or on confidence intervals. It is necessary to have a substantial number of cases to obtain some 'stability' in the CDF. In most space time clusters this is not easily established. An other precaution is that with a lot of pieces below the space cutoff the probability of finding a small ridit by chance will increase.

Even though the ridit method has some serious drawbacks, it does have some nice features. It assures that the influence of large distances won't be exaggerated by collapsing the space information into two categories (below and above the cutoff). It also collapses the time distances into ridits which prevents the effect of extreme observations on the test statistic. Because of its use of the CDF it shows similarities with the Wilcoxon test (a rank test).

II. Knox method

Again, a prior choice of a cutoff in space and time is necessary. Then all possible pairs are considered. The pairs are put into a two by two table; according to the cutoff points chosen a priori. The test statistic, Z, is the number of pairs below both the cutoffs. Knox proposed that this statistic had a Poisson distribution (Knox 1964a,b). Under the null hypothesis of no space time clustering the parameter of the distribution is estimated by multiplying the row and column total and divide this by the grand total (the familiar method of calculating expected frequencies).

David & Barton (1966) showed that the assumption of the Poisson distribution is met in the case of leukemia. However, with a different data set using a known contagious disease (measles) the mean and variance differed by a factor 2 (this violates the poisson assumption).

A generalization of the Knox method has been presented by Pike & Smith (1968). This generalization assumes that for any case two

time periods and two residing places can be defined: one set in which the disease is caught and one set in which the disease is transmitted (resp. susceptive and infective). Their test statistic is the number of pairs such that one member of the pair was in the susceptible state, and the other member was in the infective state, while both were within the set space limit. The distribution of the statistic should be obtained from a Monte-Carlo simulation or can be approximated by a normal distribution. This modification of the Knox has a drawback in that if the etiology of the disease in the study is not known, it is difficult to make a sensible guess about the susceptible and infective states. On the other hand, if this knowledge is available, the method may be more powerful than the original Knox method.

The Knox statistic can be viewed as:

$$S = \sum_k D'_k E'_k$$

$$Z \sim P(\theta) \quad \text{for small n}$$
$$Z \sim AN(\theta,\theta) \quad \text{for large n}$$

where $k = 1,2,....,N$
$D' = 1$ if space < threshold, $D' = 0$ if space > threshold
$E' = 1$ if time < threshold, $E' = 0$ if time > threshold

$E[S] = \theta$ and is estimated by $(\Sigma D_k * \Sigma E_k)/ N$.

An extension of the Knox method is given by Mantel (1967). Mantel proposed:

$$S_m = \sum_k D_k E_k$$

where D_k and E_k are the real distances in space and time.

The distribution of S under the null hypothesis can be obtained from all possible permutations, or in larger data sets by a Monte Carlo simulation. For large N, S is approximately Normal distributed.

$$S - E[S] = 2N(\Sigma DE - (\Sigma D \Sigma E/N)), \quad \text{summation over k}$$

$$\text{Var } [S] = (4/(N-1)) * (n \Sigma D^2 - (\Sigma D)^2) * (n\Sigma E^2 - (\Sigma E)^2)$$

so: $$\frac{S - E[S]}{\text{Var } [S]} \sim AN(0,1).$$

The null hypothesis is rejected if S is substantially smaller than its expectation. This modification is equivalent to the regression approach, which will be discussed later.

III. Ederer Myers & Mantel method (EMM)

It is necessary to divide the study area in sub-areas of a given size (Ederer et al. 1964). This can be blocks in a city, towns in a state or circles with radius cr (cr=critical distance) around cases as proposed by Mantel in his discussion on the Pinkel

& Nefzger method (Mantel 1967). The next step is to divide the time of the study into r sub-periods of the desired length. Only the areas with more than one case are considered. The statistic proposed is M, the maximum number of cases in a time period within a space area. This statistic was looked upon from the cell occupancy approach. For example, in an area in space there are five time periods. In this area there are five observed cases. These can be uniformly distributed over the five time periods (M = 1) but at the other extreme they can also occur within one time period (M = 5). The Expectation and Variance for M can be calculated. In the 1964 paper (Ederer et al. 1964) tables of the expectation and the variance of M were given in the case of five time periods. Further extensions were calculated by Mantel et al. (1976). Hence, it is possible to compare the actual observed M with its Expectation. To increase the power of the procedure all the M's for a space area are added and a summary Chi-Square statistic is evaluated at 1 degree of freedom:

$$S_1^2 = \frac{(\Sigma M - E[\Sigma M])^2}{V[M]} \qquad \text{with 1 degree of freedom.}$$

In the example used by EMM the space unit (a town) is still large, so if a significant space-time clustering was observed it may be necessary to verify that it were only a time clustering. Since the actual number of cases in an area are used rather than an Incidence figure, it is necessary to assume that the population at risk is similar in each unit. This technique is especially useful in the case where naturally independent units exist, i.e. towns, families or farms.

IV. Mantel regression method

This method uses all possible pairs of cases. The temporal distance is plotted against the spatial difference. A regression line is fitted through the pairs (Mantel 1967). Under the null hypothesis of no space-time clustering, a line with zero slope is expected. If there is a space-time clustering, a decreasing increasing slope is expected. To emphasize the importance of the smaller distances it was suggested to transform the distances in both time and space by taking the reciprocal. This transformation will blow up the small distances and reduce the large distances. If the absolute value of the reciprocal is used, an increasing increasing slope would be expected in case of clustering. To prevent an infinite reciprocal in cases of short distances in time or space it may be necessary to add a constant to X and Y before taking the reciprocal. The regression coefficient is defined as:

$$b_{DE} = \frac{\Sigma D_k E_k - [(\Sigma D_k \Sigma E_k)/n]}{\Sigma E_k^2 - [(\Sigma E_k)^2/n]} \qquad \text{, summation over k}$$

with Variance :

$$Var[b_{DE}] = [\Sigma(D_k - \bar{D})^2/n-1] \; / \; \Sigma(E_k - \bar{E})^2.$$

The test statistic is:

$$t = \frac{b_{DE}}{Var(b_{DE})}$$ with n-1 degrees of freedom.

Although the technique requires a uniform distribution of the population density, it has some attractive properties. It reduces the large distances and places more emphasis on small distances, but the actual values are still important. The interpretation is made easy by the approximation to the familiar regression approach. Graphical techniques can be used to explain the results. It is not necessary to choose cutoff points, although the required addition of a constant to prevent an infinite reciprocal is still a judgment call. The actual power of the method has not been worked out.

Other methods

Only the four most widely used methods were reviewed. Other statistical methods to detect space-time clustering are: the Pinkel and Nefzger exact probability test, the David and Barton methods and the Mustacchi persistent high rates method (PHR).

TWO EXAMPLES IN VETERINARY EPIDEMIOLOGY

Space-Time Clustering of Mastitis Outbreaks on Dairy Farms

Introduction and methods: Some forms of mastitis are contagious diseases, hence disease observations of cows on a given farm are not independent of each other. To study management variables, the individual cow is the sampled observation, but the farm is the unit of observation. The null hypothesis to be investigated is that farms in a study are independent of each other. Hence, no relationship is expected between a mastitis outbreak on a given farm and a mastitis outbreak on one of the nearby farms. In that case space-time clustering techniques should not give evidence of disease clustering.

Data were available for 34 dairy farms over a 4-year (48 mo) period. These farms were scattered around Ithaca, NY with some farms far from each other (up to 45 Km) and others within walking distance. These farms receive veterinary care from the ambulatory clinic of the New York State College of Veterinary Medicine. Farms close in space most likely receive veterinary service from the same practitioners. The farms cooperated in an intensive management project, encompassing frequent visits by technicians and sampling of animals and feed (Erb et al. 1985). Although sanitary precautions were secured, the project might act as a vector for transmission of disease.

Clinical mastitis was diagnosed by either the farmer or the veterinarian. No data were available on the microbiology of the mastitis cases. In this study, all first cases of mastitis per lactation are included. A mastitis outbreak was defined as: at least three times as many observed mastitis cases as expected in any 30-day period. The expected number of cases per 30-day period

was calculated using the average cumulative incidence of mastitis over the total period the farm was on the study, and the number of animals freshening in the 30-day period. It was shown previously (Schukken et al. 1988) that the great majority of cases of mastitis occur in early lactation; therefore, using the number of cows freshening as an indicator of the number of animals at risk was a reasonable assumption for the purpose of this study.

Three techniques were used to test for space-time clustering of the mastitis outbreaks: the Knox method, the ridit method and the Mantel regression method. Since three different techniques were used, the probability of finding space-time clustering, if it really was present, increased (power was high) of course, the probability of 'finding' a cluster if none truly existed (i.e. making a type I error) also was increased.

The time distance between two outbreaks was calculated as the number of months between the midpoints of the two outbreaks. The spatial distance was the length of the straight line connecting the two farms where the outbreaks occurred. This was calculated using the euclidean X and Y coordinates in the Pythagorean formula.

Results and discussion: The total number of outbreaks detected was 24 on 17 different farms. With the Knox method, the number of observed and expected pairs of farms with an outbreak close in space and time were calculated:

Table 1 Number of observed and expected mastitis outbreaks, Knox method

	< 1 month apart			< 2 month apart		
	observed	expected	p-value	observed	expected	p-value
< 5 Km apart	0	.55	1.0	0	.92	1.0
< 10 Km apart	0	1.56	1.0	1	2.61	.82

Because two threshold values for both time and space were chosen, there were four observed and four expected values. In none of these four cases did the observed and expected numbers differ significantly.

The average ridits for the five classes below 10 km and their 95% upper confidence are shown in Table 2.

Table 2 Ridits and 95% upper limit, ridit method

	Space distance (Km)				
	0 - 2	2+ - 4	4+ - 6	6+ - 8	8+ - 10
ridit	.55	.67	.63	.60	.56
upper limit	.72	.81	.80	.72	.66

None of the average ridits was lower than .5, hence the upper confidence limits were all higher than .5, indicating no relation between a short distance in time and a short distance in space.

The regression coefficients from regression of space on time and of 1/space on 1/time were not significantly different from zero. The regression equations were resp.:

Space = 20.96 * - .07(SD .06) * time (P > .2)
1/Space = .09 * - .04(SD .05) * 1/time (P > .4)
Figure 1 is a graphical representation of the space and time distances between all possible pairs of outbreaks. Note that the regression line in Figure 1 has virtually no slope.

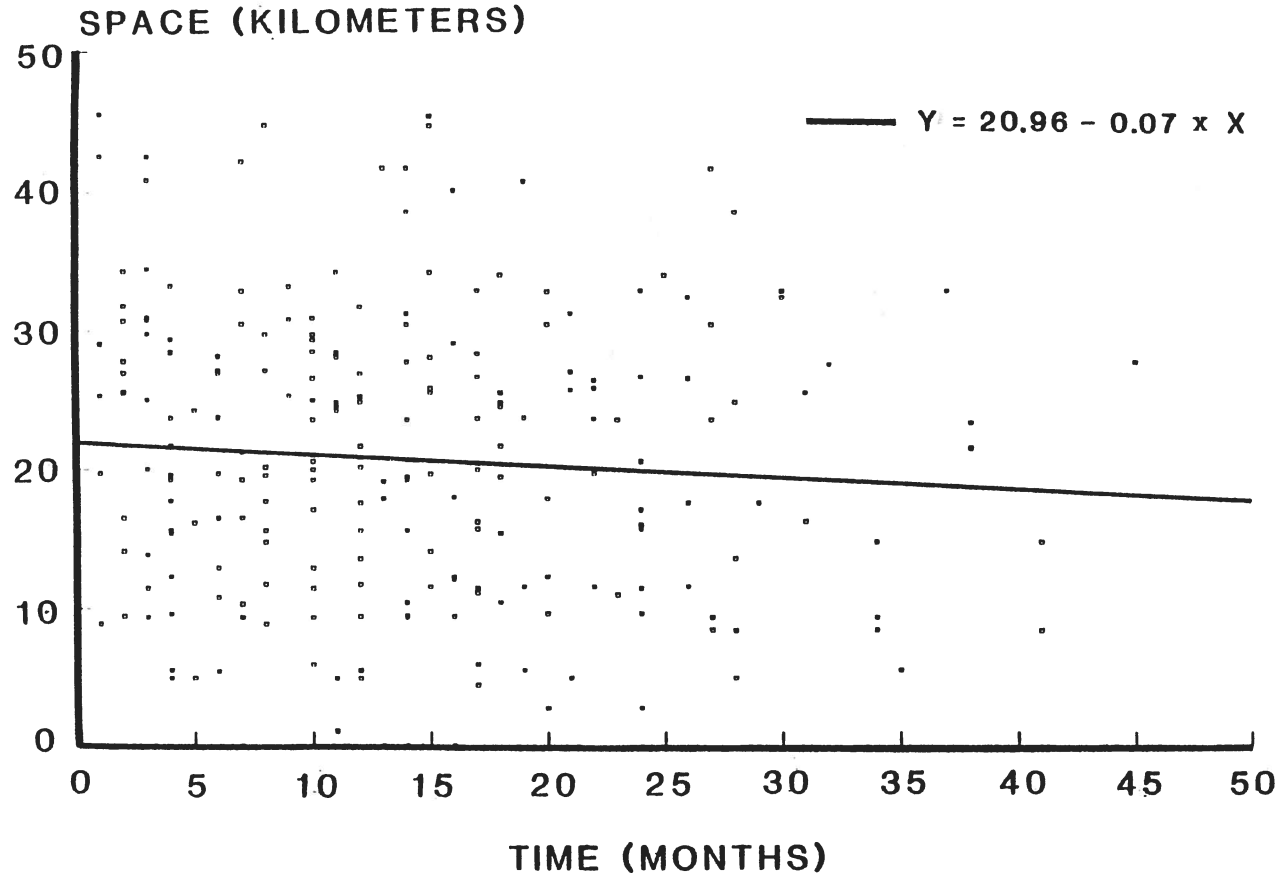

Fig. 1 Space and time distances between all possible pairs of mastitis outbreaks, Mantel regression method

The consistent result of no space-time clustering between mastitis outbreaks on dairy farms in this area is fairly strong evidence in favor of the independence of these events. The observational study in which the farms participated was not responsible for a space-time clustering of this infectious disease.

Space-Time clustering of winter dysentery outbreaks on dairy farms

Introduction and methods: Winter dysentery is an acute, highly contagious disease of adult cattle characterized by a brief,

explosive attack of diarrhea or dysentery. It usually occurs in housed cattle during the winter months and results in a moderate to marked drop in milk production. In affected herds, the attack rate may reach 100%, but fatalities are rare. The causative agent is unknown but is presently suspected to be a virus (Campbell and Cookingham 1978). Recent research has focused on corona virus as cause of this syndrome (Saif et al. 1988). If winter dysentery is due to an infectious agent such as a virus, the agent might spread between farms by traffic or airborne transmission and clusters of outbreaks in space and time might occur.

All dairy farms with 40 km of Ithaca, NY received a letter in august 1987 asking the farmers to inform us about any outbreaks. In January, a reminder was sent, and in may a last request was mailed. Farms not returning any of the forms were contacted by telephone. Space-time cluster analysis was done using the Knox method and the Mantel regression method. For the Knox method the cutoff value for time was 30 days and for space 5.5 km.

Results and discussion: Ninety-eight percent of the farms responded to the questionnaire. Thirty seven of the 113 farms reported an outbreak of winter dysentery; this was a cumulative incidence of 33%. Figure 2 shows the temporal distribution of the outbreaks.

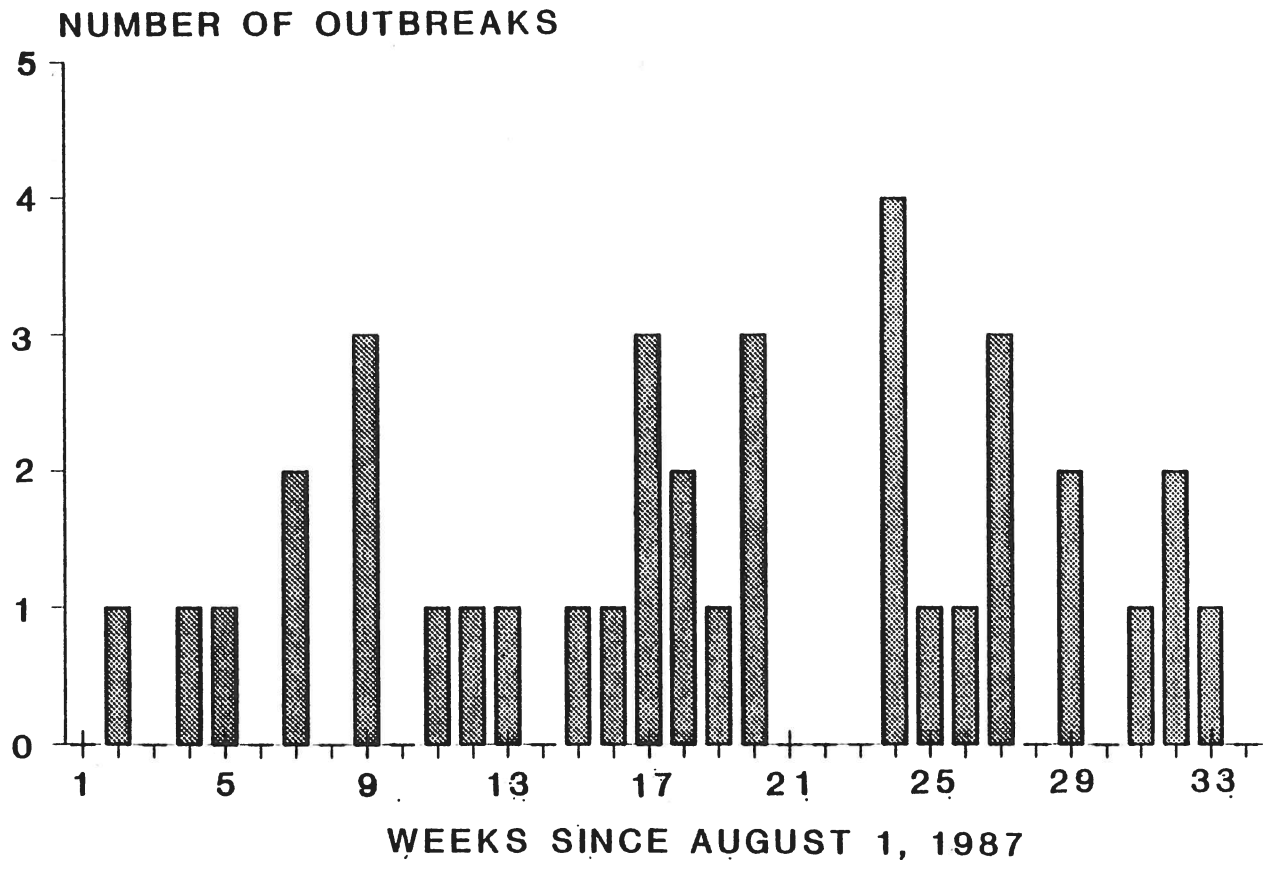

Fig. 2 Temporal distribution of winter dysentery

By the Knox method, there was significant space-time clustering of outbreaks within a 30 day time and 5.5 km space radius. Fourteen pairs of cases were below the cutoff value; if

there were no clustering, 9 would be expected (p=.041). The Mantel regression method also yielded significant clustering in space and time, the regression coefficient was .13 (SD = .088; p = .05).

It can be concluded that the syndrome of farmer diagnosed explosive diarrhea called 'winter dysentery' shows space-time clustering in the Ithaca, NY area. The data support a common cause of multiple outbreaks, consistent with the evidence that winter dysentery is due to an infectious agent.

DISCUSSION

Essentially all epidemiological studies on the occurrence of disease should use the rate of disease as the measure of outcome. An important drawback of several of the described statistical methods is the use of the epidemiological numerator (cases of the diseases) and ignoring the epidemiological denominator (population at risk). In some recent studies, Schulman, Selvin & Merill (Schulman et al. 1988, Selvin et al. 1988) introduced algorithms to generate density equalized map projections. These are geographical maps transformed to be proportional to the population density. The methodology has not yet been applied to space time clusters but may prove to be useful. Most of the methods described in this review can be applied to the density equalized maps.

In Table 3 the characteristics of the test described in this review are summarized.

Table 3 Comparison of the space time clustering techniques

	Feature:						
METHOD	1	2	3	4	5	6	7
Ridit method	yes	yes	yes	1	no	a,b	+
Knox method	yes	yes	yes	2	no	a,c	-
EMM	no	yes	yes	2	yes	a	++
Mantel Regression	yes	no/yes*	no/yes*	0,1*	no	a	-
Pinkel & Nefzger	no	yes	yes	2	no	a	-
David & Barton	no	yes	no	1	no	a	+++
Mustacchi PHR	no	yes	yes	3	yes	-	++

Features:
1 Uses all possible pairs
2 Transformation of time distances
3 Transformation of space distances
4 Number of arbitrary decision necessary (e.g. cutoff point)
5 Possibility of border problem (two close cases not recognized because of artificial borders)
6 Assumptions necessary:
  a Uniform spatial distribution of population
  b Uniform time distribution of cases within spatial distances
  c # of cases close in time and space have a poisson distribution
7 Other
  + problem of multiple comparisons
  ++ especially useful in natural sub populations (e.g. towns)
  +++ tedious calculating
* original values, reciprocals resp.

Some authors have tried to compare some of the methods described above. Aldrich (1981) could find no evidence for clustering in a sample of suspicious cases. He used the exact probability test of Pinkel & Nefzger, the Knox Method, Mantels regression approach and the EMM method. The tests agreed in their conclusions, this may be due to a lack in power of all the tests or to a real lack of space time Clustering. Mustacchi et al. (1967) compared the Ridit method, the EMM method and the Mustacchi persistent high rates (PHR) method. With all three techniques a significant space-time clustering was found; it was anticipated that the Ridit method might be the most powerful because it checks more than one small distance and it does not make arbitrary borders in the area of interest (this is necessary by both the EMM and the PHR method).

Several methods for the detection of space time clustering have been described. It is not possible to select the best method, and it may therefore be advisable to use more than one method in the analysis of possible disease clusters (Symons et al. 1983). Checking the assumptions that are necessary is an important consideration in selecting the appropriate statistical methods.

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A LONGITUDINAL STUDY OF ENTERIC PATHOGENS IN DAIRY CALVES

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Several enteric pathogens of calves are endemic to every farm and every animal is likely to become infected. Examples of such agents are rotavirus, coronavirus and Cryptosporidium. Other agents which may be included in this group, but for which insufficient data is available, include calici-viruses and verotoxin-producing E.coli (VT^+ E.coli). In experimental models, all these agents are pathogenic, causing diarrhoea, but in the field many infections occur without clinical signs (Morgan, 1990). The reasons for this discrepancy are unknown, but possible mechanisms include variation in strain virulence (Bridger & Pocock, 1986), interaction with active and passive immune mechanisms (Saif & Smith, 1985), the occurrence of multiple infections (Reynolds et al., 1986), and physiological compensation in the large intestine (Argenzio, 1984).

This study was designed to examine the excretion patterns of rotavirus, coronavirus, Cryptosporidium and VT^+ E.coli in calves up to 6 weeks of age on a normal dairy farm, with particular emphasis on the occurrence of mixed infections.

MATERIALS AND METHODS

The study was conducted on the dairy enterprise of the Institute for Animal Health, Compton, which consists of 2 herds, Cheseridge (200 cows) and Superity (150 cows); calving takes place throughout the year, but with the majority of calves born in the winter.

At Cheseridge, cows waiting to calve were placed in separate boxes, generally with adequate amounts of unsoiled bedding. A large number of calving boxes meant that they could be maintained in a hygienic condition. Calves remained with their mothers for approximately 48 hours after birth. At Superity, however, all cows due to calve were placed together in a fenced-off yard with access to limited shelter. Calves were born and nursed in the same area without adequate disinfection or changes of bedding. The calving area was in continuous use and cows waiting to calve used the area for shelter. Due to inadequate accommodation calves were separated from their mothers approximately 24 hours after birth.

After separation from their dams, calves were retained at their farm of birth for 4-14 days and fed a "colostrum pool" consisting of the surplus colostrum and reject milk available from the parlour each day. At Cheseridge,

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the follow-on calf pens were satisfactory with solid walls, adequate ventilation control and heat lamps were available for additional warmth. Calf pens at Superity were in an unenclosed area of a wide-span shed shared with older calves and the milking parlour. The pens had railed partitions and there were no facilities for heat lamps.

The main calf rearing unit, designated F17, was a purpose-built enclosed building with a forced ventilation system controlled by thermostat. Calves entered F17 earlier from Superity (their sixth day of life on average) than Cheseridge (their tenth day of life on average) due to the inadequate accommodation at Superity. Four rooms containing approximately 25 solid-sided pens each were used in rotation, thus allowing cleansing and disinfection between batches. Calves remained in F17 until one week after weaning (approximately 5 weeks of age) and then transfered to loose housing. A summary of calf movement is depicted in Fig. 1.

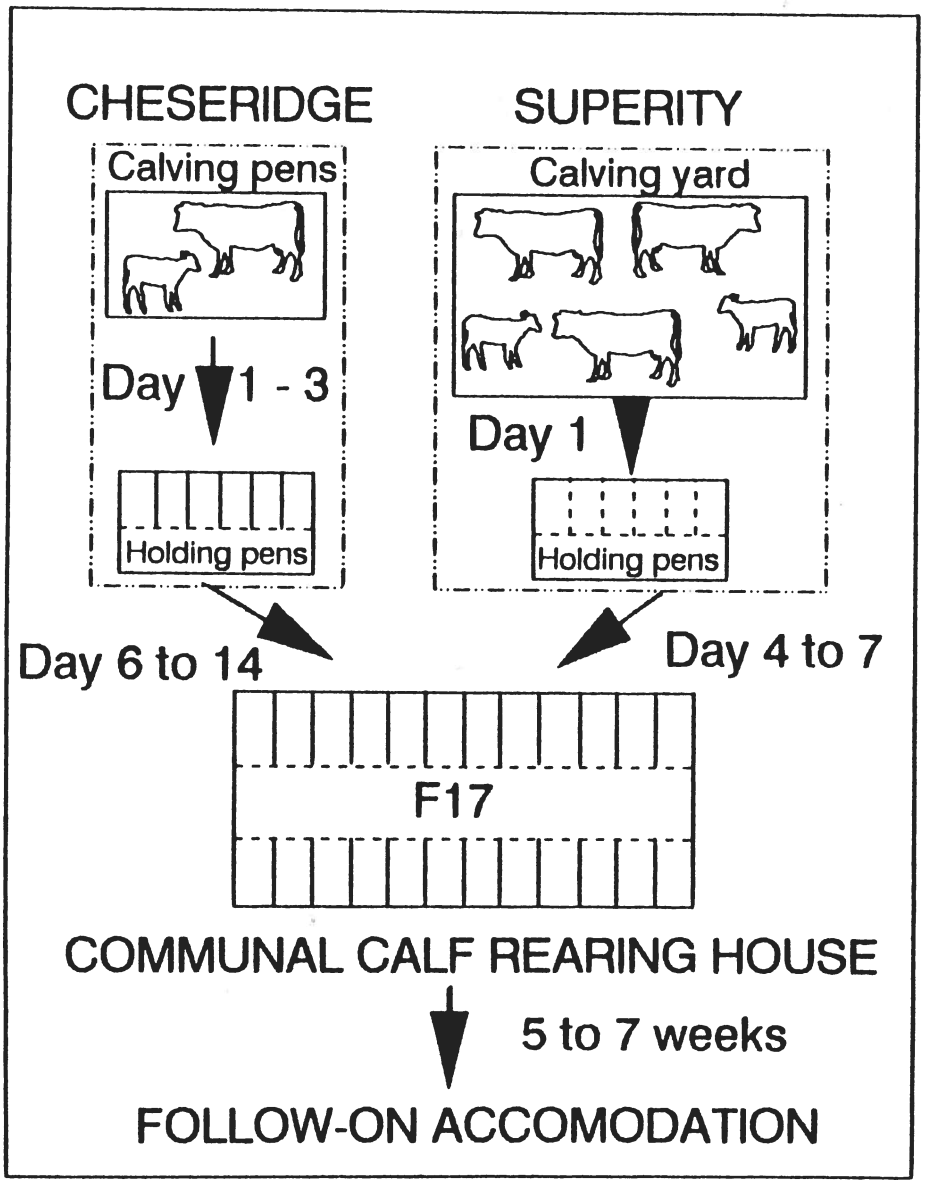

Fig. 1. Calf movements from birth to 8 weeks of age.

Calves were fed on warm milk substitute until weaning. They were offered hay and concentrates on entry to Unit F17, and weaned at 3-4 weeks of age when they were judged to be consuming adequate amounts of concentrate and hay.

A total of 96 calves were sampled over a period of 8 months from September 1987 to May 1988. These calves were sampled in three distinct batches of thirty calves chosen at random; batch 1 born between 11th September and 16th November (heifer calves only), batch 2 born between 6th January and 1st February (heifer and castrated male calves) and batch 3 born between 24th February and 9th April (heifer and castrated male calves). A further six calves (three which developed diarrhoea, three which remained healthy, born between 2nd and 21st February) were also selected. For each calf, a serum sample was taken soon after birth for ZST determinations as an indicator of absorption of colostral immunoglobulin. Calves were then observed daily from the day of separation from their dams until they were 6 weeks of age; health and appetite were recorded, and faeces samples collected. The faecal samples were assessed for consistency, colour, excessive bulk and presence of mucus and blood, each characteristic being given a numerical score. A "cumulative faecal score" was calculated by summing these scores over periods of diarrhoea. Diarrhoea was defined as mild if the cumulative faecal score was between 16 and 20; scores over 20 were classified as moderate diarrhoea.

All faeces samples were examined for rotavirus antigen by ELISA (in-house indirect test with polyclonal capture antibody, monoclonal antibody to vp6 as detector antibody, and goat anti-mouse peroxidase-linked secondary antibody) and for Cryptosporidium by modified Ziehl-Neelsen stain (Angus, 1987). Faeces from selected calves were examined for coronavirus by ELISA (Reynolds et al., 1986) and for VT^+ E.coli by the method of Karmali et al. (1985).

A small number of faeces samples from calves with diarrhoea were negatively stained and examined by electron microscopy for enteric viruses (Reynolds et al., 1984). A single calf that was destroyed in extremis was examined culturally for cytopathic and non-cytopathic bovine virus diarrhoea virus (Clarke et al., 1987).

RESULTS

Moderate diarrhoea was seen in 43 out of 96 calves (44.8%), mild diarrhoea in 17 calves (17.7%) and diarrhoea was not observed in 46 calves (37.5%). One calf in the study became moribund at 26 days of age due to diarrhoea and required euthanasia. Post-mortem examination of this calf revealed typical gross and microscopic lesions of mucosal disease. The diagnosis was confirmed by the isolation of cytopathic and non-cytopathic BVD virus from tissues. All other cases of diarrhoea were not severe, and responded to fluid therapy and treatment with astringents. Diarrhoea increased to a peak in January/February which was a period of intensive calving, and was particularly a problem in calves born at Superity. A clean up of the calf pens in the holding accommodation at this farm, and increased attention to hygiene throughout the calf rearing system lead to a decrease in cases of diarrhoea.

A negative correlation was found between cumulative faecal score and ZST value (correlation coefficient -0.226; $p < 0.05$). Hence a high ZST value is correlated to a low severity score and indicates that a high ZST value may be protective against diarrhoea.

Rotavirus excretion was detected in 92 out of 96 calves (95.8%); 26 calves (27.1%) showed a period of re-excretion. The average age at onset of the first excretion period was 10 days, ranging from 3 to 35 days. The mean duration of the first excretion period was 4.4 days (range 1 to 13 days). The periods of re-excretion occurred at a mean age of 27.3 days (range 16 to 40 days) and lasted from 1 to 4 days.

The number of animals excreting rotavirus in each batch did not fall below 93% (28/30 in batch 1). Rotavirus was present from the beginning of the sampling period to the end and the percent of calves infected did not show any seasonal variation. However, its behaviour did vary with season as calves born during January and February showed an earlier onset of excretion (mean 8.3 days of age, compared to 9.3 days in calves born September to November, and 12.5 days for calves born after the improvement in hygiene, $p < 0.05$). Re-infections with rotavirus also varied with season. Whilst only 20% (6/30) and 16.7% (5/30) of calves re-excreted rotavirus in batches 1 and three respectively, 53.3% (16/30) of animals re-excreted in batch 2.

Age at onset of rotavirus excretion was directly related to ZST value (correlation coefficient +0.202; $p < 0.05$), whereas duration of excretion was inversely related (correlation coefficient -0.244; $p < 0.02$) (Fig. 2).

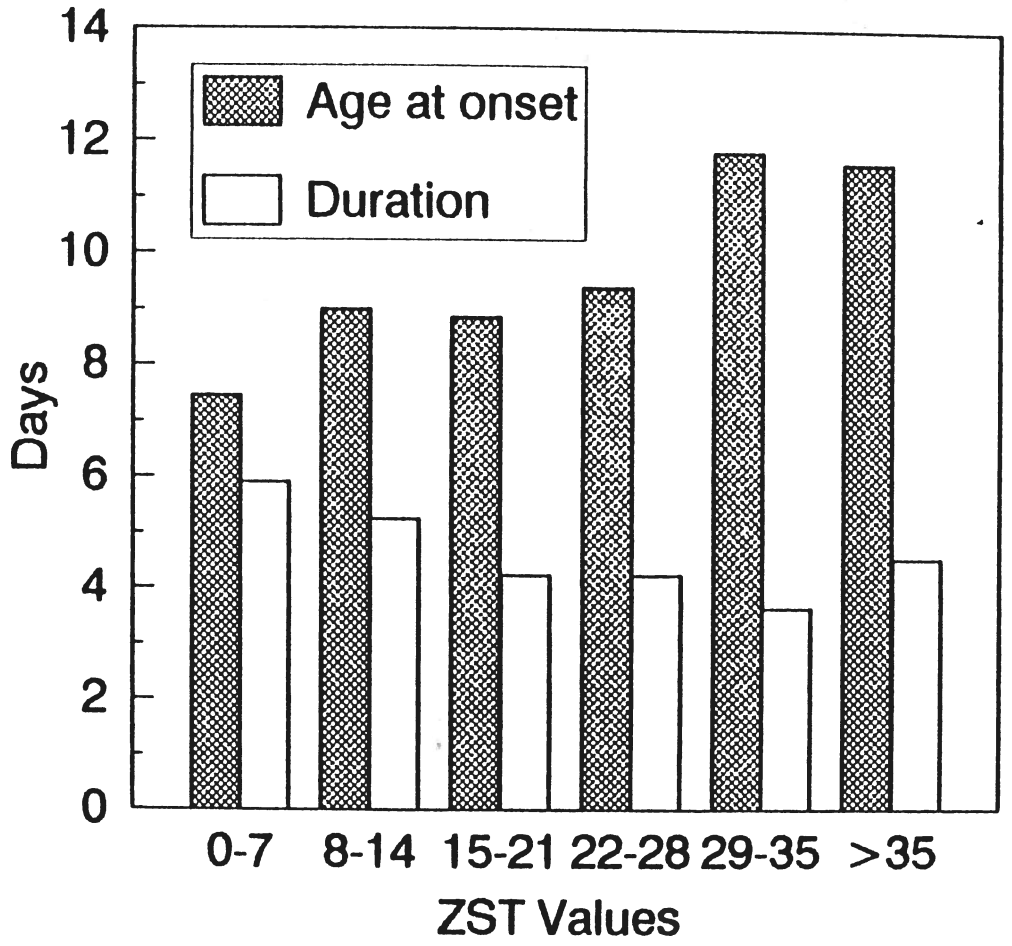

Fig. 2. Correlation of ZST values to onset and duration of rotavirus excretion.

There was a negative correlation between age at onset of excretion and severity of diarrhoea for those animals in which rotavirus excretion was not complicated by Cryptosporidium infection (correlation coefficient -0.552, $p < 0.001$), indicating that early onset is associated with more severe diarrhoea.

Cryptosporidium excretion was detected in 78 calves (81%). Average age at onset of first excretion was 12.9 days (range 7 to 26 days). The average duration of infection was 7.05 days (range 1 to 13). Re-excretion was detected in 6 calves (6.25%). Average age at onset of re-excretion was 31.3 days ranging from 22 to 41 days, and duration was 1 day in all cases. Cryptosporidium excretion was not detected in calves before mid-October but after its appearance it was detected in almost all calves (Fig. 3); 97% (64/66) of calves born between January and April were positive. Cryptosporidium infection was first found in older calves but spread rapidly to the younger ones (Fig. 3). No correlation was found between ZST and age at onset or duration of infection.

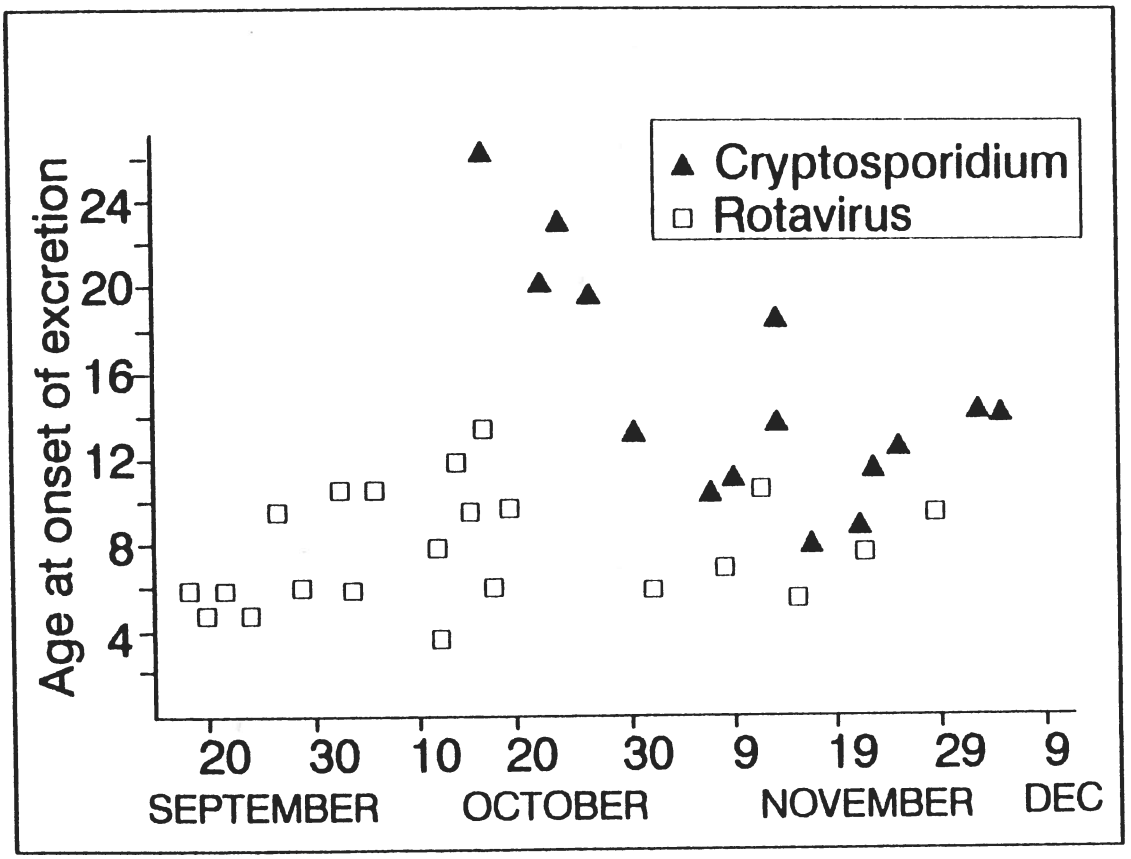

Fig. 3. Excretion of enteric pathogens in early-season calves.

Of the 78 calves that excreted Cryptosporidium, 33.3% (26/78) excreted it separately from rotavirus (i.e. at least 2 days between each infection). Most separate infections were not associated with diarrhoea (22 out of 26, 84.6%), in 2 calves, excretion was associated with mild diarrhoea, and in 2 calves, moderate diarrhoea was observed. No correlation was found between age at onset and severity of infection of calves with separate Cryptosporidium infections.

The association of diarrhoea with separate or mixed infections was studied for the rotavirus and Cryptosporidium results only (Fig. 4). Calves were

designated as having separate infections if one agent occurred in the absence of the other, or infections were separated by at least 2 days; mixed infections were defined as periods of excretion of rotavirus and Cryptosporidium overlapping by at least 2 days. Separate infections of rotavirus were more likely to cause diarrhoea (37% of these infections were associated with diarrhoea) than those of Cryptosporidium (15.4% associated with diarrhoea). Diarrhoea occurred in 75% of calves with mixed infections. Calves with diarrhoea associated with mixed infections had more severe clinical signs than calves with diarrhoea associated with rotavirus or Cryptosporidium only.

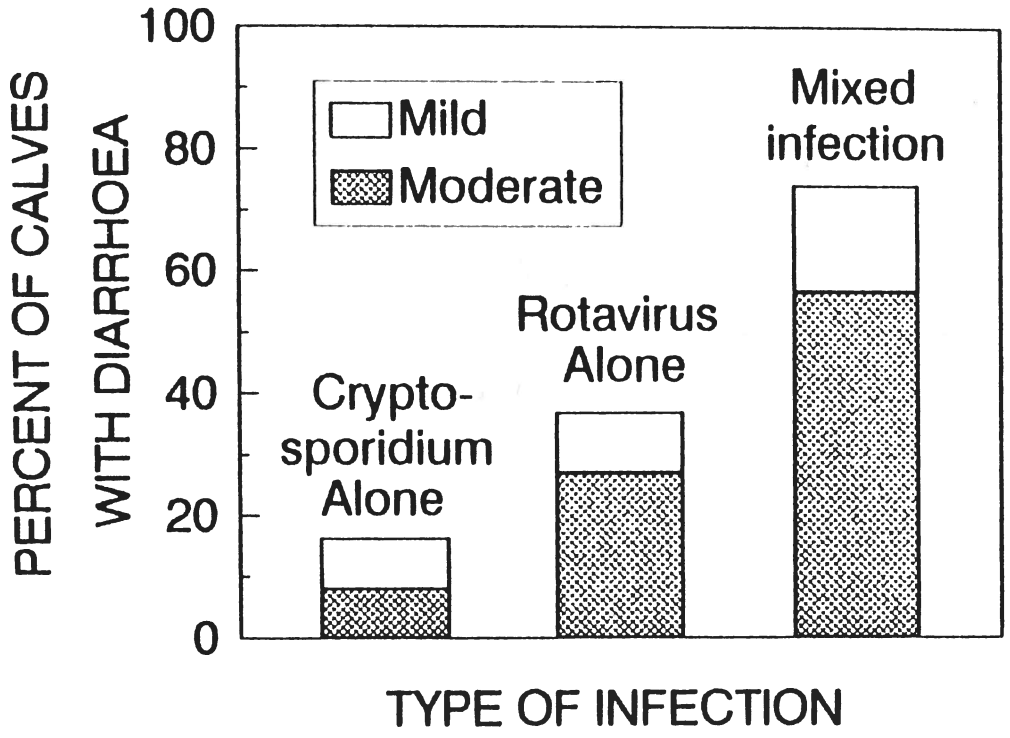

Fig. 4. Association of mixed and single infections with diarrhoea.

Using the "cumulative faecal score", 28 calves that showed no evidence of diarrhoea, and 24 calves that showed marked diarrhoea were selected as a case-control study. The number of calves from the diarrhoeic group with mixed infections was greater than the number of calves from the healthy group. Mixed infections occurred in 10 out of 24 calves with diarrhoea (41.7%) whereas only 6 out of 29 healthy calves (20.7%) suffered mixed infections.

The calves in the case-control groups were examined for coronavirus and VT^+ E.coli. Coronavirus was detected in only one calf by daily ELISA examination of faeces, and this infection was associated with watery diarrhoea. However, other calves suffering watery diarrhoea at the same time were also examined by electron microscopy, and coronavirus particles were detected in spite of negative ELISA results. Calici-viruses were detected in some of these calves.

Fifty-two calves were tested for VT^+ E.coli by examining every third daily faeces sample. They were all positive on at least one occasion; many calves

showed an intermittent pattern of excretion, which did not correlate with presence of diarrhoea.

The infections of a group of calves in adjacent pens are illustrated in Fig. 5.

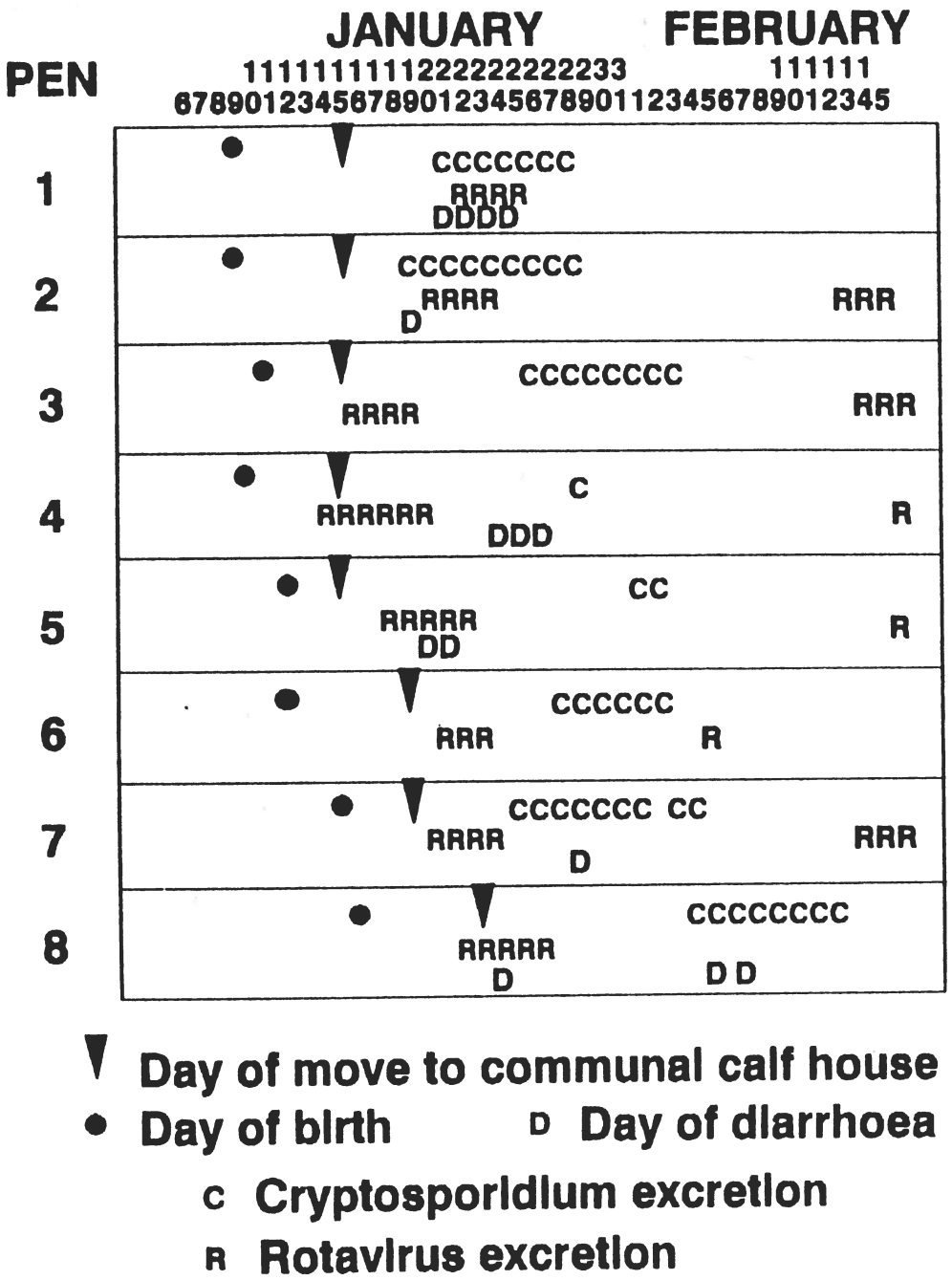

Fig. 5. The excretion of enteric pathogens in calves in adjacent pens.

No clear pattern of spread for rotavirus infection across the whole study was seen; calves often began to excrete rotavirus on the same day in different areas. However, Cryptosporidium infection apparently spread from pen to pen. Rotavirus excretion commonly occurred in the holding pens on the farms of origin, whereas most Cryptosporidium infections occurred in Unit F17, and it was rarely seen in the holding pens. The re-excretion of rotavirus is also of interest as calves in adjacent pens seem to re-excrete on the same calendar day rather than a particular day of life.

DISCUSSION

The study confirmed diarrhoea to be prevalent in a normal herd associated with the presence of endemic pathogens. Over 60% of calves experienced diarrhoea before their sixth week of life, although mortality was low and little veterinary help was deemed necessary. All but the calf with mucosal disease showed only mild degrees of clinical change even when diarrhoea was evident; however, this study is a useful model for studying the interactions of these pathogens.

Rotavirus was endemic within the herd showing no seasonality and rapid spread of infection throughout the housing. Rotavirus could be found at all times throughout the sampling period and was often identified in the calves within the holding pens. This illustrates the need for strict hygiene practices and good husbandry technique. The build up of infection in the housing was demonstrated by a decrease in the age at onset of excretion and an increase in the number of animals re-excreting before the end of their sixth week of life. This was particularly noticeable from the beginning of January until the clean up of the holding pens in mid February. The period of clean-up was beneficial to the number of cases of diarrhoea in the following weeks; this has also been noted by McNulty and Logan (1983).

Of particular interest was the correlation between ZST value and onset and duration of rotavirus excretion. Traditionally, maternally derived serum antibody was considered not to give any protection against rotavirus infection. However, the recent findings of Besser et al. (1988) have cast doubt on this and the observations of this study seem to support their findings. Hence although maternally derived serum antibodies do not prevent infection, they may affect age at onset, duration and severity of excretion.

The significance of mixed infections in determining whether or not infection with these endemic pathogens results in diarrhoea has been highlighted by this study. Calves with mixed infections are more likely to have diarrhoea, and rotavirus infection has been shown to be more severe when occurring concurrently with Cryptosporidium than in single or sequential infections. The results shown in Fig. 4 suggest a synergistic effect, rather than an additive one. Studies of outbreaks of calf diarrhoea have lead to the same conclusion (Morin et al., 1980; Reynolds et al., 1986; Hall et al., 1988). Single rotavirus infections were more severe than those of Cryptosporidium. This supports the observation of Snodgrass et al. (1980) that Cryptosporidium infection resulted in milder clinical disease.

The appearance of Cryptosporidium after the start of the winter housing period, and the change in rotavirus excretion patterns, may be contributing factors to the "build-up of infection" during the calving period, a phenomenon well recognized by veterinary practitioners, but not well documented. The

absence of Cryptosporidium infection in the autumn sampling period may indicate that the pathogen is maintained over the summer months outside the calf population. The parasite may maintain itself in the herd by transitory re-infection of yearlings and older cattle as indicated by Allen & White (1985). Cryptosporidium infection had a later onset than rotavirus, and was rarely found in the holding pens although after its appearance almost all calves excreted the parasite during the observation period; together with its absence during the autumn period, when calf numbers were low, this indicates that it is a less infectious agent than rotavirus. The brief re-excretion that occurs with rotavirus and Cryptosporidium is usually subclinical and may be missed if calves are not examined daily, but these episodes are probably of great epidemiological significance in maintaining the agent in the herd, and in boosting herd immunity. Such periods of re-excretion probably recur throughout life.

Considering the possible zoonotic nature of Cryptosporidium, its endemic nature and the lack of methods for its treatment and control, more knowledge of its epidemiology is required. The pathogen is always likely to be present in calf accommodation and the visitor is hence always at risk of infection.

The low frequency of detection of coronavirus in this study was surprising; it is known that animals in this herd seroconvert by 6 months of age and previous studies have detected this agent in 10-20% of calves of this age (Reynolds, 1983). Some problems were encountered with the ELISA test, as samples from calves with watery diarrhoea during this study were positive for coronavirus by EM but negative by ELISA. It is probable that the coronavirus ELISA is not sensitive, as a selection of these samples were also examined at CVL, Weybridge, and also found to be ELISA negative (data not presented); however, it is also possible that coronavirus is more common in older calves.

The behaviour of VT^+ E.coli in calves requires further research before their significance can be assessed. The study found that every calf examined excreted these agents intermittently. This has not been reported before as healthy calves have not been studied. The method used to detect the cytotoxin is sensitive with VT^+ E.coli being detected when they represent only 1/300 of the E.coli population (unpublished data). As this pathogen is possibly a zoonotic agent, its presence so commonly in calves is a cause for concern.

This study high-lights the problems of diagnosis of these endemic agents. Samples taken from calves in normal dairy calf rearing units on any one day are likely to reveal at least 50% positive for rotavirus in calves 7-10 days old, and similarly for Cryptosporidium in calves 10-20 days old (see Fig. 5). Diagnosis must rely on comparison of results in healthy and affected calves.

This study will form the base-line for interpretation of studies currently under way to examine the effects of husbandry manipulation on the epidemiology of enteropathogens, with particular interest in extended colostrum feeding. Eradication of these agents is not practical and control is effected by improved husbandry and by stimulating herd immunity, which require a thorough understanding of their epidemiology.

SUMMARY

Ninety-six calves were examined for faecal changes and excretion of enteric pathogens daily from birth to 6 weeks of age. Rotavirus excretion was detected in 96% of calves, and occurred throughout the study period. Cryptosporidium excretion was not detected in the early winter housing period, but after its appearance in mid-October, most of the calves became infected. Rotavirus excretion occurred at an earlier age than Cryptosporidium and was shorter in duration. Severity of diarrhoea associated with single infections of rotavirus was found to be greater than that of Cryptosporidium. Concurrent infection with both rotavirus and Cryptosporidium correlated strongly with the presence of diarrhoea. Severity of diarrhoea, and age at which calves acquired rotavirus infection, were related to colostrally-acquired antibody, but there was not such correlation with Cryptosporidium excretion. Coronavirus was only detected in a minority of calves, and excretion was associated with watery diarrhoea. Fifty-two calves were examined for verotoxin-producing E.coli; all were positive on at least one occasion, but presence of these agents did not correlate with the presence of diarrhoea.

ACKNOWLEDGEMENTS

We thank Dr. J.C. Bridger for electron microscopy, Mr. M.C. Clarke for isolation and identification of BVD viruses and Dr. G.J. Rowlands and Mrs. K. Bunch for advice on data analysis. This study would not have been possible without the full co-operation of Mr A. Bloor and his farm staff (especially the late Mr. D. Verinder). Figure 5 is reproduced with permission of the editor of In Practice.

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I wish to be elected to membership of the Society for Veterinary Epidemiology and Preventive Medicine.

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Please tick the appropriate boxes to indicate your interests:

- ☐ Computing, including data-logging
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The annual subscription is £10.

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.

4. Candidates for election must return a completed application form. The Secretary will then circulate the names of candidates on the agenda for the next general meeting. Election of candidates will be by a simple majority vote of members present at the general meeting.

5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than four ordinary elected members. However, the Committee will have powers of co-option.

ELECTION

8. The election of office bearers and ordinary committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Election will be by simple majority of members voting at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the annual general meeting and will be decided by a simple majority vote of members present at the annual general meeting.

10. The Honorary Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. He will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received

by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or the Honorary Secretary.

11. Two auditors will be appointed annually by members at the annual general meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the annual general meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendation of members. The annual general meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not be reported either in part or in whole without the written permission of the Executive Committee.

15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.

17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.

18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.

19. In case of an equal division of votes, the Chairman of the meeting will have a second or casting vote.

20. All members on election will be supplied with a copy of this constitution.

21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.

22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

April, 1982
Revised March, 1985; April, 1988