An experimental model to quantify the transmission of *Mycoplasma gallisepticum*

A. Feberwee[1], D.R. Mekkes[1], D. Klinkenberg[2], H. Vernooij[2], A.L.J. Gielkens[1] & J.A. Stegeman[2]

1. Animal Health Service Ltd., Deventer, the Netherlands * www.animalhealthservice.nl

2. Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht, the Netherlands

ABSTRACT

Transmission dynamics in a flock can be quantified by R_0 (Reproduction ratio) and ß (transmission rate). In this study the transmission dynamics of *M. gallisepticum* (Mg) was investigated experimentally. The study was carried out with different inoculation doses (10^2, 10^5 en 10^8 cfu/ml) of a recent Dutch Mg field strain. For every inoculation dose, 10 pairs of chickens were housed, each pair in a separate cage. Every pair consisted of an infected chicken (I) and a susceptible contact chicken (S1). Between pairs, 5 susceptible individually housed chickens (S2) were placed. Detection of infection was carried out by serology, quantitative PCR and culture.

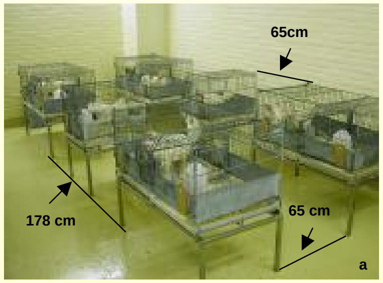
The results showed that the inoculated and contact-infected chickens were equally infectious, and that the pairs could be regarded as independent. The R_0 was estimated as ∞ (95% C.I. = 4.5-∞) and the estimated ß was 0.22 per day (95% C.I. = 0.16-0.32). There was no significant difference between inoculation doses. In conclusion, this infection model is suited to establish the quantitative effect of intervention measurements on transmission parameters R_0 and ß.

INTRODUCTION

Mg is responsible for significant economic losses in poultry industry. Current measures to control Mg includes vaccination. When the aim of vaccination is to eliminate Mg from a population transmission has to be quantified. Transmission dynamics in a flock can be quantified by two transmission parameters: i) the average number of secondary cases infected by one typical infectious case (R_0) and ii) the number of new infections that occur due to one infectious animal per time unit (ß). Until now no transmission experiments for Mg have been carried out. Before intervention measures can be tested, a suitable experimental model should be available with respect to inoculation dose, homogeneity and duration of the experiment. A pair wise design was used to link contact chickens to specific infectious chickens. Three different inoculation doses were applied. The assumptions included in the analysis were tested and R_0 and ß were estimated.

MATERIAL & METHODS

- Ø There were 3 experimental groups representing 3 different inoculation doses (10^2, 10^5 en 10^8 cfu/ml) of a recent Dutch Mg field strain. The climate was standardized.
- Ø Each experimental group consisted of 25 SPF White Layer hens of 26 weeks of age. Twenty chickens were housed in pairs. Each pair consisted of an infected (I) and a susceptible contact chicken (S1). The cages were housed at 65 and 178 cm from each other. Between pairs, at a distance of 65 cm, 5 susceptible individually housed chickens (S2) were placed to measure effect of airborne transmission.
- Ø One control group of 5 chickens (S0) was added in a separate group in order to exclude influence of the used test methods.
- Ø Detection of infection was carried out by serology (blood samples), quantitative PCR and culture (trachea swabs). The experimental period was 35 days.
- Ø The model was first validated with respect to the assumptions of independency between pairs, homogeneity of excretion of I and S1and equal infectiousness of I and S1.
- ØThe transmission parameters R_0 and ß were quantified by the use of the SIR model[1] $R_0 = -2x/(x-2)$ and $\log b = \log(2\Sigma D t_i) - \log\Sigma x_i$.



a. Housing

b. Trachea swab

c. Quantitative PCR device

d. Sample preparation

RESULTS

Fig. 1. Analysis of homogeneity and independency

Results analysis of homogeneity of excretion :

Statistical differerence of the sum of four random observations per group from the first moment excretion was measured. Statistical analysis Kruskall-Wallis Sum test (*P-value*)

	Group 1 10^3 cfu Mg	Group 2 10^5 cfu Mg	Group 3 10^8 cfu Mg
I/S1[2]	0.096[1]	0.23	0.62
I/S2[3]	0.0021	0.022	0.00082
S1/S2[4]	0.0078	0.22	0.0013

1. P <0,05 is significant; 2. Difference between infected (I) and contact chickens (S1); 3. Difference between infected (I) and airborne exposed (S2) chickens; 4. Difference between contact (S1) and airborne exposed (S2) chickens.

Results analysis of independency of excretion :

Independency of pairs was analyzed with a linear regression model : Y = C + a1 x1 + a2 x 2

Statistical Program R, version 1.6.2.

ØThe estimated regression coefficient a2 for the S2 chickens is 0.007 (*P-value* = 0.0003)

Ø The estimated regression coefficient a1 for the S1 chickens is 0.71 (*P-value* = 0.0006)

Fig. 2. Mean $^{10}\log$ excretion levels 10^2 cfu Mg

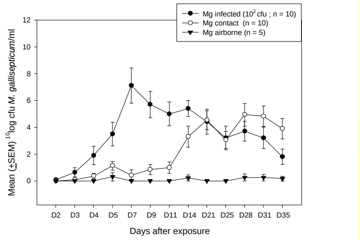
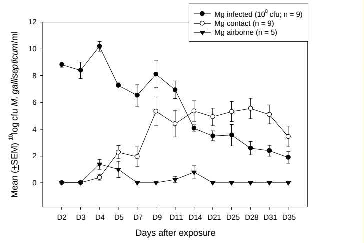


Fig. 3. Mean $^{10}\log$ excretion levels 10^8 cfu Mg



<u>i) Observation of transmission ii) analysis of infectivity, homogeneity and independency of excretion iii) estimation of transmission parameters</u>

- Ø Of all groups, only one contact chicken in the 10^8 cfu inoculation group escaped infection. There was no difference in the susceptibility between the I and S1.
- Ø In all groups airborne transmission occurred. Mean excretion curves of S2 was lower than that of S1 (Fig. 2 and 3). The S0 were negative until D35.
- Ø The epidemic process was not ended within the experimental period, I and S1 were still excreting Mg at the last sampling moment (Fig 2 and 3).
- Ø Analysis of independency showed that the pairs can be analyzed as independent observations (Fig. 1).
- Ø Analysis of homology of excretion showed that the excretion of I and S was not significantly different : I and S1 can be regarded as equally infectious (Fig. 1).
- Ø As only one contact chicken escaped infection R_0 = >1 in all groups and no significant differences were observed between the different groups.
  For group 10^2 and 10^5 cfu/ml Mg R_0 was estimated to be ∞ (95% C.I. = 4.5-∞) for group 10^8 cfu/ml Mg R_0 was estimated to be 16.0 (95% C.I. = 2.1-710).
- Ø ß did not differ significantly between three excretion thresholds and four latent periods, the estimated ß being 0.22 per day (95% C.I. = 0.16-0.32).

CONCLUSIONS

- Ø The model was validated with respect to the assumptions of homogeneity, infectiousness and independency included in the analysis.
- Ø In this infection model the conditions for the estimation of R_0 and ß were met. The estimated R_0 is ∞ (95% C.I. = 4.5-∞) and the estimated ß is 0.22 per day (95% C.I. = 0.16-0.32).
- Ø Airborne transmission does occur over short distances, however the amount of Mg excreted by S2 chickens is lower than that of S1.
- Ø On basis of the excretion levels of S1 and S2 it was possible to conclude that the excretion levels of S2 was mainly determined by the I in the same cage. Otherwise airborne transmission would have been included in the model.
- Ø The observation that airborne transmission only occurs over a short distance and at a low level cannot be extrapolated directly to the field. The ventilation rate per chicken was much higher than under field conditions.
- Ø Preventive vaccination program for Mg in layer stock will only contribute to a reduction of Mg transmission in a situation that vaccination will reduce R_0 = < 1. In this situation the infection will fade out.
- Ø The animal model meets the conditions for the establishment of transmission dynamics of Mg. Therefore this model can also be used to establish the quantitative effect of intervention measurements (e.g. vaccination).

1. Becker, N.G. (1989). Analysis of infectious Disease data. Monographs on statistics and applied probability. 1st ed., vol.1. London: Chapman and Hall Ltd, 1989.