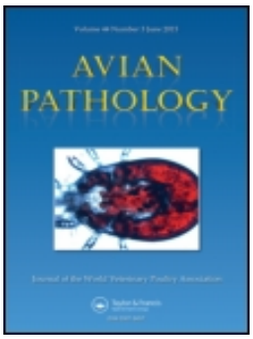

Avian Pathology

ISSN: 0307-9457 (Print) 1465-3338 (Online) Journal homepage: www.tandfonline.com/journals/cavp20

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To cite this article: Kannan Ganapathy & Janet M. Bradbury (1998) Pathogenicity of *Mycoplasma gallisepticum* and *Mycoplasma imitans* in red-legged partridges *(Alectoris rufa)* , Avian Pathology, 27:5, 455-463, DOI: 10.1080/03079459808419369

To link to this article: https://doi.org/10.1080/03079459808419369

Avian Pathology (1998) **27**, 455–463

Pathogenicity of *Mycoplasma gallisepticum* and *Mycoplasma imitans* in red-legged partridges (*Alectoris rufa*)

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Groups of 3-day-old red-legged partridges were infected intranasally either with the S6 strain of *M. gallisepticum* or with an *M. imitans* strain from a partridge with sinusitis. Starting 6–8 days post-infection (p.i.) birds in both groups developed signs of depression, nasal exudation, tracheal râles, sneezing, gasping, head shaking, watery eyes and eye scratching. The most outstanding feature was bilateral swelling of the infraorbital sinuses. Morbidity reached 100% in the *M. gallisepticum* infection and 80% in the *M. imitans* infection and mean clinical scores in the former were significantly greater than those of the latter group on days 11 and 14 p.i. There was also slower recovery in the *M. gallisepticum* infection. Necropsies at weekly intervals for 5 weeks revealed nasal and sinus exudate in both groups but tracheal exudate and cloudy airsacs were seen only in *M. gallisepticum* infection. *M. gallisepticum* was isolated from both upper and lower respiratory tract throughout the experiment while *M. imitans* was recovered less frequently from the upper respiratory tract and from the lungs and air sacs only at 7 days p.i. The numbers of isolations from eyes, tracheas, lungs and thoracic air sacs of the *M. gallisepticum* group were significantly greater than those from the *M. imitans* group. Seroconversion occurred in both groups using homologous antigen.

Introduction

Mycoplasma gallisepticum (Mg) is well known as an economically important respiratory pathogen that causes mild to severe disease in chickens and turkeys (Jordan, 1996). Upper respiratory tract disease, similar to that caused by Mg in turkeys, has been noted in partridges, and pheasants in the UK and USA for many years (Gianforte *et al.*, 1955; Wichmann, 1957; Osborn & Pomeroy, 1958; Keymer, 1958, 1961) and has also been reported in Australia (Reece *et al.*, 1986). Mg was isolated and identified in the cases reported by Reece *et al.* (1986), but the other reports preceded the availability of reliable identification methods.

The condition has been causing more concern in the UK of late (J. M. Bradbury, unpublished) and in 1994 Cookson and Shivaprasad reported a field outbreak of Mg infection in California in chukar partridges, pheasants and peafowl. The source of infection was thought to be either chickens brought onto the farm 2–4 months prior to the outbreak or breeder chukars brought in from the field. McMartin *et al.* (1996) described another outbreak of Mg infection in chukar partridges in California and were able to reproduce the disease experimentally in mature mycoplasma-free chukar partridges.

Outbreaks of upper respiratory disease (conjunctivitis and/or sinusitis) in UK pheasants and partridges have been examined for mycoplasmas over the last 14 years (Bradbury *et al.*, 1996). Mg was isolated from approximately 13 per cent of these outbreaks but several different fast-growing mycoplasma species were isolated, including *M. glycophilum*, *M. pullorum* and *M. gallinaceum*, and were thought to be interfering with Mg isolation. The number of Mg positive outbreaks increased to 28% when a PCR assay was used retrospectively on stored material. Thus, the burden of evidence still points to Mg as the one of the main causes of the clinical condition in the field although until the recent report of McMartin *et al.* (1996) there

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Received 29 September 1997. Accepted 17th December 1997.

0307-9457/98/050455-09

appeared to be no attempt to reproduce the disease in pheasants or partridges under experimental conditions using a properly characterised strain of Mg. An early attempt to reproduce the condition was reported by Keymer (1961) who produced severe sinusitis in an adult pheasant and an adult partridge (species unspecified) by inoculating them into the sinus with sinus exudate from a turkey which had been similarly inoculated with exudate from a field case in pheasants.

Other workers have reported the occurrence of mycoplasmas in game birds. Shimizu *et al.* (1979), in a survey of various birds in Japan, including pheasants and partridges, did not isolate Mg, but found *Mycoplasma pullorum* in green pheasant and *Mycoplasma gallinarum* in a bamboo partridge. Poveda *et al.* (1986) isolated *Mycoplasma synoviae* from the tracheas of red-legged partridges with respiratory signs which were in contact with infected chickens in Spain. In a cultural survey of birds in southern Spain Poveda *et al.* (1990) reported isolation of *Mycoplasma gallinaceum*, *Mycoplasma gallinarum* and *Mycoplasma iners* from pheasant. Kempf *et al.* (1991) isolated *M. pullorum* from pheasant breeders and Gerlach (1994) summarised the species found in game birds, adding *Mycoplasma glycophilum* to the list. However, little information is available on the prevalence of disease attributable to any of these species.

In 1985 a mycoplasma, now known as *Mycoplasma imitans* (Mim), was recovered from the eyes and infra-orbital sinuses of a red-legged partridge (*Alectoris rufa*) and a common partridge (*Perdix perdix*) in the UK, both of which were suffering from sinusitis. An identical organism was also isolated in France from ducks and geese (Dupiellet, 1988) and had originally been identified as Mg (Buntze *et al.*, 1986) due the strong serological relationship existing between these two mycoplasmas. Subsequently, Mim was found to share many phenotypic and antigenic properties with Mg, but to be only 40% related at the genome level (Dupiellet *et al.*, 1988). It was eventually fully characterised as a distinct new species (Bradbury *et al.*, 1993).

Mim, like Mg, possesses a tip-like structure which mediates its attachment to the respiratory mucosa (Abdul-Wahab *et al.*, 1996). Attachment is thought to be the first step in the pathogenic process (Razin, 1985). Mim causes ciliostasis of duck and chicken embryo tracheal organ cultures and mortality of duck and chick embryos (Abdul-Wahab *et al.*, 1996), properties which are considered to indicate its potential pathogenicity in avian hosts. A UK strain of Mim was found to be mildly pathogenic for turkey poults and disease was exacerbated in the presence of turkey rhinotracheitis virus (Ganapathy *et al.*, 1998). In chickens uncomplicated infection did not cause disease, but there was evidence of synergism in a dual infection with infectious bronchitis virus (Ganapathy & Bradbury, 1996). Despite the isolation of Mim from partridges no pathogenicity studies have been conducted in this host. Furthermore, no experimental studies appear to have been conducted of the pathogenicity of Mg for the red-legged partridge, which is commonly reared in the UK.

The experiment described here was carried out to investigate the pathogenicity of Mg and Mim in uncomplicated infections of young red-legged partridges. For the Mg infection the S6 strain was used since it is well characterised for its pathogenicity for turkeys and chickens under experimental conditions. For the Mim infection a UK partridge isolate was used.

Materials and Methods

Partridges

Forty-nine 2-day-old partridge chicks were obtained from a commercial farm with no history of mycoplasma infection. For further confirmation of the mycoplasma-free status oesophageal and yolk sac fluid swabs from 20 dead-in-shell embryos from the same parent flock were cultured for mycoplasma. In addition, swabs for mycoplasma culture were taken from the choanal cleft of five chicks on arrival. All these samples proved to be negative.

The partridges were housed in wire colony cages on thick plywood floors surrounded by a 0.5 m high circular wall of corrugated cardboard. Overhead electrical heaters were used which, in addition to fluorescent lights, gave some light throughout the day. On day 5, wood shavings were placed on the floor. Water was provided *ad libitum* in small partridge drinkers. Food was mixed with grit and given *ad libitum*.

Mycoplasmas

The origin of Mg S6 was described by Zander (1961). Since its arrival in our laboratory, it had undergone nine *in vitro* passages after a single *in vivo* passage through mycoplasma-free turkeys (Power & Jordan, 1976) and was known to be virulent for young turkeys at the ninth *in vitro* passage.

Mim strain B2/85 of UK origin was used. After its original isolation from the eye of a red-legged partridge with sinusitis, it had undergone 11 *in vitro* passages and five *in vivo* passages through mycoplasma-free turkey poults (Ganapathy *et al.*, 1998). It had then been passed three times through SPF chicks and the inoculum was a pool made by suspending material collected on swabs from the right sinus, upper trachea, thoracic air sac and lung of the chicks.

The inocula were checked for bacterial contamination by plating 20 μl of broth onto blood agar and a viable count was determined (Bradbury & Jordan, 1971) before and after inoculation.

Mycoplasma media

The mycoplasma broth (MB) and agar (MA) used in this experiment were as described by Bradbury (1977).

Experimental design

On arrival the partridge chicks were divided randomly into two groups of 20 and a control group of nine. On the following day, i.e. at 3 days of age, one group of 20 chicks was inoculated intranasally with 0.05 ml of Mg culture [8.0×10^4 colony-forming units (CFU)/bird], and the other with 0.05 ml of Mim culture (3.8×10^5 CFU/bird). Birds in the control group were similarly inoculated with 0.05 ml of sterile MB.

The birds were observed daily for clinical signs and every 3 or 4 days the beaks were gently squeezed, and the main clinical signs were

scored according to the following scheme: 0 = absence of clinical signs; 1 = clear nasal exudate; 2 = turbid nasal exudate; 3 = as 2 plus swollen infra-orbital sinuses and/or conjunctivitis. Mean clinical scores (MCSs) were calculated for each day of examination. Signs such as râles, sneezing, gasping for air, eye scratching and head shaking were also recorded.

Three birds from each infected group and two controls were selected at random on days 7, 14, 21, 28 and 35 post-infection (p.i.) for post-mortem examination. (Only one control bird was available for sampling on day 35 p.i.). Chicks were killed by intra-abdominal injection of barbiturate and external and internal abnormalities were recorded. Lesions in the infra-orbital sinuses and trachea were scored as none (0), mild (1), moderate (2) and severe (3). Lesions of thoracic air sacs were scored on a scale of 0–4 as described by Kleven *et al.* (1972) (0 = absence of lesions; 1 = slight cloudiness; 2 = thickened air sac membrane with small accumulation of exudate; 3 = air sac obviously thickened, meaty consistency and large accumulation of cheesy exudate confined to a single airsac; 4 = as in 3, but lesions in two air sacs or more). Mean lesion scores (MLSs) were calculated for each group.

Pieces of trachea were taken for histopathology. They were placed in Bouin's solution for 24 h then into 70% alcohol. After processing, sections of 4–5 μm were made and stained with Mayer's haemotoxylin and eosin (H&E). Samples from thoracic air sacs were also taken for histopathology on day 28 from the Mg infected group.

Swabs were taken from the eyes, infra-orbital sinuses, upper and lower trachea, thoracic air sacs and lungs for mycoplasma isolation as described below.

On day 26 p.i., blood was collected from six birds in each group and the sera examined by the rapid slide agglutination (RSA) test using stained antigen of Mg strain S6 (Intervet, Boxmeer, Holland) and Mim strain 4229 (Sanofi Diagnostics Pasteur, Inc, Watford, Herts). The procedure recommended by the manufacturer was followed in each case.

Mycoplasma isolation and identification

Swabs were plated onto MA and then inoculated into 1 ml of MB. MB was incubated at 37°C and assessed for colour change for 7 days. Broths that changed colour were immediately plated onto MA and the remaining broths were plated on day 7. MA plates were incubated in a CO_2 incubator and observed for mycoplasma growth at weekly intervals for a maximum of 3 weeks.

Representative numbers of isolates from upper and lower respiratory tract were identified by indirect immunofluorescence (Rosendal & Black, 1972).

Statistical analysis

MCSs and MLSs were analysed using the Mann–Whitney *U*-test (SPSS® for Unix™, SPSS, Chicago, IL, USA) to detect any significant differences between the two groups. The differences in total numbers of birds with macroscopic lesions and also those with positive isolation were analysed by chi-square or Fisher's exact tests, as appropriate.

Results

Clinical signs

No clinical signs were seen in the birds in the control group. In the Mg infected group, two partridge chicks were found dead (days 10 and 19 p.i.) and two others were killed by injection of barbiturate on day 15 p.i. due to severe bilateral eye occlusion. One chick in the Mim infected group was found dead on day 8 p.i.

The birds in both infected groups showed signs of depression, râles, sneezing, watery eyes (Figure 1), eye scratching, head shaking, gasping and swollen sinuses (Figures 2 and 3). Almost 10% of the Mg infected chicks showed moist nares with clear exudate as early as day 6 p.i. (data not shown). In the Mim infected chicks nasal exudation was detected from day 8 p.i. onwards. Figure 4 shows the overall percentage of birds affected in

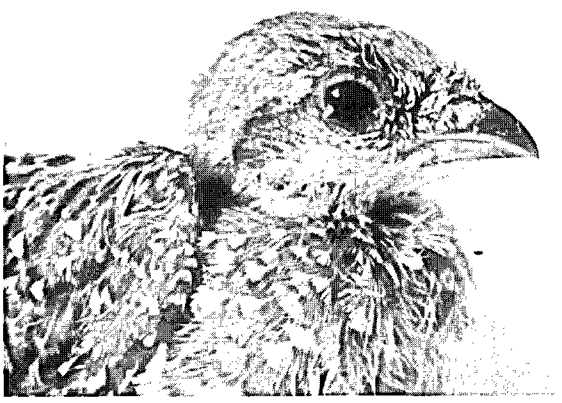

Figure 1. *Partridge showing watery eyes 11 days after infection with* M. imitans.

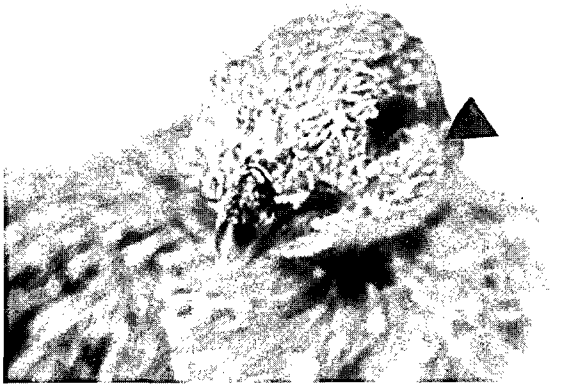

Figure 2. *Partridge with bilateral swelling of the infra-orbital sinuses (arrow) 11 days after infection with* M. gallisepticum.

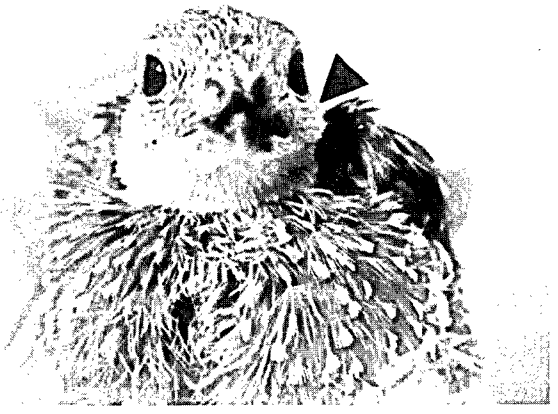

Figure 3. *Partridge with slight swelling of the infra-orbital sinuses (arrow) 11 days after infection with* M. imitans.

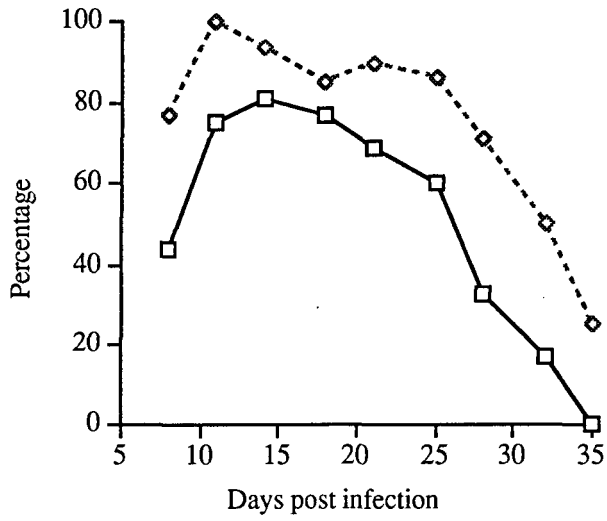

Figure 4. *Percentage of partridges with clinical signs in the mycoplasma infected groups.* □ *Mim,* ◇ *Mg.*

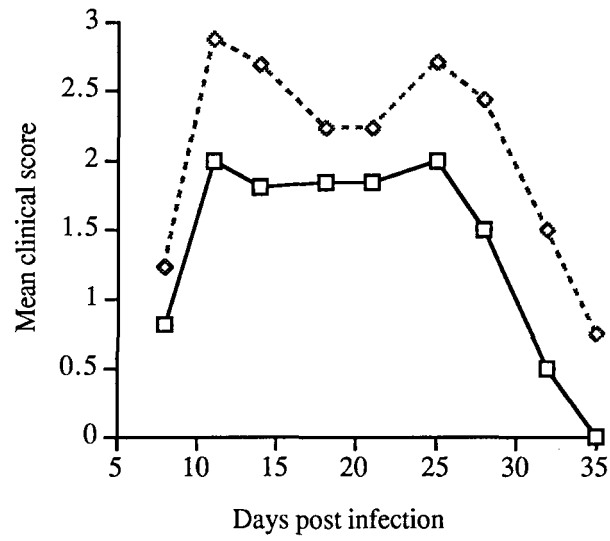

Figure 5. *Mean clinical scores of the partridges in the mycoplasma infected groups.* □ *Mim,* ◇ *Mg.*

each of the two groups and Figure 5 shows the mean clinical scores from day 8 p.i. The clinical manifestations were more severe and prolonged in the birds infected with Mg than those infected with Mim. The Mg infected birds had consistently higher MCSs than those with Mim infection however only on days 11 and 14 p.i. were they significantly ($P < 0.05$) higher.

Table 1 gives a breakdown of the percentage of birds showing certain clinical signs (râles, watery eyes or swollen sinuses) on selected days p.i. Râles occurred from day 8 to 11 in the Mim infected chicks, but in Mg infected chicks were first noted on day 11 p.i. and were detected on all subsequent examination days except day 32 p.i. The percentage of partridges with watery eyes was higher in the Mg than the Mim infection, reaching a maximum of 70% in the former, but only 17% in the latter. Swollen sinuses were seen from day 8 p.i. in both infected groups. The percentage affected was initially the same in both groups but with Mg had risen to 100% by day 11 whereas only 56% were affected in the Mim group. The percentage affected remained higher in the Mg birds until the end of the experiment at 35 days when 25% of them were still showing swollen infraorbital sinuses while the Mim group had recovered.

Macroscopic lesions

Macroscopic lesions were not seen in the birds in the control group. The numbers of infected birds with macroscopic lesions, and the MLSs are shown on Table 2. Nasal exudate was observed in a greater number of Mim infected than Mg infected chicks on day 7 p.i. It was seen on day 7, 14 and 21 p.i. in both infected groups, but also on day 28 p.i. in the Mg infected chicks. However, the total numbers of birds with nasal exudate were the same in both groups and there was no significant difference in the MLSs. There was no exudate in the infra-orbital sinuses in either group at day 7 p.i., but on day 14 p.i. it was present in all birds in both groups (Figures 6 and 7). Thereafter, more birds infected with Mg showed sinus exudate than the Mim infected chicks, but there was no statistical difference. The MLSs of sinuses on days 14, 21, 28 and 35 were greater in Mg infected chicks, but there were no significant differences.

Tracheal exudate was seen only in the Mg infected chicks and only on days 14, 21 and 28 p.i. Air sac cloudiness was also observed only in Mg infected birds and was seen on days 14, 21, 28 and 35 (Figure 8).

Histopathology

Examination of tracheal specimens revealed no damage in the control or the Mim infected chicks, but in the Mg infection lesions were observed in the tracheas of all birds examined. At day 14 p.i. many inflammatory cells, consisting mainly of macrophages and heterophils, were seen within the epithelium, and by day 21 the epithelium had thickened and contained microcysts. Subepithelial lymphoid aggregates were also conspicuous. On day 28 focal to confluent subepithelial lymphocyte accumulation was seen in all three tracheal samples, while two air sacs revealed heterophils, macrophages, lymphocytes and plasma cells in the subepithelial connective tissue. There was also marked fibroplasia and capillary growth in this tissue and the lumen was filled with necrotic inflammatory cells and fibrin. By day 35 p.i. recovery was indicated by the fact that two of the three tracheal sections appeared relatively normal with only a few focal lymphocyte aggregates. In the third sample there was still diffuse subepithelial

Table 1. *Percentage of birds with râles, watery eyes or swollen sinuses*

Signs	Group	Days post-infection								
		8	11	15	18	21	25	28	32	35
Râles	Mg	0	6	25	39	40	43	29	0	17
	Mim	6[a]	12	0	0	0	0	0	0	0
Watery eyes	Mg	0	0	50	31	70	43	43	50	25
	Mim	0	0	6	15	15	10	11	17	0
Swollen sinuses	Mg	24	100	81	69	80	71	43	25	25
	Mim	25	56	50	46	54	30	10	0	0

[a] Percentage of birds with signs of râles, watery eyes or swollen sinuses.
Mg = *M. gallisepticum.*; Mim = *M. imitans.*

Table 2. *Number of birds with macroscopic lesions at postmortem examination and the mean lesion scores*

Lesions	Group	Days post infection					
		7	14	21	28	35	Total
Nasal exudate	Mg	1 (0.333)[A]	2 (1.667)	2 (1.000)	2 (1.667)	0	7[B] (0.933)[a]
	Mim	3 (1.000)	2 (1.33)	2 (0.667)	0	0	7 (0.599)[a]
Sinus exudate	Mg	0	3 (2.667)	2 (1.333)	1 (0.667)	2 (1.333)	8 (1.200)[a]
	Mim	0	3 (1.667)	1 (0.667)	1 (0.667)	1 (0.333)	6 (0.667)[a]
Tracheal exudate	Mg	0	2 (0.333)	1 (0.667)	1 (0.333)	0	4[a] (0.200)[a]
	Mim	0	0	0	0	0	0[b]
Airsac cloudiness	Mg	0	1 (0.333)	1 (1.000)	3 (2.333)	1 (0.333)	6[a] (0.800)[a]
	Mim	0	0	0	0	0	0[b]

[A] Number of birds out of three with lesions and MLSs (in brackets).
[B] Cumulative total.
Different lower case letters indicate significant differences ($P < 0.05$).
Mg = *M. gallisepticum*; Mim = *M. imitans.*

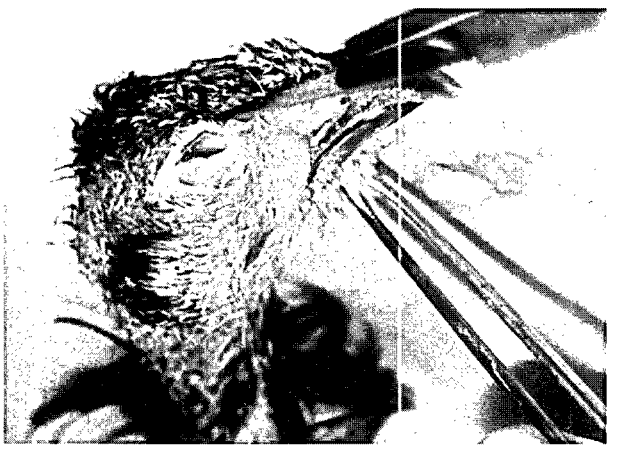

Figure 6. *Sticky turbid exudate emerging from the infra-orbital sinus of a partridge infected with* M. gallisepticum *and necropsied 21 days post-infection.*

Figure 7. *Solid cheesy exudate in the infra-orbital sinus of a partridge infected with* M. gallisepticum *and necropsied 21 days post-infection.*

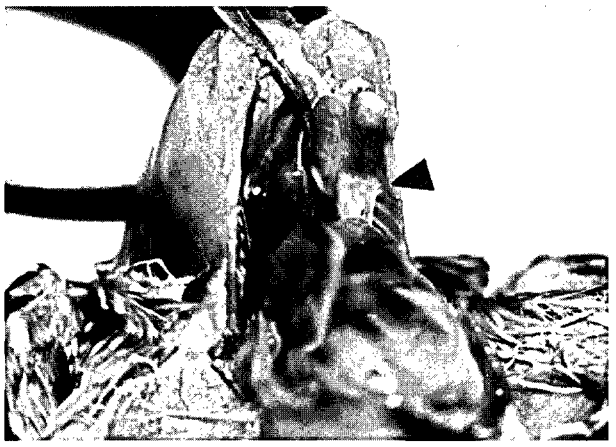

Figure 8. *Thickened thoracic air sac membrane (arrow) in a partridge infected with* M. gallisepticum *and necropsied 21 days post-infection.*

lymphocyte accumulation and the epithelium contained scattered heterophils.

Mycoplasma isolation

Mycoplasmas were not isolated from the control birds. The total numbers of isolations from eyes, upper and lower trachea, lungs and thoracic air sacs were significantly ($P < 0.05$) higher in Mg infected than Mim infected partridge chicks although there were similar numbers from the sinuses (Table 3). Mim was recovered from all the sampled sites at day 7 p.i., but thereafter it was not recovered from the lungs or thoracic air sacs, whereas these sites yielded Mg throughout.

In addition, Mg was isolated from the eyes, infraorbital sinuses, upper and lower trachea, lungs and thoracic air sacs of the two dead and two chicks which were killed, giving confluent growth in each case. Mim was recovered from the eyes, sinuses and upper trachea of the single bird which died in this group. The identities of representative isolates from the Mg and Mim groups were confirmed by indirect immunofluorescence as Mg and Mim, respectively.

Serology

Sera collected from the six remaining Mg infected birds and the six Mim infected birds on day 26 p.i. were all positive with the homologous antigen, and there was no evidence of agglutination with the heterologous antigen. The sera from the remaining control birds were all negative with both antigens.

Discussion

These studies have clearly shown that both Mg and Mim are capable of reproducing a clinical disease in red-legged partridges which is very similar to that seen in the field in chukar partridges in Australia (Reece *et al.*, 1986) and the USA (Cookson & Shivaprasad, 1994; McMartin *et al.*, 1996). Despite the greater challenge dose of *M. imitans*, the morbidity of the infection and its severity were greater with *M. gallisepticum*. However it is difficult to reach any conclusions about the relative pathogenicity of these two *Mycoplasma* species because only one strain of each was studied.

The percentage of Mg infected chicks with clinical signs was always greater than that of Mim infected chicks, as were the MCSs. On days 11 and 15 p.i., the MCSs of the Mg infected chicks were significantly ($P < 0.05$) higher than those of the Mim group. In addition, 20% of chicks were found dead or had to be culled in the Mg infected group, compared to only 5% in Mim infected group. It is possible that under the field conditions the mortality could be much higher and McMartin *et al.* (1996) reported that 60% of the partridges in a field outbreak were either found dead or had to be culled.

Mild to severe conjunctivitis was observed in red-legged partridge chicks infected with either Mg or Mim. In the Mg infection 70% of birds were affected compared to a maximum of 17% in the Mim infection. McMartin *et al.* (1996) reported 100% incidence of conjunctivitis in their experimental Mg infection of mature chukar partridges, however, their birds were inoculated intra-ocularly while ours were inoculated by the intranasal route. They also reported that the disease caused by Mg in chukar partridges was similar to infectious sinusitis in turkeys, although the birds did not appear to be as susceptible as turkeys to tracheitis, pneumonia and airsacculitis. Eight out of 12 (67%) of their experimentally infected birds developed sinusitis while it occurred in 100% of our Mg inoculated birds and in 56% of the Mim infected birds. Exudates in sinuses ranged from clear to turbid, sticky or caseous yellowish plugs which agrees with the observations of McMartin *et al.* (1996) in chukar partridges. No pneumonia was seen in our birds, but Mg S6 was capable of causing tracheitis in 20% of the birds and airsacculitis in 40%. These differences between our observations and those of McMartin *et al.* (1996) may again have been due to the route of infection, but the outcome may also have been influenced by factors such as breed and age of the birds, and/or the virulence and tropism of the particular mycoplasma strains used.

The number of birds with nasal and sinus exudate at necropsy was similar in both infected groups, but the MLSs were higher in the Mg than the Mim infection, although not significantly so. Tracheal exudate and air sac cloudiness were observed only in Mg infection indicating that the Mg S6 strain was more invasive in the lower respiratory tract than the Mim strain and also caused more damage to the respiratory tract.

Table 3. *Isolation of mycoplasmas*

Sites	Group	Days post-infection					
		7	14	21	28	35	Total[A]
Eyes	Mg	3[B]	3	3	3	2	14[a]
	Mim	1	3	2	0	1	7[b]
Sinuses	Mg	3	3	3	2	3	14[a]
	Mim	3	3	3	2	2	13[a]
Upper trachea	Mg	3	3	3	3	3	15[a]
	Mim	3	3	2	1	1	10[b]
Lower trachea	Mg	1	3	3	3	3	13[a]
	Mim	3	1	1	1	0	6[b]
Lungs	Mg	2	3	3	2	3	13[a]
	Mim	2	0	0	0	0	2[b]
Thoracic air sacs	Mg	2	3	3	2	3	13[a]
	Mim	2	0	0	0	0	2[b]

[A] Cumulative total.
[B] No. of birds out of three with positive isolation.
Different lower case letters indicate significant differences ($P < 0.05$).
Mg = *M. gallisepticum*; Mim = *M. imitans*.

In the Mg infection the microscopic lesions observed were similar to those reported by McMartin *et al.* (1996) and the lesions in the air sacs were similar to those described for chickens (Kerr & Olson, 1967). In their experimental Mg infection of chukar partridges McMartin *et al.* (1996) observed microscopic lesions in nasal and paranasal tissues, and mild tracheitis. In our experiment, only specimens of mid-trachea were examined regularly and thoracic air sacs occasionally.

Failure to observe any appreciable histological changes in the tracheas obtained from the Mim infected chicks agrees with the macroscopic findings. It should be noted that the dose of Mim inoculum was approximately 4×10^5 CFU per bird and it has been reported that in Mg infection in chickens (McMartin & Adler, 1961) and turkeys (Varley & Jordan, 1978) the disease severity is influenced by the number of organisms inoculated. It is possible that a higher infective dose might have produced more severe lesions.

The number of birds from which mycoplasmas were isolated from the infra-orbital sinuses was similar in both infected groups but the number of birds from which isolations were made from eyes, upper and lower trachea, lungs and thoracic air sacs was significantly ($P < 0.05$) greater in the Mg infection. The total number of Mg isolations from any of the sites sampled was never lower than 13 out of a possible 15 showing that Mg was able to infect, colonize and sustain colonization throughout the 35 days of the experiment. Such ability was lacking with Mim and it was recovered from the lungs and air sacs only at day 7 p.i. The similar degree of nasal and sinus exudation caused by Mg and Mim, together with the similar rates of isolation from the sinuses indicated that Mim caused its maximum damage to the nasal and paranasal passages, while causing little or no damage to the trachea and lower respiratory tract. However, it is possible that, in the field, factors such as stress, poor nutrition or secondary pathogens might enhance the invasiveness and pathogenicity of Mim.

RSA tests for Mg and Mim at 26 days were positive with the homologous antigens, but did not cross-react with the heterologous antigens although the numbers of sera were too small to rule out any possible cross-reactions between these two organisms as reported in other avian hosts (Dupiellet, 1988; Abdul-Wahab, 1991; Kempf *et al.*, 1996).

Our previous studies in turkeys (Ganapathy *et al.*, 1998) and chicks have demonstrated that Mim is a potential pathogen, especially in mixed infection. The current work has shown that a UK isolate of Mim given alone was pathogenic for young red-legged partridges even though the disease produced was considerably less severe than that produced by the virulent S6 strain of Mg. However, it is worth noting that Mim has not apparently been found in game birds since its original isolation in 1985.

Our experiment has also demonstrated for the first time the pathogenic effects of experimental Mg infection in red-legged partridges and it seems likely that this organism causes similar disease in pheasants. In many western countries an increasing number of such game birds are released into the wild and may well be a source of spread of this organism into nearby domestic poultry.

Acknowledgements

The authors thank Dr J. Beer and Mr P. W. Laing for considerable help and advice on obtaining and rearing healthy partridges and also Mr C. Perkins of Hill Game Farm, Cheltenham for his willingness to supply the chicks at the specified time. Thanks are also due to Mrs C. A. Yavari, Mrs C. M. Dare and Mr A. Jopson for technical assistance and to Professor D. F. Kelly for interpreting the histopathology. K.G. was the recipient of a scholarship from Universiti Putra Malaysia.

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RÉSUMÉ

Pathogénicité de *Mycoplasma gallisepticum* et *Mycoplasma imitans* chez des perdrix rouges (*Alectoris rufa*)

Des groupes de perdrix rouges âgées de trois jours ont été infectées par instillation nasale avec la souche S6 de *Mycoplasma gallisepticum* ou avec une souche de *Mycoplasma imitans* isolée de perdrix présentant une sinusite. Les oiseaux des deux groupes ont paru déprimés et ont présenté une exsudation nasale, des râles trachéaux, des éternuements, des difficultés respiratoires, des tremblements de la tête, des yeux humides et un grattage des yeux 6 à 8 jours après l'infection (p.i.). La caractéristique la plus frappante a été un gonflement bilatéral des sinus sous-orbitaires. La morbidité a atteint 100% et 80% respectivement pour les infections à *M. gallisepticum* et

à *M. imitans*. La moyenne des scores cliniques a été significativement supérieure dans le premier groupe comparé au deuxième groupe les 11ème et 14ème jours p.i. La guérison a été plus lente dans le groupe infecté par *M. gallisepticum*. Des examens nécropsiques réalisés hebdomadairement durant une période de 5 semaines ont révélé un exsudat sinusal et nasal dans les deux groupes, mais un exsudat trachéal et des sacs aériens légèrement épaissis n'ont été observés qu'après l'infection à *M. gallisepticum*. *M. gallisepticum* a été isolé à partir de l'appareil respiratoire supérieur et inférieur durant l'essai, alors que *M. imitans* a été moins fréquemment isolé de l'appareil respiratoire supérieur et seulement 7 jours après l'infection à partir des poumons et des sacs aériens. Le nombre des isolements obtenu à partir des yeux, de la trachée, des poumons et des sacs aériens thoraciques dans le groupe infecté par *M. gallisepticum* a été significativement supérieur à celui du groupe infecté par *M. imitans*. Des séroconversions ont été observées dans les deux groupes en utilisant les antigènes homologues.

ZUSAMMENFASSUNG

Pathogenität von *Mycoplasma gallisepticum* und *Mycoplasma imitans* bei Rothühnern (*Alectoris rufa*)

Gruppen von drei Tage alten Rothühnern wurden entweder mit dem Stamm S6 von *M. gallisepticum* oder mit einem *M. imitans*-Stamm aus einem Rothuhn mit Sinusitis intranasal infiziert. Ab 6. bis 8. Tag *post infectionem* (p.i.) kam es in beiden Gruppen zu Trauern, Nasenausfluß Röcheln, Niesen, Atemnot, Kopfschütteln, wässrigen Augen und Kratzen an den Augen. Die hervorstechendste Besonderheit war eine beidseitige Schwellung des Infraorbitalsinus. Die Morbidität erreichte 100% bei der *M. gallisepticum*-Infektion und 80% bei der *M. imitans*-Infektion, und die mittleren klinischen Scores waren am 11. und 14. Tag p.i. in der ersteren Gruppe signifikant höher als in der letzteren. Bei der *M. gallisepticum*-Infektion war außerdem die Erholung verzögert. Die 5 Wochen lang in wöchentlichen Abständen durchgeführten Sektionen zeigten in beiden Gruppen das Vorliegen von Nasen- und Sinusexsudat, aber Trachealexsudat und trübe Luftsäcke wurden nur bei der *M. gallisepticum*-Infektion festgestellt. *M. gallisepticum* wurde während der gesamten Versuchsdauer sowohl aus dem oberen als auch aus dem unteren Respirationstrakt isoliert, während *M. imitans* aus dem oberen Respirationstrakt seltener und aus den Lungen und Luftsäcken nur am 7. Tag p.i. isoliert wurde. Die Anzahl der Erregerisolierungen aus den Augen, Tracheen, Lungen und Brustluftsäcken war in der *M. gallisepticum*-Gruppe signifikant größer als die aus der *M. imitans*-Gruppe. Eine mit homologem Antigen nachweisbare Serokonversion erfolgte in beiden Gruppen.

RESUME

Patogenicidad de *Mycoplasma gallisepticum* y *Mycoplasma imitans* en perdices comunes (*Alectoris rufa*)

Se infectaron por vía intranasal grupos de perdices comunes de 3 días de edad con la cepa S6 de *M. gallisepticum* o una cepa de *M. imitans* procedente de una perdiz común con sinusitis. A los 6-8 días postinfección (p.i.) las aves de ambos grupos presentaban signos de abatimiento, secreción nasal, estornudos, boqueo, agitación de la cabeza, lacrimeo y rascado ocular. El hecho más significativo era una tumefacción de los senos infraorbitarios. La morbilidad fue del 100% en el grupo infectado por *M. gallisepticum* y del 80% en el infectado con *M. imitans*, y la media de signos clíinicos fue significativamente mayor en el primer gru po que en el segundo los días 11 y 14 p.i. La recuperación, en el grupo de aves infectadas con *M. gallisepticum* fue también más lenta. En las necropsias, realizadas a intervalos semanales, durante 5 semanas, se observó exudado en cavidad nasal y seno infraorbitario en ambos grupos aunque sólo se pudo observar exudado y opacidad en sacos aéreos en el grupo infectado con *M. gallisepticum*. Se aisló *M. gallisepticum* a partir de vías respiratorias altas y vías respiratorias bajas a lo largo de todo el experimento, mientras que el aislamiento de *M. imitans* fue menos frecuente en vías respiratorias altas; y en pulmones y sacos aéreos, únicamente el día 7 p.i. El número de aislamientos a partir de ojos, tráquea, pulmones y sacos aéreos torácicos, fue significativamente mayor en el grupo de aves infectadas con *M. gallisepticum* que en el de las infectadas con *M. imitans*. Se produjo seroconversión, detectable con un antígeno homólogo, en ambos grupos de aves.