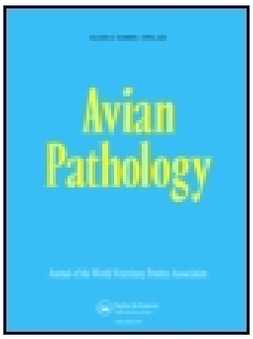

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MYCOPLASMA IOWAE INFECTION IN YOUNG TURKEYS

JANET M. BRADBURY[1], AINI IDERIS[2] and TIN TUN OO[3]

[1] *Department of Veterinary Pathology, University of Liverpool, Leahurst Neston, South Wirral L64 7TE, England*

[2] *Department of Veterinary Clinical Studies, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia*

[3] *Institute of Veterinary Science, Yozin, Pyinmana, Burma*

SUMMARY

A comparison was made of the effect of five different strains of *M. iowae* after inoculation of one-day-old poults via the thoracic air sac and foot pad. Three strains appeared to be more virulent and more invasive than the other two, causing stunting, poor feathering and leg abnormalities including chondrodystrophy. One of these three strains was used in a second experiment in which three routes of infection were compared. Infection *in ovo* caused a severe generalised disease in hatched poults with high mortality. The only two birds which survived into the third week developed chondrodystrophy. One group was infected orally at one day of age and some birds developed bone and joint abnormalities but another group, infected via both the thoracic air sac and the foot pad, also at one day of age, developed a higher incidence of these abnormalities, which included chondrodystrophy, rotated tibia, deviated toes and, in a few cases, erosion of the articular cartilage of the hock joint. Some of the control uninfected birds developed leg abnormalities but never chondrodystrophy, rotated tibia or cartilage erosion. *M. iowae* was most widely disseminated in tissues following *in ovo* infection and least after oral infection. Isolations became less frequent with age and no organisms were recovered in birds sampled at 12 weeks. In neither experiment could antibodies to *M. iowae* be detected by rapid agglutination.

INTRODUCTION

Mycoplasma iowae has been reported to kill experimentally infected chicken and turkey embryos (Yoder and Hofstad, 1962, 1964; El-Ebeedy and Stipkovits, 1975; McClenaghan *et al.*, 1981; Rhoades, 1981; Bradbury and McCarthy, 1983). It may also cause mild to moderate airsacculitis in turkeys (Yoder and Hofstad, 1962,

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1964; Dierks *et al.*, 1967; Rhoades, 1981) and experimental infections have given rise to leg lesions in both chickens and turkeys (Yoder and Hofstad, 1962, 1964; Bradbury and McCarthy, 1981, 1984). In a preliminary communication Bradbury and Ideris (1982) reported bone and tendon abnormalities in young turkeys following infection with five strains of *M. iowae* via the air sac and foot pad. This paper provides a detailed account of these studies (Expt 1) and also describes a further investigation in which one strain of *M. iowae* was used to compare the effect of different routes of infection (Expt 2).

MATERIALS AND METHODS

Mycoplasma *strains*

The type strain (695) of *M. iowae* and four UK isolates (B10/80, B11/80, B16/80 and M4/77) were used to infect turkeys in Expt 1 and strain B11/80 was used in Expt 2. The sources of these strains, and details of their identification and purification have been described (Bradbury and McCarthy, 1983). Strains B11/80 and M4/77 were of turkey origin and strains B10/80 and B16/80 were from chickens. Other reference strains used were avian serovar J DJA, *M. gallisepticum* PG31, *M. synoviae* WVU 1853 and *M. meleagridis* 17529.

Antisera

Rabbit antisera to *M. iowae* 695, avian serovar J DJA, *M. gallisepticum* PG31, *M. synoviae* WVU 1853 and *M. meleagridis* 17259 were all prepared in this laboratory (Bradbury and McClenaghan, 1982).

Mycoplasma *media*

Conventional broth and agar media (Bradbury, 1977) were employed except that thallium acetate was omitted from the broth cultures that were used to infect embryos and poults.

Preparation of inocula

These were prepared as described previously (Bradbury and McCarthy, 1983) and the stock cultures were diluted to provide the desired number of colony-forming units (CFU)/ml.

Source of eggs

Fertile turkey eggs were from a flock that was culturally free of *M. iowae* and culturally and serologically free of evidence of *M. gallisepticum*, *M. synoviae* and *M. meleagridis* infection.

Inoculation procedures

Experiment 1. Sixty-three poults were hatched and randomly divided into six groups and each group was housed in a fibreglass isolator. The number of birds in each group is indicated in Table 1.

Each poult was inoculated at one day of age via the right thoracic air sac with 0.1 ml, and via the right foot pad with 0.05 ml of a broth culture of the appropriate *M. iowae* strain. Control birds were inoculated similarly with sterile mycoplasma broth. Five different strains of *M. iowae* were used as indicated in Table 1. The number of viable mycoplasmas in the inocula were: B10/80, 6.6×10^6 CFU/ml; B11/80, 3.7×10^6 CFU/ml; B16/80, 4.2×10^5 CFU/ml; M4/77, 1.5×10^6 CFU/ml and 695, 2.1×10^5 CFU/ml.

Table 1. Expt.1: Summary of main observations in poults infected with five different strains of M. iowae.

Strain	Age of bird (weeks)	No. of birds examined	No. of birds showing			
			Stunting	Diarrhoea	Swollen kidneys	Bone, tendon or joint abnormalities+
B10/80*	3	5	5	1	3	5
	6	6	6	1	4	6
B11/80*	3	5	5	0	4	5
	6	6	6	3	3	6
B16/80*	3	5	0	0	1	3
	6	5	0	0	2	5
M4/77*	3	6	2	0	2	6
	6	6	1	0	1	5
695	3	5	0	0	2	4
	6	6	0	1	1	1
Broth controls	3	4	0	0	0	0
	6	4	0	0	0	0

* Poor feathering was seen in these groups.

\+ For further detail see Table 2.

Experiment 2. Four experimental groups were established from a single batch of fertile turkey eggs. Group 1 comprised 60 viable turkey embryos inoculated via the yolk sac at 21 days of incubation with 0.1 ml of *M. iowae* B11/80 culture. They were hatched in a Curfew incubator (Curfew Incubators, Chertsey, England) and 18 hatched poults were transferred to an isolator. In addition to these 18 poults seven late-hatched birds were removed from the Curfew incubator and sampled for mycoplasmas as described below. Group 2 comprised 18 poults infected orally at one day of age with 0.15 ml of *M. iowae* B11/80 culture and transferred to a separate isolator, while group 3 contained 19 poults which were inoculated with *M. iowae* B11/80 via the air sac and footpad at one day of age, as in Expt 1, and then placed in a separate isolator. Group 4 comprised three control sub-groups, all within one isolator. Ten poults were inoculated *in ovo* as for group 1 but with sterile mycoplasma broth, 10 were inoculated with broth orally as for group 2 and 10 were inoculated with broth via the air sac and foot pad. The number of viable mycoplasmas in the strain B11/80 inocula were 1.9×10^5 CFU/ml for embryos and 1.9×10^7 CFU/ml for the poults.

The birds in both experiments were given commercial diets *ad libitum* and the management and lighting were similar for each group.

Observations and sampling procedures

Experiment 1. Poults were examined daily for 6 weeks for clinical signs and at 3 and 6 weeks about half the birds in each group were killed by intravenous injection of pentobarbitone and examined for abnormalities. Samples of tissues with gross lesions were taken for histopathological examination and samples were also taken

from the same sites in control birds for comparison.

Swabs for mycoplasma culture were taken from the oesophagus, right and left thoracic air sac, right and left tibiotarsal-tarsometatarsal (hock) joint region (Bradbury and McCarthy, 1984), right foot pad and cloaca. Swabs were also taken from the hock joint region of two birds from each group for bacteriological culture and, at 6 weeks, samples of cartilage and tendon were taken for virus isolation from the hock joint region of four birds. Faecal samples were collected from six birds for the same purpose.

At 6 weeks blood was taken from each poult prior to killing and the sera were collected after 24 hours.

Experiment 2. The birds were checked daily and any dead ones were removed, examined for gross lesions and, unless too decomposed, were cultured for mycoplasma.

At one week, either four or five poults from each group were cultured for mycoplasma from swabs taken from the oesophagus and cloaca. At 3 weeks, about half the remaining birds in each group were killed and sampled as described for Expt 1 except that additional tissues (spleen, bursa of Fabricius, duodenum, ileum, caecal tonsils) were taken for histological examination and mycoplasma culture from the foot pad was omitted. At 4 weeks the remaining birds in groups 2, 3 and 4 were transferred to separate wooden houses (there were no birds remaining in group 1). At 6 weeks blood samples were taken from all the infected birds and from half of the controls, and oesophageal and cloacal swabs were taken from three birds per group for mycoplasma culture.

The turkeys were kept under observation for 12 weeks when they were bled, killed and examined as above.

Isolation and identification of mycoplasmas, bacteria and viruses
The methods were the same as those described in an earlier publication (Bradbury and McCarthy, 1984) except that cloacal swabs for mycoplasma isolation were also inoculated into broth. Broths showing colour change were inoculated on to agar and all other broths were thus inoculated after one week of incubation.

Serological tests
Rapid agglutination tests were conducted using stained antigens prepared from *M. iowae* 695 and serovar J DJA by the method of Bradbury and Jordan (1971). Commercially-produced stained antigens (Intervet, Boxmeer, Holland and Wellcome Reagents Ltd., Beckenham, England) were used to examine the sera for antibodies to *M. gallisepticum*, *M. synoviae* and *M. meleagridis*. Agar gel precipitation tests were carried out using reovirus strain R_2 as antigen (Jones *et al.*, 1980).

RESULTS

Experiment 1
Clinical signs. All birds appeared normal during the first week of life but by the second week some poults in the groups infected with strains B11/80 and M4/77 seemed depressed. Growth was retarded compared with the controls, and poor feathering was apparent. By the third week birds in the group infected with strain B10/80 were similarly affected (Fig.1). Leg and toe deformities were also noted in

Table 2. Expt 1: Details of bone, joint and tendon abnormalities in poults infected with five different strains of M. iowae.

Strain	Age of bird (weeks)	No. of birds examined	No. of birds showing:								
			Chondro-dystrophy	Rotated tibia	Splayed legs	Deviated toes	Excess fluid in joint cavity	Pitted articular cartilage	Wrinkled df[a] tendons	Sigmoid folding of df[a] tendon	Ruptured df[a] tendon
B10/80	3	5	2	1	0	1	5	0	4	0	0
	6	6	2	0	1	2	3	2	6	2	2
B11/80	3	5	3	0	0	1	4	0	3	1	1
	6	6	4	1	3	5	5	1	2	0	0
B16/80	3	5	0	0	0	1	3	0	0	0	0
	6	5	0	1	0	4	4	0	4	0	0
M4/77	3	6	3	1	0	2	6	1	2	0	0
	6	6	0	1	0	3	4	1	3	0	0
695	3	5	0	1	0	1	4	0	1	0	0
	6	6	0	0	0	1	0	0	0	0	0
Broth-inoculated controls	3	4	0	0	0	0	0	0	0	0	0
	6	4	0	0	0	0	0	0	0	0	0

a df = digital flexor.

some birds in all the infected groups from about 3 weeks of age (Table 1). The control birds remained normal throughout.

Postmortem and histological lesions. Some of the postmortem findings were summarised elsewhere (Bradbury and Ideris, 1982). However, Tables 1 and 2 indicate these findings in more detail. The birds infected with strain B10/80 and B11/80 were most markedly affected with the majority of them showing not only stunting but also swollen kidneys and bone and/or tendon abnormalities. The abnormalities included chondrodystrophy, rotated tibiotarsus, splayed legs and deviated toes (Figs.2 to 5).

Some of these abnormalities were also seen in the other three infected groups but at a reduced incidence. On dissection of the legs, further abnormalities were revealed (Table 2). There was excess fluid in the hock joint cavity of many poults and, in five birds, the articular surface of the tibiotarsus appeared to be pitted (Fig.6). The digital flexor tendons were also affected, many showing a finely wrinkled appearance and in three birds there was sigmoid folding of one of the digital flexor tendons associated with rupture of that tendon (Fig.7). In two advanced cases of chondrodystrophy the gastrocnemius tendon had slipped.

Histological sections of a few selected tissues revealed no microscopic changes in the kidneys. There was evidence of mild tenosynovitis of the digital flexor tendons, with infiltration of lymphocytes and scattered heterophils. There was thickening of some tendon sheaths. No obvious abnormalities could be seen in a section of pitted cartilage.

Microbiological survey. Mycoplasmas, subsequently identified by immunofluorescence as *M. iowae*, were recovered from all infected groups but not from the control birds (Table 3). Recoveries were most frequent at the first sampling at 3 weeks from the birds infected with strains B10/80, B11/80 and M4/77. There were very few isolations at 6 weeks. The oesophagus and thoracic air sacs yielded the most isolates although the organism was also found in the cloaca of some birds. Only two birds were culturally positive in the hock region or inoculated foot pad, and no bacteria or viruses were isolated.

Serological tests. There were no positive serum agglutination reactions with the five different stained antigens although control specific antisera gave agglutination in all cases. Antibodies to reovirus were not detected by gel precipitation tests.

Experiment 2

Clinical signs. A large proportion of the poults that had been infected *in ovo* failed to hatch and those that did were depressed and markedly stunted. Their appetites were poor and the droppings were whitish and liquid. Feathering was abnormal with most birds having "helicopter" wings. Of the 18 poults in this group, 14 died in the first week and another two by 16 days. During the second week the abnormal feathering became more noticeable in the surviving birds as the sheath of the primary feathers was still present on part of the shaft (Figs.8 and 9). The two birds that survived into the third week developed chondrodystrophy and showed intermittent tremor.

The orally-infected poults appeared initially to be slightly stunted compared with the controls but after about 2 weeks this was less apparent. Poor feathering was obvious and the droppings were watery, but otherwise the birds seemed bright

Table 3. Expt 1: Isolation of M. iowae *from poults infected with five different strains.*

M. iowae strain	Age of birds (weeks)	No. of birds examined	No. of birds positive	No. of birds in which *M. iowae* was isolated from						
				Oesophagus	Rt. thoracic air sac	Lt. thoracic air sac	Rt. hock region	Lt. hock region	Rt. foot pad	Cloaca
B10/80	3	5	5	3	4	3	0	0	0	2
	6	6	1	1	1	1	0	0	0	0
B11/80	3	5	5*	4	5	5	1	1	1	3
	6	6	3	3	3	2	0	0	0	0
B16/80	3	5	2	1	0	1	0	0	0	2
	6	5	0	0	0	0	0	0	0	0
M4/77	3	6	6*	6	5	5	1	1	1	3
	6	6	0	0	0	0	0	0	0	0
695	3	5	1	0	1	0	0	0	0	0
	6	6	1	1	1	1	0	0	0	0

No mycoplasmas were isolated from the control birds.

* All sites were positive for one bird in the group.

and alert. There were four deaths by 18 days in this group of 18 poults and two of these had shown leg weakness with swollen hock joints. Two birds developed splayed legs and several had rotated tibias. In general the group appeared less mobile than their control counterparts.

Of the 19 birds infected via the air sac and foot pad three died during the first week. The majority of remaining birds were stunted with poor feathering and the droppings were white and watery. They were depressed, uneven in growth rate and the food intake appeared to be less than that of the control group. By about 10 days leg problems had become apparent and by 2 weeks more than half the birds had developed leg or toe abnormalities.

In the control group of 30 birds there were some early deaths which were mainly due to birds becoming trapped in the wire mesh floor. The remainder of the birds were bright and active although a small proportion had poor feathering and four developed deviated toes. Later in the experiment three birds developed splayed legs. One of these was considerably undersized and died at 5 weeks. As there was no apparent correlation between abnormalities and the administration of sterile broth by different routes, the controls were treated as a single group.

Postmortem and histological lesions. Although abnormalities were seen in birds in all groups the incidence was generally higher in the infected birds (Tables 4 and 5). Abnormalities of the internal organs were more often seen in birds of 3 weeks than in those examined later (Table 4) and consisted of pallor of the kidneys, liver, spleen and heart. A few birds also had cloudy air sacs and congested lungs.

Leg lesions tended to become manifest in birds of 2 to 3 weeks of age and then more abnormalities developed with time (Table 5). A few control birds had leg or toe problems but none of them developed chondrodystrophy. This was of greatest incidence (37%) in the group infected via the air sac and foot pad. Other abnormalities included excess fluid in the hock joint cavity and this was seen in birds in all groups but particularly in groups 2 and 3. The incidence of deviated toes was also higher in these two groups than the others.

On histological examination a number of tissues from infected birds in all three groups showed signs of an inflammatory response although insufficient samples were examined to enable comparison of response in the different groups. Spleen sections showed proliferation of reticular cells and the presence of macrophages, plasma cells and heterophils in the parenchyma. In the bursa of Fabricius there was localised congestion with infiltration of plasma cells, heterophils and reticular cells (Fig.10). Macrophages, lymphocytes, heterophils and plasma cells were seen in the lamina propria of the duodenum, ileum and caecal tonsils. Sections of swollen kidney from a bird in group 3 sampled at 21 days revealed hyperaemia and haemorrhage and many localised accumulations of heterophils (Fig.11). There was little obvious change in cartilage and tendon sections except for oedema in the sheaths of wrinkled digital flexor tendons.

Microbiological survey. Mycoplasmas were isolated from all infected groups in the first three weeks but thereafter only one more culturally positive bird was found (Tables 6 and 7). This was a bird in group 3 that died at 5 weeks of age. The organisms, which were identified by immunofluorescence as *M. iowae*, were recovered from more sites and in greater numbers from those birds that had been infected

Table 4. Expt 2: Summary of main post-mortem findings in poults infected with M. iowae *B11/80 by different routes.*

Group[a]	Age of bird (weeks)	No. of birds examined	No. of birds with abnormalities of					
			Kidneys	Liver	Spleen	Heart	Bone, tendon or joint	Respiratory tract
1	0 – 3*	18 (16)+	5	7	5	0	6	4 (2AS, 2LU)
2	0 – 3	11 (4)	5	2	2	1	8	1 (AS)
	12	7 (0)	0	1	0	0	7	0
3	0 – 3	11 (3)	3	2	3	1	8	3 (LU)
	5 – 8	5 (5)	1	0	0	0	5	4 (2AS, 2LU)
	12	3 (0)	0	1	0	0	3	0
Broth-inoculated controls	0 – 3	19 (7)**	1	0	0	0	4	2 (1AS, 1LU)
	5	1 (1)	0	0	0	0	1	0
	12	10 (0)	0	1	0	0	5	0

a Group 1 was inoculated *in ovo*; Group 2 orally at one day of age; group 3 into the air sac and foot pad at one day of age.

* There were no survivors beyond 3 weeks in this group.

** Six of these birds died as a result of becoming trapped.

\+ The figure in parenthesis gives the number of natural deaths.

AS Cloudy air sacs.

LU Congested lungs.

Table 5. Expt 2: Details of bone, joint and tendon abnormalities in poults infected with M. iowae *B11/80 by different routes.*

Group[a]	Age of bird (weeks)	No. of birds examined	No. of birds showing						
			Chondro-dystrophy	Rotated tibia	Splayed legs	Deviated toes	Excess fluid in joint cavity	Pitted articular cartilage	Wrinkled df[b] tendons
1	0 – 3	18	2	0	0	2	5	1	1
Percentage			11	0	0	11	28	5.5	5.5
2	0 – 3	11	1	2	1	5	6	0	0
	12	7	0	3	1	3	6	0	0
Percentage			5.5	28	11	44	67	0	0
3	0 – 3	11	4	4	0	4	8	1	0
	5 – 8	5	3	0	3	2	4	2	0
	12	3	0	2	0	2	3	2	0
Percentage			37	32	16	42	74	26	0
4	0 – 3	19	0	0	0	2	2	0	0
Broth-	5	1	0	0	1	1	1	0	0
inoculated	12	10	0	0	2	1	5	0	1
controls									
Percentage			0	0	10	13	27	0	5.5

a See corresponding footnote to Table 4.

b df = digital flexor.

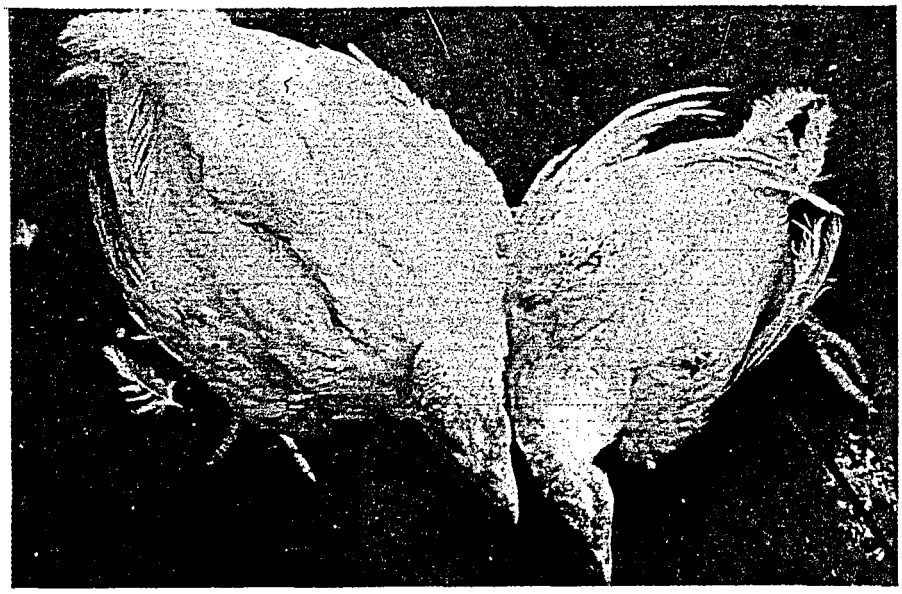

Fig. 1. Three-week-old poult from group B10/80 showing stunting compared with control of same age (Expt 1).

Fig. 2. Three-week-old poult from B10/80 group with chondrodystrophy (Expt 1).

Fig. 3. Three-week-old poult from B10/80 group with rotation of the tibiotarsal bone (Expt 1).

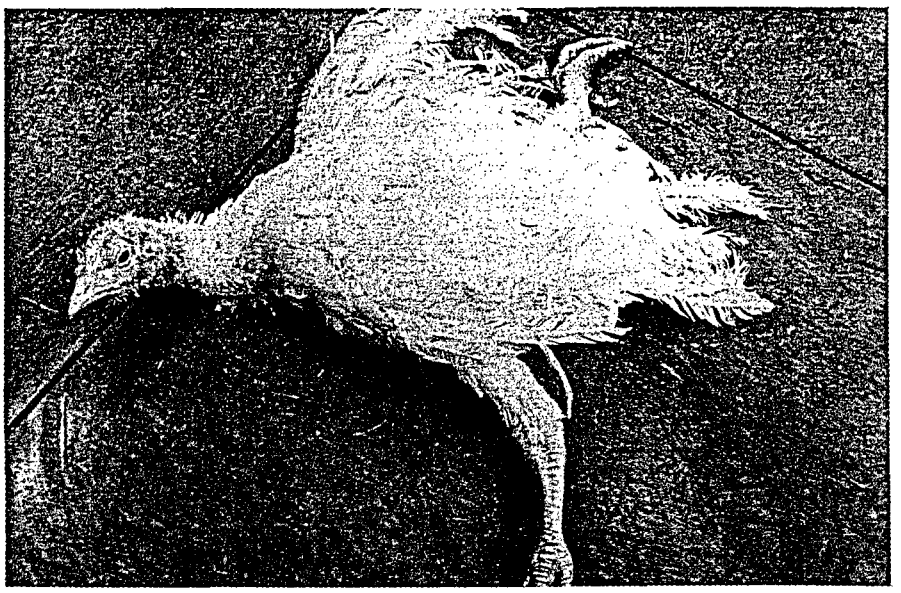

Fig. 4. Six-week-old poult from B10/80 group showing splayed legs (Expt 1).

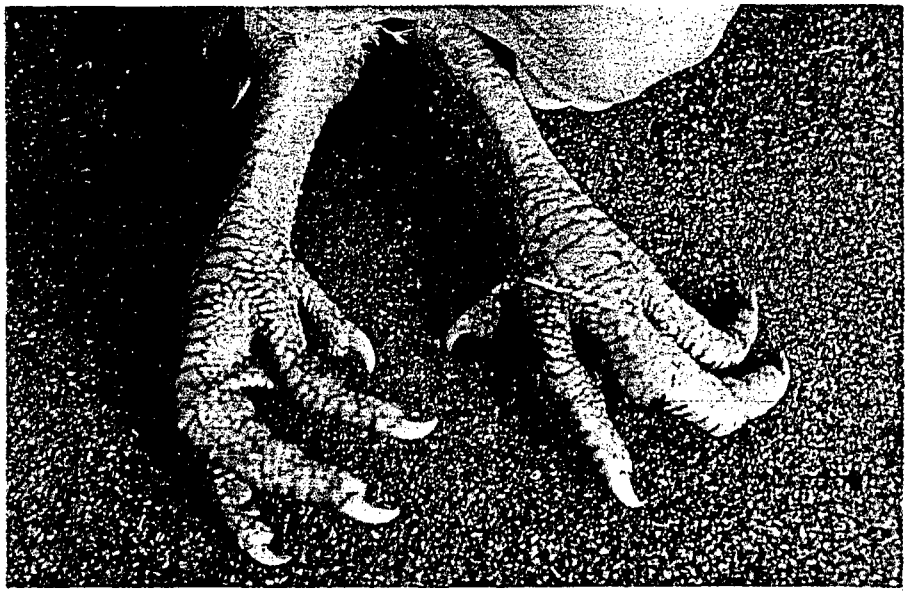

Fig. 5. Six-week-old poult in B11/80 group showing bilateral deviation of the toes (Expt 1).

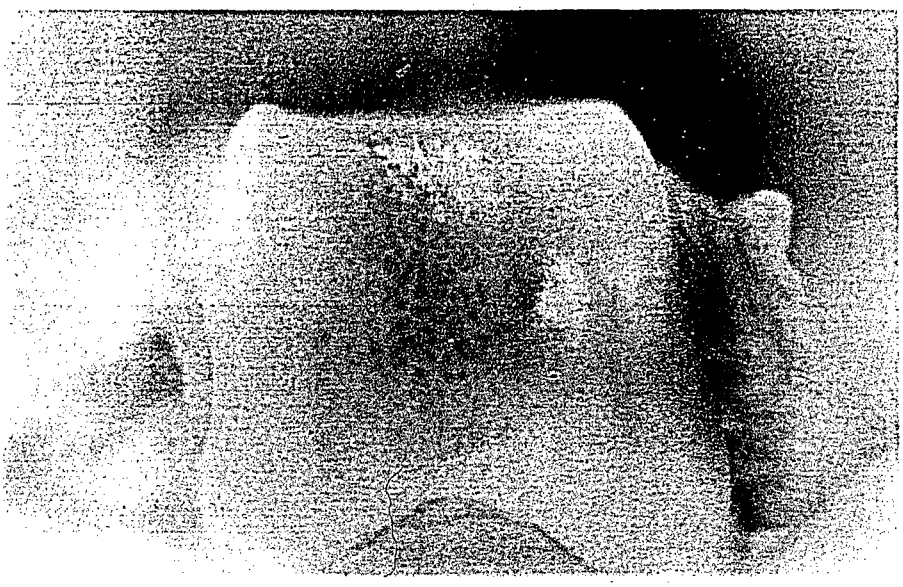

Fig. 6. Pitted articular cartilage on the distal end of the tibiotarsal bone of a six-week-old poult from group M4/77 (Expt 1).

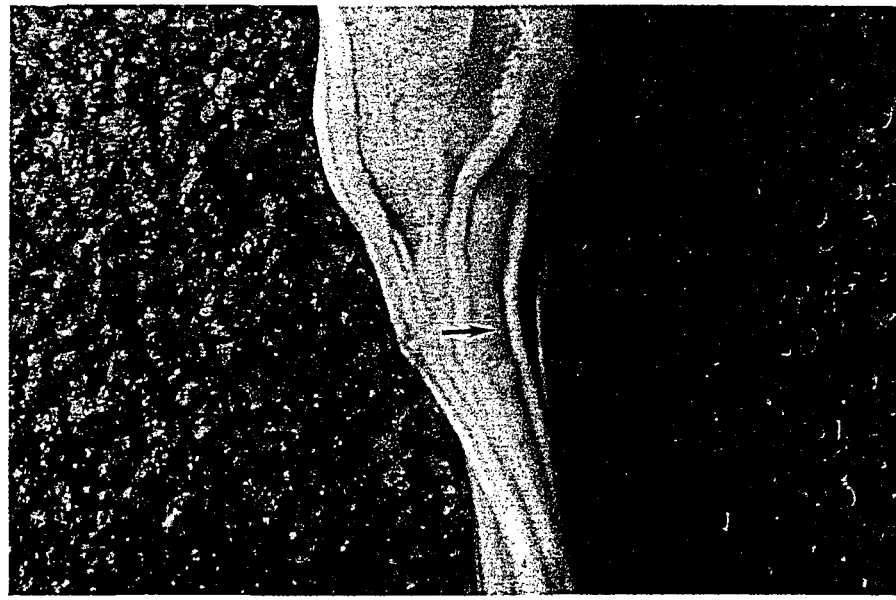

Fig. 7. Sigmoid folding of the digital flexor tendon (arrowed) of a three-week-old poult in group B11/80 (Expt 1).

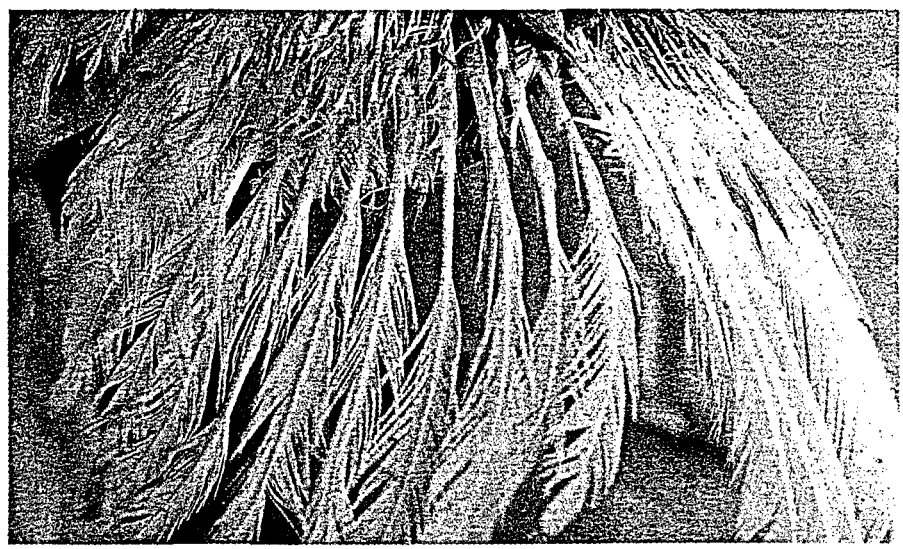

Fig. 8. Abnormal primary wing feathers in a three-week-old poult infected in ovo *with strain B11/80 (Expt 2).*

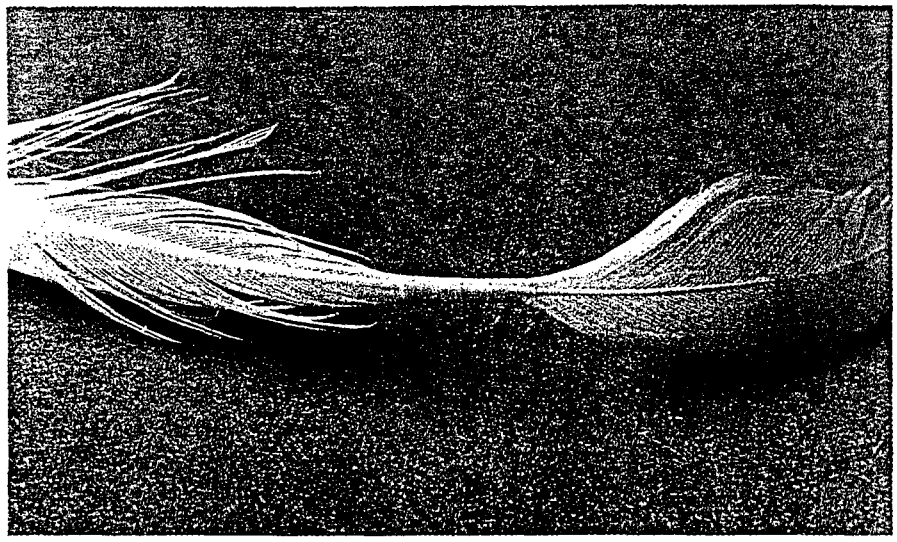

Fig. 9. Primary wing feather of a three-week-old poult infected in ovo *with strain B11/80. Retention of the sheath can be seen (Expt 2).*

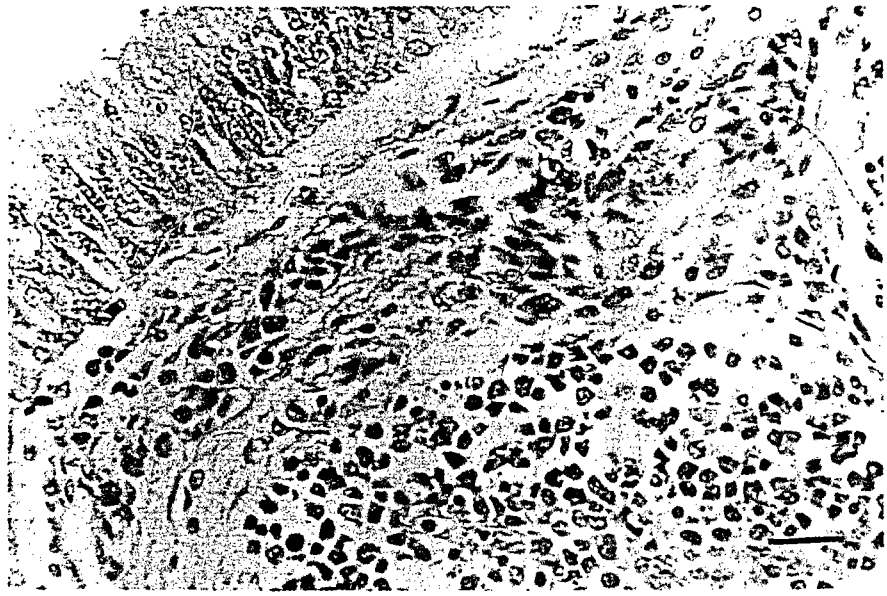

Fig. 10. Micrograph of bursa of Fabricius of a three-week-old poult infected with B11/80 (Expt 2). There is localised congestion with infiltration of plasma cells, heterophils and reticular cells. Bar = 20 μm.

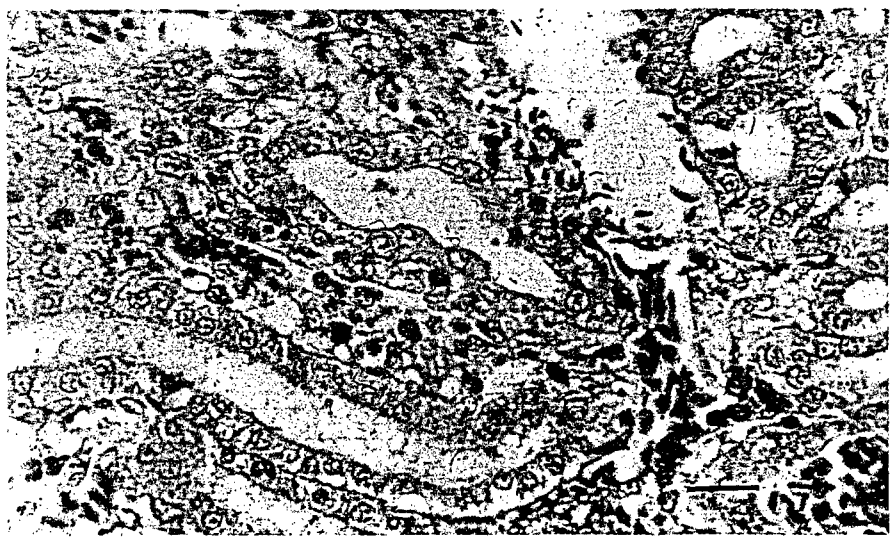

Fig. 11. Micrograph of kidney of a three-week-old poult infected with B11/80 (Expt 2). There is congestion and haemorrhage with infiltration of heterophils. Bar = 20 μm.

Table 6. Expt 2: Isolation of M. iowae *B11/80 from dead or killed poults infected by different routes.*

Group[a]	Age of birds (weeks)	No. of birds examined	No. of birds positive	No. of birds with isolation of *M. iowae* from					
				Oesophagus	Right thoracic air sac	Left thoracic air sac	Right hock region	Left hock region	Cloaca
1	0*	7	7	7	7	7	7	5	7
	0 – 3	15	15	14	11	11	7	7	12
2	0 – 3	11	3	3	0	0	0	0	0
	12	7	0	0	0	0	0	0	0
3	0 – 3	11	10	7	4	4	4	2	6
	5 – 8	5	1	1	1	0	0	0	0
	12	3	0	0	0	0	0	0	0

a See corresponding footnote to Table 4.

* The group of 7 late-hatched poults.

No mycoplasmas were isolated from the broth-inoculated controls.

Table 7. Expt 2: Isolation of M. iowae *from live birds.*

Group[a]	Age of bird (weeks)	No. of birds sampled	No. of birds with isolation of *M. iowae* from	
			Oesophagus	Cloaca
1	1	4	3	4
	6	ND*		
2	1	5	0	0
	6	3	0	0
3	1	5	2	3
	6	3	0	0

a See corresponding footnote to Table 4.

* There were no survivors in this group.

in ovo than from the other infected birds. The late-hatched *in ovo*-infected poults gave the highest percentage of positive cultures. Only three poults infected orally subsequently yielded *M. iowae.*

Mycoplasmas were not isolated from the control birds and no bacteria or viruses were isolated in this experiment.

Serological tests. No antibodies were detected to *M. iowae* 695, serovar J DJA, *M. gallisepticum, M. synoviae* or *M. meleagridis* by the rapid serum agglutination test on sera collected at 6 and 12 weeks nor were there any positive reactions for reovirus antibody.

DISCUSSION

These studies have demonstrated that early experimental infection of turkeys with *M. iowae* can give rise to impaired growth and poor feathering and a number of abnormalities of the skeletal system. Of the five strains used in Expt 1, B10/80 and B11/80 gave rise to the most severe clinical signs and lesions, but it could be argued that this was dose-related, since these two strains were inoculated at higher dose levels than the other three. This was unintentional and was probably due to uneven loss of viability during storage of the inocula at -70°C. In earlier experiments with the same strains of *M. iowae* in chick embryos (Bradbury and McCarthy, 1983) and in young chicks (Bradbury and McCarthy, 1984) the inoculation doses were more uniform and yet a similar variation in pathogenic effects was seen between strains. As in the earlier studies the *in vitro* passage level of the four field strains was between 9 and 12 and was considered unlikely to account for differences in virulence. It is possible, however, that the very high passage level of the type strain was responsible for its low virulence.

The stunting, poor feathering and chondrodystrophy seen in these experiments were clinically indistinguishable from turkey syndrome '65 (TS 65), a condition of young turkeys first reported in Great Britain in 1965 (Working Party, 1965). It was subsequently ascribed to early infection with *M. meleagridis* (Peterson, 1968; Wise *et al.*, 1974) and it was also reproduced experimentally with *M. gallisepticum* by Wannop *et al.* (1971) and by Wise and Fuller (1975). It is interesting to note

Wise and Fuller (1976) described a similar syndrome in turkeys and pheasants artificially infected with a mycoplasma designated "W8". Although this mycoplasma was reported to be unrelated to *M. iowae* (Windsor *et al.*, 1977) it now seems likely, from subsequent serological testing, that "W8" belongs to the *M. iowae* serogroup (F.T.W. Jordan and J.M. Bradbury, unpublished findings).

TS 65 has been likened to nutritional perosis (Wise *et al.*, 1973) and, with all three *Mycoplasma* species mentioned above, the condition seems to be a result only of early infection (*ie* of the embryo or very young bird). This would explain why other workers, who used older birds, did not observe the syndrome in experimental *M. iowae* infections (Yoder and Hofstad, 1962, 1964, Rhoades, 1981). With *M. meleagridis* it was suggested that the leg lesion was caused by an impairment of the supply of nutrients to the growth plate (Wise *et al.*, 1973) and it is possible that the fast-growing turkey is particularly susceptible to chondrodystrophy if subjected to a minor stress such as a *Mycoplasma* infection. However, three other avian *Mycoplasma* species (*M. lipofaciens, M. cloacale* and *M. glycophilum*) inoculated into the yolk sac of 7- or 14-day-old turkey embryos or into the thoracic air sac of one-day-old poults did not give rise to any clinical signs in hatched birds kept up to 3 weeks (J.M. Bradbury and M. Forrest, unpublished observations).

There is no ready explanation for the poor feathering that was observed in these and the earlier studies and it is not known if it was a direct result of the mycoplasma infection or due to a secondary effect such as impaired nutrition or metabolism. Retention of an abnormally long sheath on primary and secondary feathers of chicks is characteristic of certain nutritional deficiencies such as amino acids or vitamins (Deschutter and Leeson, 1986). It is possible that *M. iowae* infection interferes in some way with the uptake or metabolism of some of these nutrients. Certain toxins, such as mycotoxins may also cause depressed growth and poor feathering (Macpherson, 1982) and the possibility that *M. iowae* produces a toxin cannot be ruled out.

When routes of infection were compared in the second experiment it was apparent that birds in the group inoculated via the air sac and foot pad were more likely to develop poor feathering and chondrodystrophy than those inoculated orally, while infection via the developing embryo resulted in a severe generalised condition of high mortality. Leg problems usually became apparent in the second week and it was probable that most of the poults that had been inoculated *in ovo* died before there was time for leg abnormalities to develop, so that only the two survivors at 3 weeks were thus affected.

These experiments did not establish whether it was inoculation via the air sac or via the foot pad or both that predisposed to chondrodystrophy, but in another experiment (Bradbury, 1984) one-day-old turkeys were infected with *M. iowae* strain B11/80 via the air sac only and they developed a similar incidence of chondrodystrophy to those in this study. Thus it seems likely that infection via the respiratory route, like the *in ovo* route, gives rise to a generalised infection. This was borne out by the mycoplasma recoveries in Expt 2.

A low incidence of cartilage erosion of the hock joints was encountered in both experiments, but only in those birds inoculated via the air sac and foot pad and in one bird infected *in ovo*. Ruptured tendons were seen in three birds in the first experiment but were not a feature in the second experiment. Other workers have

not reported cartilage erosion or ruptured tendons in *M. iowae* infections of turkeys although Yoder and Hofstad (1962, 1964) produced tendovaginitis by inoculating the organism directly into the hock joint. The turkeys used by these workers were older than the ones used in our experiments, being inoculated between 4 and 16 weeks of age and the connective tissue may have been less vulnerable to damage by this time. Thus, the age of the bird and route of inoculation both appear to play a part in determining the outcome of *M. iowae* infection. The birds infected orally at one day of age were the least affected of all the groups and isolation attempts suggested that the organism did not become widely disseminated.

The limited histological studies revealed inflammatory cells in a number of tissues including the lymphoid organs and the intestinal tract. The observations were similar to those observed in *M. synoviae* infection in chickens by Kerr and Olson (1970) but have not been reported hitherto for *M. iowae*. There was no obvious microscopic change in the articular cartilage and thus no ready explanation for the gross lesions. It would be interesting to examine the growth plate for histological lesions in future studies in order to compare the findings with those already reported for TS 65 (Wise *et al.*, 1973).

M. iowae recoveries did not necessarily correlate with gross lesions although the organism was usually recovered from some site in the most severely affected birds. The results of Expt 1 suggested that strains B10/80, B11/80 and M4/77 were more invasive than the other two strains although again it could be argued that this was due to the inadvertent difference in doses. However, in earlier studies using the same strains in chicks (Bradbury and McCarthy, 1984), recoveries were only made from those birds infected with strains B10/80, B11/80 and M4/77 even though the doses of B16/80 and 695 were of the same order in that experiment.

In Expt 1 the mycoplasma did not apparently persist in the inoculated foot pad, and hock isolation was rare. Only two poults (out of a total of 55 inoculated) yielded *M. iowae* from the hock joint region whereas 11 of 65 chicks were positive in the earlier study with these strains (Bradbury and McCarthy, 1984). Culture was attempted from only the surface structures of the hock joint and tendon region and it is possible that the organism is sequestered within the connective tissue. Kohn *et al.* (1982) have demonstrated the presence of *M. pulmonis* in the matrix and lacunae of the articular cartilage of rats.

Expt 2 showed that, following *in ovo* infection, *M. iowae* was widespread in the tissues in the first few days of life after hatching and that the majority of hock joints were culturally positive at this time. After 10 days hock joint isolations became less frequent but the mycoplasma persisted in the oesophagus, air sacs and cloaca in the birds surviving to 3 weeks. The oral route of infection resulted in only three isolations, all from the oesophagus, while the combined air sac and foot pad inoculation resulted in a greater dissemination of the organism but little persistence, with no isolations at all being made after 5 weeks.

The lack of serological response to *M. iowae* in agglutination tests using two antigens of this serogroup (strains 695 and J DJA) was not entirely unexpected because Yoder and Hofstad (1962) reported a lack of detectable antibodies following infection of turkeys with the 695 strain via the hock and foot pad or by respiratory routes. In 1964 these authors detected agglutinins in tube tests in some infected

turkeys but with less consistency than those detected following *M. gallisepticum* infection. A similar poor response to *M. iowae* has been noted in chickens (Yoder and Hofstad, 1962, 1964, Bradbury and McCarthy, 1981, 1984) and it is unfortunate that an agglutination test cannot be reliably used to monitor turkey flocks for this infection.

These studies have brought into focus some of the unsolved questions about *M. iowae* infection. For example, despite its obvious pathogenicity in experimental infections, there is little field evidence of this mycoplasma causing clinical problems apart from losses in turkey hatchability. It seems likely that the rate of egg transmission is low and that heavily infected embryos may not hatch, and, from these experiments it would appear that it is very early infection, either through the egg or via the respiratory tract that would be more likely to cause disease in the hatched bird. If such infected birds do survive, our results suggest that, by the time any leg problems might be seen, the organism would not be readily isolated, nor would there be a detectable antibody response. Thus proper assessment of the economic importance of *M. iowae* must await the development of better diagnostic techniques.

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RESUME

Infection du dindonneau par *Mycoplasma iowae*

Une comparaison a été faite sur les effets de l'inoculation de cinq souches différentes de *M. iowae* chez des dindonneaux d'un jour en utilisant l'injection dans le sac aérien thoracique ou dans le coussinet plantaire. Trois souches sont apparues plus virulentes et envahissantes que les deux autres, entraînant une croissance réduite, un mauvais emplumement et des anomalies des pattes de type chondrodystrophie. Une de ces trois souches a été utilisée dans une seconde expérience destinée à étudier différentes voies d'infection. L'infection *in ovo* a entraîné une maladie généralisée sévère à l'éclosion avec une forte mortalité. Les deux seuls oiseaux qui ont survécu ont présenté de la chondrodystrophie au cours de la troisième semaine. Un groupe, qui avait été infecté par voie orale à l'âge d'un jour, n'a présenté que quelques anomalies de l'os et des articulations alors que le dernier groupe infecté par injection dans le sac aérien thoracique et le coussinet plantaire, également à un jour d'âge, a développé ces anomalies en plus grand nombre: chondrodystrophie, déformation du tibia, déviation des doigts et dans certains cas érosion du cartilage du jarret. Quelques-uns de cas animaux témoins non infectés ont présenté quelques anomalies de poids mais jamais de chondrodystrophie, de déviation du tibia ou d'érosion de cartilage. *M. iowae* était le plus disséminé dans les tissus lors d'infection *in ovo* et le moins lors d'infection orale. Les isolements sont devenus moins fréquents avec l'âge et aucun organisme n'a été retrouvé sur les prélèvements faits à 12 semaines. Dans aucune des expériences, des anticorps

vis-à-vis de *M. iowae* n'ont pu être décelés par agglutination rapide.

ZUSAMMENFASSUNG

Mycoplasma iowae — Infektion junger Puten

Die Wirkung von fünf verschiedenen Stämmen von *M. iowae* nach einer Inokulation von Eintagsputen in den thorakalen Luftsack und in die Fußsohle wurde vergleichend untersucht. Drei Stämme erschienen virulenter und invasiver zu sein, als die anderen zwei. Sie verursachten Kümmern, schlechte Befiederung und Beinabnormalitäten inklusive Chondrodystrophie. Einer der Stämme wurde in einem zweiten Versuch benutzt, um drei Infektionswege zu vergleichen. Eine *in ovo* Infektion verursachte eine generalisierte Krankheit bei frischgeschlüpften Putenküken mit hoher Mortalität. Die einzigen beiden überlebenden Küken entwickelten in der dritten Woche Chondrodystrophie. Von einer am ersten Lebenstag oral infizierten Gruppe, zeigten einige Küken Knochen- und Gelenksveränderungen, dagegen wurden von einer anderen Gruppe, die am ersten Lebenstag via thorakalem Luftsack und Fußsohle infiziert worden war, diese Abnormalitäten inklusive Chondrodystrophie, Tibiadrehung, Zehenverkrümmung und einigen Fällen von Erosion des Gelenksknorpels am Sprunggelenk in größerem Umfang entwickelt. Einige der nichtinfizierten Kontrolltiere zeigten ebenfalls Beinabnormalitäten, aber niemals Chondrodystrophie, Tibiadrehung oder Knorpelveränderungen. *M. iowae* was nach *in ovo* Infektion am intensivsten in den Geweben verstreut, am wenigsten nach oraler Infektion. Isolationsversuche glückten mit zunehmenden Alter wenig häufig. In der 12. Woche konnten in den Tieren keine Mycoplasmen nachgewiesen werden. In keinem Versuch konnten durch Schnellagglutination Antikörper gegen *M. iowae* entdeckt werden.

RESUMEN

Infección por *Mycoplasma iowae* en pavos jóvenes

Se hizo una comparación del efecto de cinco cepas diferentes de *M. iowae* después de la inoculación de pavitos de un día de edad por vía saco aereotorácico y en el cojinete plantar. Tres de las cepas mostraron ser más virulentas y más invasivas que las otras dos, causando enanismo mal emplumado y anormalidades de las patas incluyendo condodistrofia. Una de estas tres cepas fue empleada en un segundo experimento en el cual se compararon tres rutas de infección. La infección *in ovo* causó un cuadro severo generalizado en pavitos recién nacidos con alta mortalidad. Solamente dos de las aves que sobrevivieron hasta la tercera semana desarrollaron condodistrofia. Un grupo fue infectado oralmente a un día de edad y algunas aves desarrollaron anormalidades de hueso y articulaciones, sin embargo otro grupo infectado por ambas vías, es decir, por saco aéro torácico y por cojinete plantar igualmente a un día de edad presentaron una mayor incidencia de este tipo de anormalidades, las cuales incluían condrodistrofia, rotación de tibia, dedos desviados y en algunos casos, erosión del cartílago articular de la articulación del tarso. Algunas de las aves controles no infectadas desarrollaron abnormalidades de las patas, pero nunca mostraron condodistrofia, rotación de tibia o erosión del cartílago. El *M. iowae* estaba ampliamente diseminado en los tejidos como consecuencia de la infección *en ovo* y en menor escala después de la infección oral. Los aislamientos se hicieron menos frecuentes con la edad hasta que ya no se aislaron más hacia las 12 semanas de edad de las aves muestreadas.

En ninguno de los experimentos se detectaron anticuerpos contra el *M. iowae* por medio de la aglutinación rápida.