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EPIDEMIOLOGICAL AND PATHOLOGICAL STUDIES ON *MYCOPLASMA IOWAE* INFECTION

(With 9 Tables and 6 Figures)

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دراسات وبائية وباثولوجية عن العدوى بالميكوبلازما أيوا

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في دراسة لاستبيان مدى انتشار العدوى بالميكوبلازما أيوا في الرومي والدجاج والبط والحمام، تم فحص عدد ١٣٧ بيضة غير مخصبة، و ١٨٠ بيضة كابسة،و ٤٠٥ طائر. مثلت الميكوبلازما أيوا ٧,١٤% من عترات الميكوبلازما المعزولة من أجنة الرومى ، ١,٨٥% من العترات المعزولة من الرومي النامى، ٢,٧% من العترات المعزولة من أمهات الرومي، و ٠,٩% من العترات المعزولة من الدجاج. ولم يعزل الميكروب من البط أو الحمام. بدراسة العترات المعزولة بواسطة الميكروسكوب الإلكتروني تبين تغير شكل الميكوبلازما أيوا مع زيادة النمو، وكانت العترات متشابهة في ذلك حيث ظهرت في اشكال خيطية وأخرى حويصلية. و بإجراء التحليل الكهربائي بإستخدام هلام سلفات الصوديوم-بولى أكريل أميد، تم الكشف عن وجود اختلافات بسيطة ولكن واضحة و متكررة بين العترات في نماذج حزم البروتين. عند إجراء إختبار الحساسية المعملى للعترات المعزولة من الميكوبلازما أيوا، وجد أن العترات كانت عالية الحساسية لمجموعة الفلوروكينولون وخصوصاً الإنروفلوكساسين. أثبتت إختبارات العدوى الاصطناعية أن أجنة بيض الرومي أكثر تأثراً من أجنة بيض الدجاج، وأدت العدوى الاصطناعية لكتاكيت الرومي عمر يوم إلى التقزم وشذوذ نمو الريش، وكذلك إصابات الأرجل. وعلى الجانب الأخر كان التأثير المرضى للعترات قليلاً في كتاكيت الدجاج المحقونة في عمر يوم. وقد اختلفت العترات في ضراوتها سواء للأجنة أو كتاكيت الرومى والدجاج عمر يوم. كما أظهر الفحص بالميكروسكوب الإلكتروني النافذ التصاق خلايا الميكوبلازما بالخمائل الدقيقة لخلايا الأمعاء مما يؤكد ميل الميكوبلازما أيوا نحو النسيج الطلائى للأمعاء. كان اختبار التلازن المصلى على الشريحة غير كاف للكشف عن الأجسام المناعية المضادة للميكوبلازما ايوا في مصل الطيور المعدية اصطناعيا أو طيور الحقل.

SUMMARY

An investigation of *Mycoplasma iowae* (*M. iowae*) infection in turkeys, chickens, ducks and pigeons was carried out on samples collected from a total number of 137 infertile eggs, 180 dead-in-shell and pipped embryos and 405 birds. *M. iowae* constituted 7.14% of the mycoplasma flora in turkey embryos, 1.85% in growing turkeys, 2.7% in turkey breeders and 0.9% in chickens. No *M. iowae* isolates were recovered from ducks or pigeons. Scanning electron microscopy (SEM) revealed pleomorphism of *M. iowae* and absence of significant morphological differences among isolates. Filamentous and coccal forms were observed. Sodium dodecyle sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed minor but distinct and reproducible variation in the protein banding pattern of isolates. *In-vitro* sensitivity of isolates to antimycoplasmal agents indicated that *M. iowae* was highly sensitive to fluoroquinolones, particularly enrofloxacin. Pathogenicity testing indicated that turkey embryos were more susceptible than chicken embryos. Experimental infection of one-day-old turkey poults resulted in stunting, abnormal feathering and leg abnormalities. Minimal pathogenicity was detected in one-day-old chicks. Pathogenicity varied among isolates for either embryos or one-day-old turkey poults and chicks. Mycoplasma cells were detected with transmission electron microscopy (TEM) in the intestine of embryos adhering to the microvilli of enterocytes, emphasizing the proclivity of *M. iowae* to the intestinal epithelium. serum plate agglutination (SPA) test was inefficient in the detection of antibodies to *M. iowae* in sera collected from experimentally infected birds and field birds.

Key words: *Mycoplasm iowae , Turkeys , Epidemiology , Electron , Microscopy*

INTRODUCTION

M. iowae species encompasses the former serotypes I, J, K, N, Q and R. The first report on serotype I (Iowa 695) in the literature was that of Yoder and Hofstad (1962) who described strains of this new serotype isolated from turkeys and chickens. In the 1960s, 1970s and early 1980s, several works proved the relatedness of these serotypes, their uniqueness and distinctness from other avian mycoplasmas. Eventually, the group was given the species status with the name of *M. iowae* (Jordan et al., 1982).

Although it has been well known that *M. iowae* is an egg-transmitted poultry pathogen (Bradbury, 1984-b; Baxter-Jones, 1991 & 1993; Jordan, 1996; Stipkovits and Kempf, 1996), there is little field evidence of clinical problems due to this organism apart from reduced turkey hatchability and poor quality poults (Jordan, 1996; Stipkovits and Kempf, 1996; Kleven and Baxter-Jones, 1997), and disease problems due to *M. iowae* have been largely determined by experimental infections rather than by the observation of natural field cases.

Only few sporadic attempts have been made to study *M. iowae* infection in Egypt. It was felt that serious attempts should be made to study *M. iowae* infection so that the economics of the losses due to this organism in the poultry industry in Egypt could be brought to light.

MATERIALS and METHODS

Samples:

Samples for isolation and sera were collected from turkey, chicken, duck and pigeon flocks. This inculded breeders, birds of different ages and hatcheries. Swabs for isolation of *M. iowae* were collected from albumen, yolk, oropharynx, oesophagus, trachea, air sacs, intestine, cloaca, ovary and oviduct.

Isolation and identification:

Collected samples were cultured in Brain-heart infusion (BHI) broth (Yoder, 1980), then subcultured and purified on BHI agar (Dierks et al., 1967).

Colonies were checked for bacterial irreversibility (Adler et al., 1958) and digitonin sensitivity (Clyde, 1964). Glucose fermentation and arginine utilization tests were conducted on media recommended by Enro and Stipkovits (1973). Serological identification was done by growth inhibition test (Dighero et al., 1970), growth precipitation test (Krogsgard-Jensen, 1972) and indirect immunofluorescent test (Baas and Jasper, 1972).

Whole cell protein profile using SDS-PAGE:

BHI broth culture was centrifuged at 12,000 Xg for 15 minutes. The pellet was washed 3 times with 0.02 M PBS (pH 7.2), suspended 1:1 (w/v) in 2X sample buffer, and then placed in boiling water bath for 10 minutes.

Electrophoresis was carried out by the application of 20 µl of the sample using electrophoretic cell (Mini-PROTEAN II, Bio-Rad, Richmond, USA) by the method of Laemilli (1970).

SEM for *M. iowae* grown in BHI broth:

SEM was done according to the method described by Gallagher and Rhoades (1979 & 1983) with some modifications.

Two groups of *M. iowae* cultures were distinguished, one grown up to 24 hr and the other up to 48 hr. For each of 3 ml of growth media from the two groups, 6 ml of 2.5% buffered glutaraldehyde were added, mixed properly and kept for 18 hr at 4°C. The fixed media were then centrifuged at 12,000 Xg for 10 minutes to harvest mycoplasmas. The resultant pellets were washed 3 times in PBS (pH 7.2) and fixed again in glutaraldehyde for further 3 hr. Sample pellets from each group of fixed preparations were processed for SEM.

Experimental infection of *M. iowae* isolates to embryonated turkey and chicken eggs:

One hundred and eighty 7-day-old embryonated turkey eggs, proved free from mycoplasma after cultural examination, were divided into 9 equal groups. Eggs of the first 8 groups were inoculated into the yolk sac with 0.2 ml of broth culture (without inhibitors) containing approximately 10^6 CFU of corresponding *M. iowae* isolate, while eggs in the 9^{th} group were inoculated with sterile broth as control.

Eggs were properly incubated at 37°C and candled daily for viability. Any dead embryo, together with a control embryo for comparison, was removed for examination as described below. Starting 4 days after inoculation and every fourth day thereafter, 2-3 inoculated eggs from each group and 2-3 control eggs were opened aseptically so that equal number of eggs was examined in each group by the 19^{th} day of incubation. The embryos were examined for external abnormalities and then opened for detection of organ abnormalities.

Samples from the intestine, trachea and lungs were fixed in 3% buffered glutaraldehyde for TEM (Coulter, 1967). Samples collected from oesophagus, trachea, air sacs, intestine and yolk were cultured on BHI agar for re-isolation of the inoculated organism.

Hatched poults were kept in isolation on a commercial starter diet up to 4 weeks of age and observed for signs and lesions. Samples for re-isolation trials were collected by the end of the 4^{th} week of age from the oesophagus, trachea, cloaca and air sacs.

The same experimental design was applied to 180 six-day-old embryonated chicken eggs.

Experimental infection of *M. iowae* isolates to one-day-old turkey poults and chicks:

Two hundred and seventy one-day-old turkey poults (native breed) were obtained from a private hatchery. They were randomly divided into three equal groups. Each group was further subdivided into 9 subgroups separately housed in isolation, 8 of which were challenged with the corresponding *M. iowae* isolate and the 9th served as non-infected control.

Poults in the first group were inoculated into the right lung after Jordan (1990) with 0.1 ml of appropriate broth culture; poults in the second group were inoculated into the right thoracic air sac (0.1 ml) and right foot pad (0.05 ml); and poults in the third group were challenged orally with 0.25 ml of broth culture on two successive days. The number of viable organisms in the inoculum was approximately 10^8 CFU/ml. Controls received equivalent inoculations of sterile broth.

Poults in all groups were given commercial starter diet *ad libitum* and kept under similar management conditions and observed daily for clinical signs for 6 weeks (end of the experiment). Samples for re-isolation trials were collected from the oesophagus, trachea, cloaca and air sacs.

At 3 weeks of age, leaving 5 poults in each group, the remaining poults were sacrificed for detection of post-mortem lesions, re-isolation trials and electron microscopy on the intestine, trachea and lungs as well as serum collection for SPA test. This was done to the remaining poults at the end of the experiment at 6 weeks of age.

The same experimental design was applied to 270 one-day-old chicks.

***In vitro* sensitivity of *M. iowae* isolates to antimycoplasmal agents:**

This test employed broth cultures of the isolates containing approximately 10^7 CFU/ml that were cultured by running drop technique (Clyde, 1964) on BHI agar.

Rapid SPA test:

SPA antigen was prepared from I 695 reference strain of *M. iowae* according to the method described by the U.S. Dept. of Agriculture (1972), and the test was done after Adler and Yamamoto (1956).

RESULTS

Isolation and identification of *M. iowae*:

Results are shown in Table 1. The sites from which these isolates were recovered included trachea and air sacs of dead-in-shell turkey

embryos (isolates designated A1, A2 and A3), intestine and cloaca of a dead-in-shell turkey embryo (isolate designated B), air sac of a turkey poult under 4 weeks of age (isolate designated C), trachea of a turkey poult over 4 weeks of age (isolate designated D), oviduct of a turkey breeder (isolate designated E) and trachea of a chick under 4 weeks of age (isolate designated F). *M. iowae* was not recovered from any of the samples collected from ducks or pigeons, either from eggs or birds.

Whole cell protein profile of *M. iowae* isolates using SDS-PAGE:

The major protein bands in the lower part of the gel (approximately below the 61-kilodalton region) of all *M. iowae* isolates resembled each other closely. However, minor but distinct variations among the isolates were observed.

Densely stained bands unique for the isolates A2 and A3 (lanes 4 and 5) were observed nearly at the 128-kilodalton level. Similarly, the isolates D and E (lanes 8 and 9) showed a distinct band nearly at the 116-kilodalton level. Bands at approximately the 110-kilodalton level occurred in all isolates but were dense in the isolates A2 and A3 (lanes 4 and 5) (Fig.1).

SEM for *M. iowae* grown in BHI broth:

Filamentous forms were predominant in the 24 hr-growth culture. Filaments (1.3 to 2 µm in width) were curved or undulating and usually branched. Bulbous swellings measuring 1 to 2.5 µm were observed directly attached to or sprouting from the filaments or the branches (Fig.2).

Prevalence of coccal forms was noticed in the 48 hr-growth culture. In this growth phase, mycoplasmas appeared as clustered rounded cells (0.5 to 2.5 µm). Some coccal forms showed budding-like strucutures measuring 1 to 1.3 µm. Also, some coccal mycoplasma cells were arranged in short chains or in linear beaded configurations in which the cells were joined to each other with short narrow connections (Fig. 3). The surface of coccal mycoplasma cells was unevenly covered with "coating" material.

Experimental infection of *M. iowae* isolates to embryonated turkey and chicken eggs.

As shown in Tables 2 and 3, the pathogenicity of *M. iowae* isolates to turkey embryos was higher than to chicken embryos, and differences in virulence between isolates were minor with the exception of isolates A3 and D which were less virulent in both turkey embryos and chicken embryos.

Lesions such as subcutaneous haemorrhages congestion. oedema around the head and neck and stunting (Fig 4) were observed.

Birds hatched from infected eggs appeared depressed and most of them suffered from locomotor disturbances. Some of the turkey poults that were reared up to 4 weeks after hatching developed abnormal feather and chondrodystrophy, while chicks were not severely or frequently affected.

By TEM, mycoplasma cells were seen in the intestinal lumen in close opposition to the lumenal surface of enterocytes or adhering to the microvilli. Microvilli to which mycoplasmas were attached appeared swollen and blunt (Fig. 5).

M. iowae was recovered from turkey and chicken embryos and hatched poults and chicks that were reared up to 4 weeks.

Experimental infection of *M. iowae* isolates to one-day-old turkey poults and chicks.

Results of pathogenicity testing (Tables 4-9) indicated that all of the *M. iowae* isolates proved pathogenic to turkey poults by different routes. The organism was less pathogenic to chicks.

Intrapulmonary inoculation was the most effective route to induce infection. Moreover, the isolates A3 and D were less pathogenic to poults than other isolates.

High incidence of leg abnormalities occurred mainly in poults reared to the end of the experiment (Fig. 6).

Besides leg abnormalities, liver lesions and airsacculitis were the only prominent lesions observed in turkey poults at necropsy. Similar liver lesions were observed in chicks.

In birds examined within the first 3 weeks PI, mycoplasma cells were observed in association with the epithelium of trachea and parabronchial infundibula of lungs in poults inoculated intra-air sac or intrapulmonary, and in association with the epithelium of the intestine in orally infected poults.

The recovery rate of *M. iowae* from experimentally infected birds varied with the route of infection and age.

Agglutination reaction was demonstrable in few serum samples collected 6 weeks PI.

***In vitro* sensitivity of *M. iowae* isolates to antimycoplasmal agents:**

M. iowae isolates showed more or less similar sensitivity patterns. Isolates were highly sensitive to enrofloxacin, ciprofloxacin and norfloxacin. They also were sensitive to Lincospectin®, danofloxacin and doxycycline.

On the other hand, slight sensitivity to flumequine, oxytetracycline, spectinomycin and spiramycin was detected. Isolates were poorly sensitive to erythromycin and tetracycline, and almost resistant to gentamicin and streptomycin.

SPA test on sera collected from field birds:

Only 9 serum samples collected from turkeys showed positive agglutination reaction.

DISCUSSION

In the present study, a survey of *M. iowae* infection in turkeys, chickens, ducks and pigeons was made on tissues from a variety of sources and birds of various ages, and from pipped and dead-in-shell embryos.

Four isolates of *M. iowae* were recovered from trachea, air sacs and intestine of dead-in-shell turkey embryos. In Egypt, recovery of *M. iowae* from hatching turkey eggs was reported by Fatma (1994).

The recovery of *M. iowae* from the air sac and trachea of turkey poults is supported by the reports of Dierks et al. (1967), Jordan and Amin (1980) and Shah-Majid and Rosendal (1987). Our isolates were not associated with the presence of lesions in the respiratory system. The isolation of *M. iowae* from grossly affected respiratory system of turkeys and chickens was reported by Fabricant (1970) and Shimizu et al. (1979).

The lower incidence of infection in turkeys than in turkey embryos may be due to death of infected embryos before hatching. Moreover, the frequent use of antimycoplasmal drugs, to which *M. iowae* might be susceptible, may have reduced infection in birds.

Isolation of *M. iowae* from the trachea of chickens in the present study together with the reports of Yoder and Hofstad (1962), Shimizu et al. (1979) and Bencina et al. (1987) points to the role which could be played by chickens in the epidemiology of infection. Chickens may be a potential source of infection for turkeys. This is particularly important in the Egyptian poultry industry because some poultrymen used to raise chickens together with or close to turkeys. Moreover, the incorrect custom of incubating chicken eggs together with turkey eggs in the same incubator may contribute to lateral transmission of *M. iowae* not only among turkeys but also from turkeys to chickens or probably vice versa.

Infected embryos may hatch and spread the infection to the in-contact mates.

Our SDS-PAGE results agree to some extent with those of Rhoades (1984) who reported minor variation in location and intensity of protein patterns of *M. iowae* strains. The variation in major and minor protein bands between *M. iowae* strains was also reported by Zhao and Yamamoto (1989), Grau et al. (1991) and Panangala et al. (1992). It is suggested that SDS-PAGE is a useful procedure in epidemiological studies where reproducible minor but unique differences in protein patterns may be used to identify a particular strain of *M. iowae*.

SEM confirmed the pleomorphism of *M. iowae*. The fine morphology of isolates closely resembled that reported by Gallagher and Rhoades (1983). The occurrence of the relatively large filamentous form of *M. iowae* was reported by Yoder and Hofstad (1964).

The observation of material attaching to the exterior of the limiting membrane of *M. iowae* was a consistent finding in SEM and TEM of the organism in both culture and tissue. Observation of such material was previously reported by Jordan et al. (1982). Bradbury (1984-b) suggested that the presence of such material may contribute to the relatively high resistance of *M. iowae* to physical and chemical agents.

The unusual amount of the surface material observed in isolates may be attributed to their wild nature since these recently isolated organisms were kept to the minimum level of passage. Multiple passages in liquid medium decreased the virulence of *M. pulmonis* and caused the capsule to become thinner than in the original strain (Taylor-Robinson et al., 1981).

The inoculation of *M. iowae* isolates in either chicken embryos or turkey embryos via yolk sac confirmed that the organism has the potential to reduce hatchability (Grant, 1987; Jordan, 1996) and that field strains could be highly pathogenic (Kempf et al., 1994).

As far as embryonic stunting, oedema and congestion are concerned, our results entirely agree with those reported in turkey embryos (McClenaghan et al., 1981; Bradbury et al., 1988; Mirsalimi et al., 1989; Kempf et al., 1994) and chicken embryos (Yoder and Hofstad, 1964; Bradbury and McCarthy, 1983; Fatma, 1994).

High incidence of leg abnormalities was observed in infected embryos. Periarticular caseous lesions were observed in chicken embryos inoculated with *M. iowae* by Yoder and Hofstad (1962). Bradbury and McCarthy (1983) described curled toes, while Fatma (1994) reported bilateral deviation of the toes in chicken and turkey embryos inoculated with *M. iowae*.

It seems that leg abnormalities may contribute to the poor hatchability due to *M. iowae* infection where embryos that do not die become unable to hatch.

Liver lesions in inoculated embryos and hatched survivors seem to be in agreement with those reported by Mirsalimi et al. (1989).

Varying degrees of hepatitis and discolouration of the liver due to *M. iowae* infection were reported in chicken embryos (Yoder and Hofstad, 1962 & 1964; Bradbury and McCarthy, 1983).

It was noticed that some embryos had wrinkling or delayed eruption of feathers compared to the controls. It is suggested that *M. iowae* multiplies extensively in the embryo and spreads to all tissues including the feather follicles. Baxter-Jones (1993) mentioned that embryos naturally infected with *M. iowae* had swollen down plumules abnormality of the feathers.

The present electron microscopic observations on the intestine of inoculated embryos confirmed the results reported in turkey embryos by Mirsalimi et al. (1989). However, the morphology of the adhering mycoplasma cells in the present study differed from that reported in the intestine of turkey embryo (Mirsalimi et al., 1989) and in the cloaca and vagina of female turkeys (Sharcef et al., 1990-a & b).

The difference in morphology of mycoplasma may be associated with the mycoplasma cytoskeleton which is responsible for changes in cell shape (Razin, 1986). Morphological changes may represent adaptive responses which are probably a requisite for survival and propagation of mycoplasma (Panangala et al., 1992).

The demonstrated swollen microvilli at the attachment site may be due to the direct tissue damage which follows the utilization of cellular nutrients by the mycoplasma (Jordan, 1985) and/or the production of damaging metabolic products (Razin, 1985 & 1986; Almagor et al., 1986). So, it is possible that intestinal colonization could interfere with the absorptive function of the intestine and indirectly with the growth of both embryos and young growing birds (Mirsalimi et al., 1989).

On experimental infections, no mortality could be ascribed to *M. iowae* but chondrodystrophy appeared to be the main lesion and it usually accompanied other forms of skeletal abnormalities. Our findings confirmed those reported by Bradbury and Ideris (1982) and Bradbury et al. (1988).

The actual cause(s) of chondrodystrophy and abnormal feathering noticed in poults experimentally infected with *M. iowae* is not

known. Further investigations are needed to find out if it was a direct effect of mycoplasma or due to interference of mycoplasma with bone and feather nutrition or uptake and metabolism of certain nutrients such as amino acids and vitamins (Wise et al., 1973; Bradbury et al., 1988).

M. iowae and *M. gallisepticum* were occasionally isolated from the pulp of feathers from experimentally infected turkey poults (Jordan et al., 1991).

In agreement with our results, the oral route was associated with low incidence of skeletal abnormalities in the experiment performed by Bradbury et al. (1988). In other studies (Jordan et al., 1992; Shah-Majid and Rosendal, 1987), signs and lesions were absent in poults inoculated with *M. iowae* via various routes.

The infrequent occurrence and mildness of airsacculitis in poults experimentally infected with *M. iowae* seem to agree with the reports of Yoder and Hofstad (1962 & 1964), Dierks et al. (1967) and Rhoades (1981-a). Severe air sac lesions may occur in mixed infection of *M. iowae* with *M. meleagridis* (Rhoades, 1981-b).

Liver lesions were observed in experimentally infected birds and varied with the isolate, route of inoculation and infected species. Bradbury et al. (1988) reported liver abnormalities in turkey poults hatched after *in ovo* inoculation with *M. iowae* at 21 day of incubation.

It would be presumed that *M. iowae* may have reached the liver through generalized infection that may result from the respiratory routes of inoculation (Bradbury, 1984-b) or by ascending infection from the gastrointestinal tract.

Inconsistencies between chicken embryos and chickens may reflect differences in susceptibility between embryos and chickens (Lockaby et al., 1999). Therefore, although embryo inoculation may reveal some aspects of *M. iowae* infection, it can not reliably predict pathogenicity of a particular strain of *M. iowae* in chickens.

Isolation of *M. iowae* diminished with time suggesting that the organism may not persist in large numbers in infected turkeys, and infected chicks readily eliminate the infection. Similar observations were reported by Bradbury and McCarthy (1984), Bradbury et al. (1988) and Bradbury and Kelly (1991).

It is worth noting that since most of the infected embryos die (McClenaghan et al., 1981; Rhoades, 1981-a), the disease patterns produced in experimentally infected turkey poults in the present study are unlikely to occur naturally in the field. Nevertheless, Trampel and Goll (1994) reported an outbreak of *M. iowae* infection in turkey poults

in which 1.4% of the flock was culled because of leg problems. It seems that the actual field losses due to *M. iowae* arise from mixed infection with *M. meleagridis* which acts synergistically with *M. iowae* in embryos (Carpenter et al., 1981) and turkey poults (Fabricant, 1970; Rhoades, 1981-b).

Concerning the *in vitro* sensitivity of *M. iowae* isolates to antimycoplasmal agents, our results were similar to those reported by Eissa (1996). The isolates were highly sensitive to the fluoroquinolones particularly enrofloxacin. Enrofloxacin was used efficiently as an egg dip (Baxter-Jones, 1993) and as water medication for treating poults (Jordan et al., 1991) and reducing vertical transmission of *M. iowae* in laying turkeys (Jordan et al., 1993).

The SPA test appears to be not suitable for screening antibodies to *M. iowae* either in naturally or experimentally infected birds. This test was not efficient in detecting antibodies in birds experimentally infected with *M. iowae* via different routes of inoculation.

There is no ready explanation for the poor immunogenicity of *M. iowae*. It seems to be less able to provoke humoral antibody response than other mycoplasmas (Bradbury, 1983; Bradbury and McCarthy, 1984) and may even cause a transient immunosuppression (Bradbury, 1984-a). Bradbury and McCarthy (1984) suggested that birds may have eliminated the organism before they achieve a sufficient level of immunocompetence to develop agglutinins. Antigenic variation contributes to the ineffective serodiagnosis of *M. iowae* infections (Rhoades, 1984).

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Table 1. Isolation and identification of *M. iowae* isolates.

Species	Source of sample	No. of isolates	Digitonin sensitivity	Biogrouping Glucose +ve, Arginine +ve	*Serological identification			%
					GI	GP	IIF	
Turkeys	Infertile eggs	8	4	-	-	-	-	-
	Dead-in-shell & pipped embryos	56	46	6	4	4	4	7.14
	Under 4 weeks	54	48	2	1	1	1	1.85
	Over 4 weeks	46	40	3	-	-	1	2.17
	Breeders	37	28	3	1	1	1	2.7
Chickens	Infertile eggs	5	2	-	-	-	-	-
	Dead-in-shell & pipped embryos	8	5	1	-	-	-	-
	Under 4 weeks	40	31	6	1	1	1	2.5
	Over 4 weeks	32	29	3	-	-	-	-
	Breeders	28	24	2	-	-	-	-
Ducks	Infertile eggs	7	3	-	-	-	-	-
	Dead-in-shell & pipped embryos	20	11	1	-	-	-	-
	Under 4 weeks	14	10	1	-	-	-	-
	Over 4 weeks	20	13	2	-	-	-	-
	Breeders	21	15	4	-	-	-	-
Pigeons	Infertile eggs	3	1	-	-	-	-	-
	Dead-in-shell & pipped embryos	6	3	-	-	-	-	-
	Under 4 weeks	12	9	2	-	-	-	-
	Over 4 weeks	6	5	1	-	-	-	-
	Breeders	17	15	2	-	-	-	-
Total	-	440	342	39	7	7	8	1.8

* GI: growth inhibition; GP: growth precipitation; IIF: indirect immunofluorescence.

Table 2. Abnormalities observed in embryos inoculated with *M. iowae* isolates and examined up to the 19th day of incubation.

Infected species	Isolate	*No. examined	**No. of embryos with lesions						
			St	Od	Cong	Hge	Leg abnormalities	Liver abnormalities	Feather abnormalities
Turkey embryos	A1	9	7	4	7	6	5	7	2
	A2	9	8	3	8	7	5	7	1
	A3	9	5	1	3	4	4	4	-
	B	9	6	2	5	7	6	5	1
	C	9	7	3	6	6	5	7	2
	D	9	4	2	2	3	5	5	-
	E	9	7	3	5	6	5	6	-
	F	9	6	2	5	6	4	6	-
	Control	9	-	-	1	-	-	1	-
Chicken embryos	A1	9	5	3	5	5	5	6	3
	A2	9	4	3	6	5	6	5	1
	A3	9	3	2	3	3	3	3	1
	B	9	4	1	4	5	5	4	2
	C	9	5	2	5	4	6	5	2
	D	9	2	2	3	1	2	2	-
	E	9	3	3	4	3	5	5	1
	F	9	3	3	5	5	5	4	-
	Control	9	-	-	1	-	-	-	-

* 2-3 inoculated eggs from each group were examined every 4 days PI to leave 11 eggs until hatching.
**: St: stunting; Od: oedema; Cong: congestion; Hge: haemorrhage

Table 3. Hatchability of embryos inoculated with *M. iowae* isolates and left after the 19th day of incubation.

Infected species	Isolate	*No. of eggs	No. of hatched embryos	No. of unhatched birds		No. of embryos and hatched birds showing abnormalities	
				Dead-in-shell	Pipped	Leg abnormalities	Liver abnormalities
Turkey embryos	A1	11	3	5	3	2	4
	A2	11	2	4	5	3	5
	A3	11	5	2	4	1	2
	B	11	3	4	4	-	3
	C	11	3	5	3	2	6
	D	11	6	2	3	-	2
	E	11	2	5	4	2	5
	F	11	3	3	5	2	4
	Control	11	10	1	-	-	-
Chicken embryos	A1	11	5	2	4	2	3
	A2	11	4	4	3	2	4
	A3	11	7	1	3	-	2
	B	11	5	3	3	1	4
	C	11	4	4	3	1	3
	D	11	8	1	2	-	1
	E	11	3	5	3	1	3
	F	11	3	4	4	2	3
	Control	11	10	-	1	-	-

* Equal number of eggs was left to hatch after examination of 2-3 eggs from each group every 4 days PI.

Table 4. Pathogenicity of *M. iowae* isolates to one-day-old turkey poults inoculated via intrapulmonary route.

Isolate	Age (weeks)	No. examined	*No. of birds showing :							
			Stunting	Abnormal feathering	Chondro-dystrophy	SL	RT	DT	AS	LA
A1	0-3	5	2	3	-	-	1	3	-	2
	3-6	5	1	1	3	2	2	3	-	-
A2	0-3	5	3	4	1	1	-	3	1	3
	3-6	5	1	1	4	2	1	-	-	1
A3	0-3	5	2	2	-	-	-	2	-	2
	3-6	5	-	-	1	1	1	1	-	-
B	0-3	5	2	3	1	-	-	3	-	2
	3-6	5	1	-	3	3	-	1	-	1
C	0-3	5	3	2	2	1	-	4	-	-
	3-6	5	1	1	3	1	1	-	-	-
D	0-3	5	1	2	-	-	-	2	-	1
	3-6	5	1	-	1	-	1	1	-	-
E	0-3	5	1	3	1	1	-	3	1	1
	3-6	5	2	1	4	4	2	1	-	-
F	0-3	5	1	2	-	-	-	2	-	2
	3-6	5	-	2	-	1	1	-	-	1
Control	0-3	5	-	-	-	-	-	-	-	-
	3-6	5	-	-	-	-	-	-	-	-

* SL: splayed leg; RT: rotated tibia; DT: deviated toes; AS: airsacculitis; LA: liver abnormalities.

Table 5. Pathogenicity of *M. iowae* isolates to one-day-old turkey poults inoculated into the right thoracic air sac and right foot pad.

Isolate	Age (weeks)	No. examined	*No. of birds showing :							
			Stunting	Abnormal feathering	Chondro-dystrophy	SL	RT	DT	AS	LA
A1	0-3	5	1	3	1	-	-	3	1	1
	3-6	5	1	-	2	1	2	1	-	-
A2	0-3	5	-	2	-	-	-	1	2	2
	3-6	5	-	1	3	-	1	1	-	-
A3	0-3	5	2	1	-	-	-	2	-	1
	3-6	5	-	-	-	-	-	-	-	-
B	0-3	5	1	1	-	-	-	1	-	1
	3-6	5	1	-	3	2	-	1	-	-
C	0-3	5	2	-	1	-	1	3	1	-
	3-6	5	1	-	1	2	1	1	-	-
D	0-3	5	1	-	-	-	-	1	-	1
	3-6	5	-	-	2	-	-	-	-	1
E	0-3	5	2	3	1	-	-	2	-	-
	3-6	5	1	1	3	-	1	-	-	-
F	0-3	5	1	-	-	-	-	-	-	2
	3-6	5	-	1	1	-	-	-	-	1
Control	0-3	5	-	-	-	-	-	-	-	-
	3-6	5	-	-	-	-	-	-	-	-

* SL: splayed leg; RT: rotated tibia; DT: deviated toes; AS: airsacculitis; LA: liver abnormalities.

Table 6. Pathogenicity of *M. iowae* isolates to one-day-old turkey poults after oral inoculation.

Isolate	Age (weeks)	No. examined	*No. of birds showing :							
			Stunting	Abnormal feathering	Chondro-dystrophy	SL	RT	DT	AS	LA
A1	0-3	5	1	1	-	-	1	1	-	2
	3-6	5	-	-	-	-	-	-	-	1
A2	0-3	5	-	-	-	-	-	-	-	5
	3-6	5	-	-	1	-	-	-	-	1
A3	0-3	5	-	-	-	-	-	1	-	2
	3-6	5	-	-	-	-	-	-	-	-
B	0-3	5	-	-	1	-	-	-	-	3
	3-6	5	-	-	2	1	1	-	-	-
C	0-3	5	1	-	1	1	-	1	-	4
	3-6	5	-	-	-	1	-	-	-	1
D	0-3	5	-	-	-	-	-	-	-	2
	3-6	5	-	-	-	-	-	-	-	-
E	0-3	5	1	2	-	-	-	-	-	3
	3-6	5	1	-	1	-	-	-	-	1
F	0-3	5	-	-	-	-	-	-	-	4
	3-6	5	-	-	-	-	-	-	-	2
Control	0-3	5	-	-	-	-	-	-	-	-
	3-6	5	-	-	-	-	-	-	-	-

* SL: splayed leg; RT: rotated tibia; DT: deviated toes; AS: airsacculitis; LA: liver abnormalities.

Table 7. Pathogenicity of *M. iowae* isolates to one-day-old chicks inoculated via intrapulmonary route.

Isolate	Age (weeks)	No. examined	No. of birds showing:			
			Stunting	Abnormal feathering	Airsacculitis	Liver abnormalities
A1	0-3	5	1	1	-	1
	3-6	5	-	-	-	-
A2	0-3	5	1	-	1	2
	3-6	5	-	-	-	-
A3	0-3	5	-	-	-	1
	3-6	5	-	-	-	-
B	0-3	5	1	-	-	2
	3-6	5	1	-	-	1
C	0-3	5	-	-	-	-
	3-6	5	-	-	-	1
D	0-3	5	-	-	-	1
	3-6	5	-	-	-	1
E	0-3	5	-	-	-	1
	3-6	5	-	-	-	-
F	0-3	5	1	-	-	2
	3-6	5	-	-	-	-
Control	0-3	5	-	-	-	-
	3-6	5	-	-	-	-

Table 8. Pathogenicity of *M. iowae* isolates to one-day-old chicks inoculated into the right thoracic air sac and right foot pad.

Isolate	Age (weeks)	No. examined	No. of birds showing:			
			Stunting	Abnormal feathering	Airsacculitis	Liver abnormalities
A1	0-3	5	-	-	-	1
	3-6	5	-	-	-	-
A2	0-3	5	-	-	2	1
	3-6	5	-	-	-	-
A3	0-3	5	-	-	-	-
	3-6	5	-	-	-	-
B	0-3	5	-	-	-	-
	3-6	5	-	-	-	-
C	0-3	5	-	-	-	1
	3-6	5	-	-	-	1
D	0-3	5	-	-	-	-
	3-6	5	-	-	-	-
E	0-3	5	2	-	-	-
	3-6	5	-	-	-	-
F	0-3	5	1	-	-	-
	3-6	5	-	-	-	-
Control	0-3	5	-	-	-	-
	3-6	5	-	-	-	-

Table 9. Pathogenicity of *M. iowae* isolates to one-day-old chicks after oral inoculation.

Isolate	Age (weeks)	No. examined	No. of birds showing:			
			Stunting	Abnormal feathering	Airsacculitis	Liver abnormalities
A1	0-3	5	-	-	-	1
	3-6	5	-	-	-	1
A2	0-3	5	-	-	-	1
	3-6	5	-	-	-	1
A3	0-3	5	-	-	-	-
	3-6	5	-	-	-	-
B	0-3	5	2	-	-	1
	3-6	5	-	-	-	-
C	0-3	5	-	-	-	-
	3-6	5	-	-	-	1
D	0-3	5	-	-	-	-
	3-6	5	-	-	-	-
E	0-3	5	-	-	-	-
	3-6	5	-	-	-	-
F	0-3	5	1	-	-	1
	3-6	5	-	-	-	-
Control	0-3	5	-	-	-	-
	3-6	5	-	-	-	-

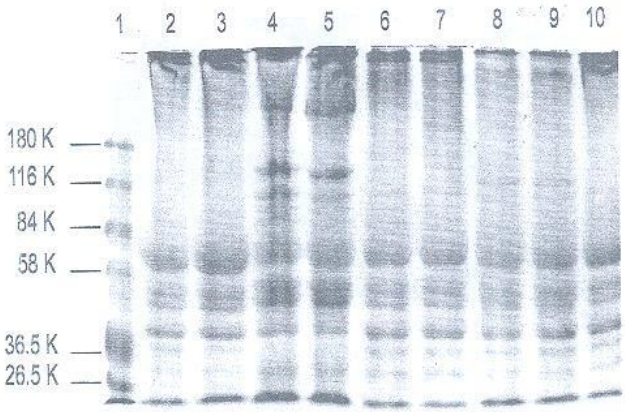

Fig.1. SDS-PAGE of whole cell protein of *M. iowae* isolates in 10% separating gel. Lane 1 = pre-stained molecular weight standard marker (K = Kilodalton); Lane 2 = *M. iowae* reference strain I 695; Lane 3 = isolate A1; Lane 4 = isolate A2; Lane 5 = isolate A3; Lane 6 = isolate B; Lane 7 = isolate C; Lane 8 = isolate D; Lane 9 = isolate E; Lane 10 = isolate F.

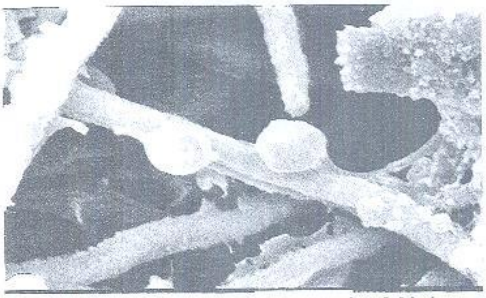

Fig.2. Scanning electron micrograph of *M. iowae* showing bulbous swellings sprouting from or attached to the filamentous forms. 24-hr growth period. X5,000.

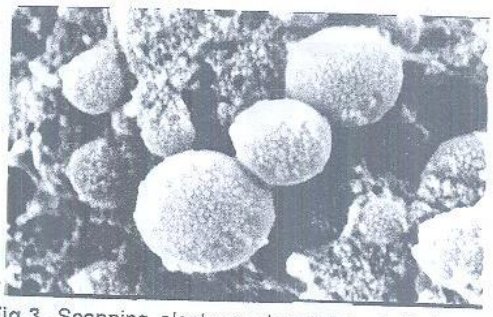

Fig.3. Scanning electron micrograph of *M. iowae* showing linear configuration of coccal forms that attach to each other with narrow connections. Note the coating material on the mycoplasma cell surface. 48-hr growth period. X15,000.

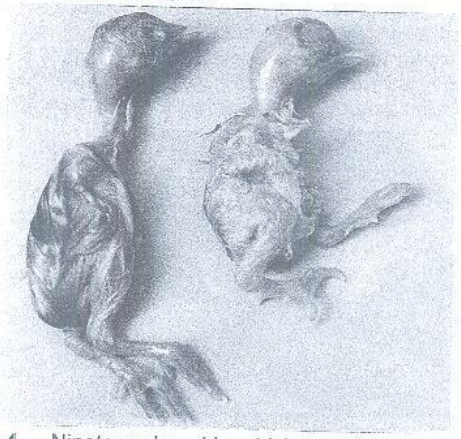

Fig.4. Nineteen-day-old chicken embryo (right) showing stunting, oedema of the head and neck, leg deformity and abnormal feather. Control embryo is at the left.

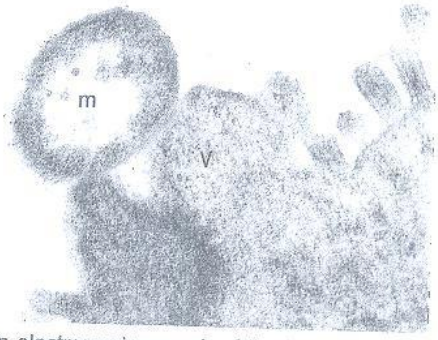

Fig. 5. Transmission electron micrograph of the intestine of 18-day-old experimentally infected turkey embryo. Mycoplasma cell (m) is attaching to the microvilli (v) of enterocytes which appeared swollen as a result. X40,000.

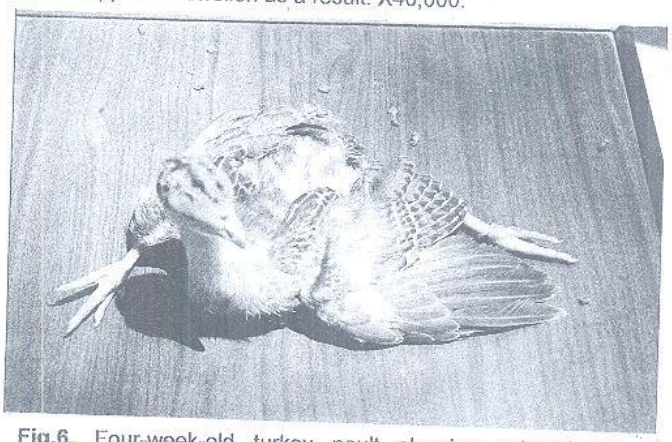

Fig.6. Four-week-old turkey poult showing splayed leg after experimental infection with *M. iowae*.