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with 1 table and 1 figure in the text.

Mycoplasma iowae (Mi) serovar (I): Recovery and Antibody Detection Post-infection of Turkey Hens

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ملخص البحث

أجريت تجربه لتقييم أعادة عزل وإنتاج مستضد ومعرفة الأجسام المضادة لمفطورة أيوا (*Mycoplasma iowae*) ذات النوع الطرز ط (Serovar 1) وذلك بعد حقنها في قناة البيض لدواجن روميه خاليه من ممرض محدد المجمع المايكوبلازما. بعد الحقن تم عزل المفطورة أيوا من كشطات أخذت من قناة البيض والشرج ولم يتم عزلها من كشطات القصبة الهوائيه. أعيد عزل 87,50% من المفطورة أيوا بواسطة الزراعة المباشره على منابت المايكوبلازما الهلاميه مقارنة بـ 78,2% بالزراعة غير مباشره علي منابت تحتوي على منبت سائل مع هلام. بالرغم من إستمرار عملية أخذ الكشطات لمدة 6 أسابيع بعد إجراء التجربه, عزلت مفطورة أيوا خلال الثلاثة أسابيع الأولى فقط. كشف عن وجود مستضد تلك المفطورة بواسطة إختبار المقايسة مناعية المضاده الامتصاصيه المرتبطة بالانزيم (ELISA) وليس اختبار التلازن المصلي السريع التراص.

Summary

An experiment was conducted to assess the re-isolation and antibody detection of *Mycoplasma iowae* (Mi) serovar I. Specific-pathogen-free (SPF) turkey hens were inoculated via the oviduct. Post-infection recovery of Mi serovar I was obtained from oviduct and cloacal swabs but not from tracheal swabs. A recovery of 87.5% was revealed by direct culturing onto mycoplasma agar while 78.2% recovery was obtained by indirect culturing through an overlay medium. Although swabbing and culturing was continued for six weeks, isolation of Mi was encountered only in the first three weeks. Anti Mi antibodies were detected by Enzyme Linked Immunosorbent Assay (ELISA) test but not by the Rapid Serum Agglutination(RSAT) test .

Introduction

Mycoplasma iowae (Mi) is known to cause reduced hatchability of turkey and chicken eggs (McMlenagham *et al.*, 1981) and generalized disease in very young poults (Bradbury and Idris, 1982). Similar to other mycoplasmas, Mi has a complex demands for isolation. In turkey poult, Mi can be isolated from the gastro-intestinal and cloacal swabs.

It is known that among commercial breeds of turkeys M**i** may be isolated from the oviduct but not from the trachea. With increased age, re-

isolation is less frequent and a combined oviduct and cloacal swabs were useful for detection of Mi infection during the final stage of eradication programme (Bradbury *et al.,* 1988). Mi strains give the poor antigenic response in chicken and turkey and little is known about cellular immune response. The immune response involved is suggested by determining the growth and metabolic inhibiting antibodies in sera of hens .The RSA test will not detect antibodies to Mi unless the birds are hyper-immunized however, the ELISA test was found more sensitive to Mi than RSA (Amal, 1984) .

To confirm these observations it was considered worthy to infect specific pathogen free (SPF) laying turkeys by Mi into the oviduct and monitor the birds for six weeks by culturing from the cloaca, the oviduct and the trachea as well as by serological tests using Enzyme Linked Immunosorbent Assay (ELISA) and Rapid Serum Agglutination(RSAT) test .

Materials and Methods

Turkeys:

Eighteen, white, broad breasted, one-year-old turkey hens from the British United Turkey (BUT) Company at the last quarter of lay, were used. They were monitored and found free from mycoplasmas as were their parents for several generations.

Mycoplasma iowae (Mi):

A broth culture of M**i** (serovar I) containing $1X10^7$ CFU/ml was used. This was obtained from the Institute of Medical Microbiology, Aarhus, Denmark as one ml lyophilized vial.

Mycoplasma Media:

Mycoplasma agar and broth (Power and Jordan, 1976) and an overlay medium (Jordan and Amin, 1975) were used (Difco Laboratories, UK).

Experimental procedure:

Before infection, each of the 18 female turkeys was swabbed from the trachea, caloaca and oviduct. These swabs were examined for mycoplasmas and a blood sample was also taken and the serum was screened for presence of antibodies to avian mycoplasma. Afterwards, and on the same day, the bird's oviducts were infected with 0.1 ml broth culture of Mi containing 10^7 CFU/ml through the vagina. At weekly intervals thereafter and for six subsequent weeks monitoring was undertaken by swabbing as mentioned above. In addition, two birds were randomly killed by intravenous injection of pentobarbitone sodium each week, necropsied and the oviduct was immediately ligated at the fimbria and vagina extremities. It was then removed from the carcass and

following loosening of one of the ligatures, 10 ml of mycoplasma broth were injected into the oviduct. The ligature was then tightened and the oviduct massaged. The ligature was loosened again and the contents were emptied into a universal bottle. A loopful was transferred to a Mycoplasma Agar plate and to the rest five ml of mycoplasma broth were added. This was then incubated at 37^0 C. Five days later a loopful was transferred to a Mycoplasma Agar plate. Whilst this was satisfactory for the first four weeks, by the 5^{th} and 6^{th} weeks the oviducts of the surviving birds had atrophied since they were out of lay. Such oviducts were aseptically removed and cut into small pieces with scissors and the tissue was placed into 10 ml of mycoplasma broth, was then incubated at 37^0 C for 5 days and sub-cultured as above.

Serological tests:

The ELISA test was undertaken as indicated by Ali (1984) using flat bottom polystyrene plates, coated with 64 μg/ml of Mi antigen; Antisera was diluted at 1/100. Anti-turkey IgG-conjugated with horseradish peroxidase was used. Ortho-phenylene diamine (OPD) was used as a substrate. Absorbencies were recorded using a multiskan spectrophotometer at 492 nm. A volume of 200 ul per well were used for all reactants. For detection of antibodies to the other avian mycoplasmas, the RSA test with a commercial antigen and a laboratory prepared Mi antigen were used.

Culture and identification of mycoplasmas:

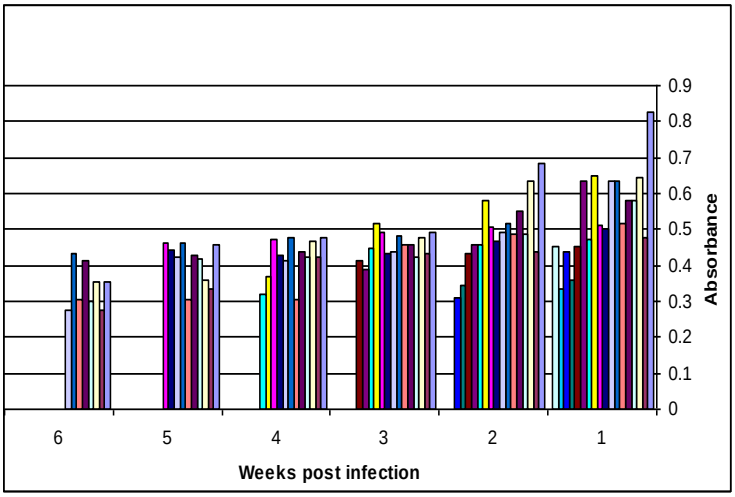
Cotton swabs were used. Tracheal, cloacal and oviduct swabs were taken at weekly intervals from the birds and were sown onto Mycoplasma Agar plates and then placed into an overlay medium and incubated at 37^0 C. Pathogens with no evidence of mycoplasma growth at day 28 were discarded as negative and also those that did not show colour change in the overlay media within five days were similarly discarded. However, if colour change occurred within five days, a loopful was sub-cultured onto Mycoplasma Agar. *Mycoplasma iowae* was identified and confirmed morphologically by the appearance of very small flat colonies, with a dense central elevation of 0.1-0.3 mm in diameter and biochemically by giving positive reaction both to glucose and arginin and negative to film, spot and tetrazolium reduction. Particular attention was paid to the isolation of Mi from the oviduct, which was removed from the carcass after bird death.

Results

Serology:

Before inoculation of Mi, no antibodies were detected for all avian mycoplasmas. Post-infection-antibodies were determined by the ELISA test and the the cut off point was found to be o.4 nm. It was taken as the mean of 100 negative SPF turkey sera plus two standard deviation (X+2SD). In the first week post infection, 16 out of the18 birds were positives. Fourteen out of 16 were positive in the second week, in the third week most of the birds gave absorbencies above the cut of point,

while 8 out of 12 were positive in the 4th week. At week 5, 5 out of 10 and at week 6 only 2 out of 8 were positives (Fig.1). Mi was not detected by the RSA test.



ELISA absorbance of antibodies to mycoplasma iowae 1-6 weeks post inoculation
Each column represents a bird.

Fig 1: ELISA absorbance of antibodies to *Mycoplasma iowe* 1–6 weeks pos-inoculation

***Mycoplasma* isolation:**

No mycoplasmas were isolated before the experimental infection. However, one week post infection and from the 18 birds**,** Mi was recovered from 14 and 16 oviducts and cloacae respectively. It was recovered from the oviduct of 2 birds and 4 from the cloacae with a total recovery of 16 out of the 18 tested birds.

At two weeks post-infection, Mi was recovered from all the 16 remaining birds; 14 from the oviduct and two from the one. Three weeks latter, Mi was recovered from the oviduct and cloacae of 8 out of the 14 surviving birds, but no recoveries were made at the 4th weeks or afterwards. The

organism was also isolated from all oviducts that were subjected to massage for the first three weeks but not afterwards (Table 1).

Table 1: Recovery of Mi from oviducts and cloacae of 18 laying turkey birds

+= Mycoplasmas was isolated;

Birds No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1 wk post-infection																		
Oviduct	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Cloaca	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
2 wks post-infection																		
Oviduct	k	k	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Cloaca	k	k	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3 wks post-infection																		
Oviduct	k	k	k	k	+	+	+	+	+	+	+	+	-	-	-	+	-	-
Cloaca	k	k	k	k	+	+	+	+	+	+	+	+	-	-	-	+	-	-

- = Mycoplasmas was not isolated;

K = killed

Forty-two Mi were isolated by direct culture from both oviducts and cloacae of 48 swabs over the first three weeks of the experiment. These include four samples in each case of isolation from the oviducts or cloaca alone. Eighteen and 20 isolations of Mi were made from the oviducts and cloacae, respectively by indirect (enriched) culture. On four occasions, the organism was recovered from either oviduct or the cloacae alone. On re-isolation mycoplasma was proved to be Mi by its biochemical characteristics and morphological appearance.

Discussion

In this experiment, Mi was introduced into the oviduct of laying turkeys and its re-isolation was attempted by oviduct, and cloacal swabs and no recovery was achieved from tracheal swabs. This finding is in agreement with that of Bradbury and Kleven (2004) who found that combined oviduct /cloacal swabs are useful for the isolation of Mi. The failure to isolate Mi from the tracheal swab may be attributed to the fact

that the organism was introduced directly into the oviduct. Generally, little is known about pathogenesis of Mi (Shareef *et al*., 1990). At onset of infection, Mi invades the epithelial surface and larger dose might be needed for dissemination of infection to different tissues.

Although Mi was introduced into the oviduct of laying turkeys through the vagina, thirty-five isolates were made from oviduct swabs, while forty-one were obtained from the cloacal swabs .It seems that swabbing of the cloaca is more suitable for isolation of Mi**.** It would seem that only a transitory infection was produced since Mi was recovered only for three subsequent weeks and then from 9 of the 14 surviving birds. These comparable to Wilding and Grant (personal communication) in which they infected turkey hens with Mycoplasma using vaginal swabs. Seroconversion using RSA test occurred in this study in 16 out of 18 birds by the 11^{th} day and at the 18^{th} at the termination of the experiment. Mm was recovered from 7 of 20 birds. Although the dose of Mm given by these authors was not known (swabs were used), it is unlikely to have exceeded the dose of Mi given in this study. If this is so then it would seem that Mi is less immunogenic than Mm for the turkey when introduced into the oviduct. It is not known, however, whether long duration infection or larger number of organisms that have produced detectable serological response. The chances of recovery of Mi seem to be more successful by direct culturing than by indirect through broth as shown by this study (42/48 recovery by direct culturing in comparison to 38 /48 recovery through broth). This was in agreement with Amin and Jordan (1978) who found better recovery was by direct culturing. Immunity of Mi was better detected by ELISA. However, non-specific reactions was enountered (Jordan *et al*., 1987). To exclude this, the ELISA results were interpreted by a cut-off-point determined by the value of the mean of absorbencies of 100 SPF turkey sera plus two standard deviation (X+2SD). Value above this figure was considered to be positive and vice versa. This method reduced non-specific reaction to the minimum (Ali, 1984), Similar interpretation was also made by Jordan *et al*. (1987) in a comparative study by which non-specific reaction was reduced or defused completely. In conclusion, Mi serovar I can easily be diagnosed by the ELISA test considering that non-specific reaction should be minimized by using highly purified reactants and of high quality.

References

Ali, Amal M. M. (1984). Ph.D. Thesis. University of Liverpool, UK.
Amin, M. M. and Jordan, F. T. W. (1978). *Avian Pathol.*, **7**: 55 - 470
Bradbury, J. M. and Idris, A. (1982). *Vet. Rec*., **110**: 559 - 560.
Bradbury, J. M.; Idris, A. and Oo, T.T. (1988) *Avian Pathol*., **17**: 149 - 171.
Bradbury, J. M. and Kleven, S. H. (2004). Poultry Diseases. 1st edn Pp. 760 -771.
Jordan, F. T. W. and Amin, M. M. (1975). *Vet. Sci*., **28**: 96 -100.
Jordan, F. T. W.; Yavari, C. and Knight, D. L. (1987). *Avian Pathol*. **16**: 307 - 318.
McMlenagham, M. J. M.; Bradbury, J. M. and Howse, J. N. (1981).*Vet. Rec.,* **108**: 459 - 460.
Power, J. and Jordan, F. T. W. (1976). *Res. Vet. Sci*., **21**: 41-46.
Shareef, J.; Wilcox, J. and Kumar, P. (1990). *Zent. Bact., Suppl* **20**: 872 -874.