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To cite this article: J.B. Poveda , A. Fernandez , J. Carranza , M. Hermoso & J.A. Perea (1986) Isolation of *Mycoplasma synoviae* from the red-legged partridge (*Alectoris rufa*), Avian Pathology, 15:4, 797-802, DOI: [10.1080/03079458608436343](https://doi.org/10.1080/03079458608436343)

To link to this article: <https://doi.org/10.1080/03079458608436343>



Published online: 02 Jan 2008.



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CASE REPORT

**ISOLATION OF MYCOPLASMA SYNOVIAE FROM THE  
RED-LEGGED PARTRIDGE (*ALECTORIS RUFA*)**

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SUMMARY

Following an outbreak of respiratory disease in a group of red-legged partridges (*Alectoris rufa*) and chickens (*Gallus domesticus*) housed in the same unit, a mycoplasma was isolated. Its biochemical profile and the growth-inhibition test enabled it to be identified as *Mycoplasma synoviae*.

Histopathological analysis revealed an intense inflammatory reaction in the respiratory tract, together with epithelial hyperplasia and lymphocyte infiltration.

The rapid serum plate agglutination test, and the haemagglutination-inhibition test, revealed the presence of humoral antibodies to *Mycoplasma synoviae* in the partridges studied.

INTRODUCTION

*Mycoplasma synoviae* (*M. synoviae*) is held to be the agent responsible for synovitis in chickens (Olson *et al.*, 1954) and turkeys (Frey, 1967). More recently, it has been shown that this microorganism is also capable of producing airsacculitis (Kleven *et al.*, 1972; King *et al.*, 1973). Kleven *et al.* (1975) reported a close link between the strain type and the route of infection in the development of synovitis or airsacculitis.

*M. synoviae* has been isolated from a natural outbreak of synovitis in guinea fowl (Pascucci *et al.*, 1976). The white pekin duck (Yamada and Matsuo, 1984), pheasants and geese (Sevoian *et al.*, 1958) have been shown to be susceptible to experimental infection, whereas the house sparrow has been found to be resistant (Kleven and Fletcher, 1983).

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Received 27 March 1986

Accepted 30 June 1986

Partridges have also been found to be susceptible to mycoplasma infection (Jungherr *et al.*, 1953; Yoder and Hofstad, 1964).

Wichman (1957) was able to isolate *M. gallisepticum* from a natural outbreak in chukar partridges (*Alectoris graeca*) and Keymer (1961) described a natural mycoplasma infection in red-legged partridges with sinusitis, but no work has been published on the susceptibility of partridges to *M. synoviae* infection.

#### CASE REPORT

Respiratory disease was observed in a group of 23 decoy partridges 1 week after being in contact with chickens housed in the same units. Partridges were between 1 and 3 years old, while chickens were about 12 weeks old.

The partridges were housed in individual cages at differing heights from the floor. The chickens were able to reach the cages, and fed from the partridges' feeding-troughs.

The partridges stopped singing, and showed signs of diarrhoea, dyspnea and anorexia. The chickens showed retarded growth and slight râles.

#### MATERIAL AND METHODS

Cotton swabs were obtained from the tracheas of 23 partridges and six chickens, and inoculated into mycoplasma broth medium (Frey *et al.*, 1968). After 5 days they were streaked on to PPLO agar with swine serum, and incubated for 7 days at 37°C in a moist atmosphere. The individual colonies were picked and inoculated into mycoplasma broth medium. After 5 days they were streaked on to PPLO agar again. The process was repeated three times in order to ensure the purity of the culture.

The biochemical characteristics of the isolations were tested using the methods described by Freundt *et al.* (1979).

Mycoplasma isolates were identified by a growth-inhibition (GI) test (Clyde, 1964), using filter-paper discs impregnated with antisera to both *M. gallisepticum* and *M. synoviae* (supplied by the Regional Animal Health Centre, Granada).

Rapid serum plate agglutination (RSP) tests were performed using fresh serum and commercial antigens.

Partridge and chicken sera were also subjected to the haemagglutination-inhibition (HI) test with *M. gallisepticum* and *M. synoviae* antigens prepared in our laboratory according to the procedure described by Yoder and Hofstad (1964) and Vardaman and Yoder (1969), using *M. gallisepticum* strain PG31 and *M. synoviae* strain WVU 1853 (supplied by Prof. Dr. E.A. Freundt, Institute of Medical Microbiology, Denmark).

Histopathological examination was made of killed chickens and partridges. Samples of different organs (trachea, lung and air sacs) were fixed in 10% buffered formalin. Tissues were processed and embedded in paraffin, sectioned, and stained with haematoxylin and eosin.

Indirect immunofluorescence (IF) tests were carried out on histological sections of lung tissue, using *M. gallisepticum* and *M. synoviae* antisera.

## RESULTS

From the samples obtained from the partridges, we obtained four mycoplasma isolates. They were inhibited by specific antiserum to *M. synoviae* using the GI test.

Seven mycoplasma isolates were obtained from the chicken samples, three of which were inhibited by *M. synoviae* specific antisera. The other four mycoplasma isolates were not inhibited by either *M. synoviae* or *M. gallisepticum* antisera, and their biochemical profile suggested that they may be *Mycoplasma gallinarum*.

The biochemical characteristics of the two types of isolations are shown in the Table 1.

*Table 1. Biochemical characteristics of mycoplasma isolated from partridges (P1), and chickens (C1, C2).*

	P1 <sup>a</sup>	C1 <sup>b</sup>	C2 <sup>c</sup>
Digitonin sensitivity	+	+	+
Urea hydrolysis	-	-	-
Glucose catabolism	+	+	-
Arginine hydrolysis	-	-	+
Phosphatase activity	-	-	-
Film and spots reaction	+	+	+
Tetrazolium reduction (Aerobic/anaerobic)	-/-	-/-	+/-

<sup>a</sup> P1 4 isolations

<sup>b</sup> C1 3 isolations

<sup>c</sup> C2 3 isolations

Table 2 shows the results obtained from the RSP tests and the HI tests, which confirmed the presence of *M. synoviae* antibodies in both species. With regard to HI titres for *M. synoviae* antigen, we considered as positive all HI titres above 1/40. Five of the 23 partridges and four of the six chickens showed HI titres of between 1/160 and 1/320.

*Table 2. Results of rapid serum plate agglutination tests and haemagglutination inhibition tests.*

	Rapid serum plate agglutination		Haemagglutination inhibition	
	<i>M. gallisepticum</i>	<i>M. synoviae</i>	<i>M. gallisepticum</i>	<i>M. synoviae</i>
Chickens	2/6 <sup>a</sup>	6/6	0/6 <sup>a</sup>	4/6
Partridge	0/23	9/23	0/23	5/23

<sup>a</sup> Number of birds positive/number tested

Histopathological examination of tissues from both species revealed tracheitis,

with epithelium loss and some necrotic-haemorrhagic areas, pneumonitis and hyperplasia of the lymphoid tissue around the secondary bronchii, perivascular and peribronchial accumulations of lymphocytes and a large number of plasma cells, hyperplasia of the bronchial and bronchiolar epithelium with excessive production from secreting cells, and airsacculitis with marked hyperplasia of epithelial cells and diffuse infiltration of the connective tissue by heterophils and mononuclear cells.

Positive immunofluorescence was detected in lung macrophages with *M. synoviae* antiserum.

#### DISCUSSION

The evidence indicates that the chickens housed in the same units as the partridges acted as carriers and transmitters of *M. synoviae*. However, the development of the respiratory disease observed in the partridges probably depends on the pathogenicity of the strain of *M. synoviae*, the susceptibility of the species and the environmental conditions in which they were confined. It is well known that free-range poultry do not succumb to certain diseases as readily as birds kept under certain conditions of confinement. Experimental reproduction of mycoplasma infections in poultry has sometimes proved unsuccessful. Yoder and Hofstad (1964) reported that none of the 12 bobwhite quails inoculated with *M. gallisepticum* developed signs of infection or HI antibodies. However, Madden *et al.* (1967) isolated *M. gallisepticum* from a natural outbreak of chronic respiratory disease in the same species. In our studies, titres obtained by the *M. synoviae* HI test were greater than 1/80 in five of the 23 partridges studied. It is likely that the chicken and partridge isolates are of the same strain of *M. synoviae* and were responsible for the clinical disorders observed, and this strain seems to show a tendency to cause lesions in the respiratory system. This is consistent with the findings of Kleven *et al.* (1975), who showed that some strains seem more prone to produce airsacculitis whereas others tend to cause synovitis.

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#### RESUME

##### Isolement de *Mycoplasma synoviae* à partir de perdrix rouges (*Alectoris rufa*)

A la suite d'un foyer de maladie respiratoire dans un groupe de perdrix rouges (*Alectoris rufa*) et de poulets (*Gallus domesticus*) élevés dans la même unité, un mycoplasme a été isolé. Le profil biochimique et le test d'inhibition de croissance ont conduit à identifier *Mycoplasma synoviae*.

L'analyse histopathologique a révélé une réaction inflammatoire intense du tractus respiratoire ainsi qu'une hyperplasie épithéliale et une infiltration lymphocytaire.

Des anticorps vis-à-vis de *Mycoplasma synoviae* chez les perdrix étudiées ont été décelés par réaction de séro-agglutination rapide et d'inhibition de l'hémagglutination.

#### ZUSAMMENFASSUNG

##### Isolierung von *Mycoplasma synoviae* aus einen Rothuhn (*Alectoris rufa*)

Während des Ausbruches einer respiratorischen Krankheit in einer Gruppe von Rothühnern (*Alectoris rufa*) und Hühnern (*Gallus domesticus*), die im gleichen Abteil gehalten wurden, wurden Mycoplasmen isoliert. Gemäß dem biochemischen Verhalten und dem Wachstumshemmtest konnten sie als *Mycoplasma synoviae* identifiziert werden.

Die histopathologische Untersuchung ergab eine intensive entzündliche Reaktion im Respirationstrakt zusammen mit einer Epithelhyperplasie und Lymphozyteninfiltration.

Mit dem Serumschnellobjektträgeragglutinationstest und dem Haemagglutination-

shemmtest konnte die Anwesenheit humoraler Antikörper gegen *Mycoplasma synoviae* bei den untersuchten Rebhühnern nachgewiesen werden.

#### RESUMEN

##### **Aislamiento de *Mycoplasma synoviae* de la perdiz de patas rojas (*Alectoris rufa*)**

Se aislaron mycoplasmas de un grupo de perdices de patas rojas (*Alectoris rufa*) y de pollos (*Gallus domesticus*) alojados en la misma caseta como consecuencia de un brote respiratorio, el cual fue identificado como *Mycoplasma synoviae* por medio de su perfil bioquímico y de la prueba de inhibición del crecimiento (IC).

El análisis histológico reveló una intensa reacción inflamatoria en el tracto respiratorio así como hiperplasia epitelial e infiltración linfocitaria.

La prueba de suero aglutinación rápida en placa y la prueba de la inhibición de la hemoaglutinación mostraron la presencia de anticuerpos humorales contra *Mycoplasma synoviae* en las perdices estudiadas.