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THE ISOLATION OF *MYCOPLASMA SYNOVIAE* FROM CHICKENS WITH INFECTIOUS SYNOVITIS AND AIR-SACCULITIS IN THE REPUBLIC OF SOUTH AFRICA

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ABSTRACT

BUYS, S. B., 1976. The isolation of *Mycoplasma synoviae* from chickens with infectious synovitis and air-sacculitis in the Republic of South Africa. *Onderstepoort Journal of Veterinary Research* 43 (2), 39–42 (1976).

Mycoplasma synoviae was isolated from the trachea of chickens showing either typical infectious synovitis lesions or air-sacculitis. *M. synoviae* was identified by means of the direct plate fluorescent antibody technique and its growth-dependence on nicotine-amide-adenine dinucleotide.

This is the first documented report on the isolation and identification of *M. synoviae* in the Republic of South Africa.

Résumé

ISOLEMENT DE MYCOPLASMA SYNOVIAE *À PARTIR DE POUSSINS ATTEINTS DE LA SYNOVITE INFECTIEUSE ET DE L'AÉROCYSTITE EN RÉPUBLIQUE SUD-AFRICAINE*

L'auteur a pu isoler Mycoplasma synoviae *de la trachèe de poussins avec manifestations cliniques soit de la synovite infectieuse soi de l'aérocystite.* M. synoviae *a pu être identifiée par la technique d'immuno-fluorescence directe sur plaque et en raison de sa dépendance du dinucleotide nicotinamide adénique dans sa croissance.*

Ceci est la première fois que l'on a rapporté l'isolement et l'identification de M. synoviae *dans la République Sud-africaine.*

INTRODUCTION

Yoder (1972) classified 19 serotypes (A–S) of *Mycoplasma* isolated from poultry. *M. gallisepticum*, *M. gallinarum*, *M. meleagridis* and *M. synoviae* are designated A, B, H and S respectively, while the other serotypes (C–R) have not been named.

M. gallisepticum, *M. meleagridis* and *M. synoviae* are considered to be pathogenic for certain avian hosts under various conditions, but the status of pathogenicity of the other serotypes is less clear (Yoder, 1972). *M. synoviae* is the cause of infectious synovitis in both chickens and turkeys (Olson, 1972) and of air-sacculitis (Kleven, King & Anderson, 1972; Vardaman, Reece & Deaton, 1973; Vardaman, Deaton & Reece, 1974; Gilchrist & Cottew, 1974; Gillespie, 1974).

In 1968, Du Preez (unpublished observation) isolated an organism suspected of being *M. synoviae* from the hock joints and footpads of broilers showing typical lesions of infectious synovitis. The isolation was done in embryonated eggs, and typical synovitis lesions could be reproduced in susceptible chickens infected with embryo material. This isolate was not identified, however.

For the isolation of *Mycoplasma*, both solid and fluid media are normally used, the more common being C- agar and C- biphasic medium (Chalquest, 1962), as modified by Olson, Kerr & Campbell (1963), *Mycoplasma* agar and broth (Bradbury & Howell, 1974) PPLO agar and broth (Timms, 1967) and medium FM -4 (Frey, Hanson & Anderson, 1968) as modified by Vardaman & Yoder (1969).

Frey *et al.* (1968) showed that nicotine-amide-adenine dinucleotide (NAD) is essential for the growth of *M. synoviae* and distinguishes it from other avian *Mycoplasma* species. Olson & Meadows (1972) gave optimum and minimum concentrations of NAD: viz. for isolation—(0,01 and 0,00125%), for antigen production—(0,00125 to 0,01% and 0,000625%), and for maintenance—(0,00125 and 0,000312%).

Olson & Kerr (1969) found that the use of embryonated eggs and C-medium was equally reliable for the isolation of *M. synoviae*, but that C-medium was better for the isolation of *M. gallisepticum.*

Olson & Kerr (1967) were able to isolate *M. synoviae* and *M. gallisepticum* from the footpad, hock joints, liver, spleen, trachea and blood of chickens. Bradbury & Howell (1974) isolated *M. synoviae* from the trachea, lung, airsacs, kidney, liver, heart, spleen and bursa of Fabricius of chickens hatched from infected eggs.

The identification of mycoplasmae is done by means of the fermentation of carbohydrates, complement fixation tests (Frey & Hanson, 1969), growth inhibition (Fabricant, 1960; Dierks, Newman & Pomeroy, 1967), polyacrylamide electrophoresis (Razin, 1968) and agar gel diffusion (Too, 1967). The staining of colony imprints on cover slips with fluorescent antibodies was used by Corstvet & Sadler (1964) while Del Giudice, Robillard & Carski (1967) stained *Mycoplasma* colonies on agar with fluorescent antibodies, using incident light for illumination.

The purpose of this article is to report the isolation and identification of field strains of *M. synoviae* from cases of synovitis and arthritis as well as cases of air-sacculitis in chickens, by the direct fluorescent antibody (FA) technique.

MATERIALS AND METHODS

Culture media and isolation procedure

Use was made of C-biphasic medium (Chalquest, 1962), modified by Olson *et al.* (1963), and further adapted by Du Preez (unpublished observations, 1973) by the omission of phenol red from the agar medium and the addition immediately before use of NAD* to the broth of the biphasic medium to a concentration of approximately 0,01%. C-agar plates with and without NAD at a concentration of 0,01% were also prepared.

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Routine cultures on C-agar plates and in C-biphasic medium were made from tracheal swabs, airsacs and/or joints in cases where a *Mycoplasma* infection was suspected.

The plates were incubated in humidified candle jars for a period of 14–28 days.

Mycoplasma strains

The avian *Mycoplasma* serotypes used in this study are given below:

Serotype	Isolate	Origin	Culture received from
A........	801.........	Turkey airsac..	Yoder
B........	1504........	Chicken trachea	Yoder
C........	859.........	Chicken trachea	Frey
D........	8...........	Chicken trachea	Frey
E........	860 (DPR-2)	Chicken trachea	Yoder
F........	1197 (SA)....	Turkey trachea	Yoder
I........	695.........	Turkey airsac...	Yoder
H........	886 (N)......	Turkey airsac...	Frey
L........	694.........	Pigeon turbinate	Yoder
S........	1853........	Turkey........	Olson

The serotypes listed above were again cloned and then used for production of hyperimmune sera (Du Preez, unpublished data, 1973). Conjugation with fluorescein was done by A. P. Schutte of this institute according to a method previously described (Schutte, 1969).

The staining method of Del Giudice *et al.* (1967) was modified by Schutte (personal communication, 1975) as follows: 10 blocks of the C-agar plate bearing typical *Mycoplasma* colonies with a surface area of approximately 0,5 cm^2 were cut out and each block was stained with a different serotype of fluorescein-conjugated rabbit antiserum. After staining, a thin horizontal slice of each block bearing the colonies was removed with a sharp blade and placed on a microscope slide. This was covered with a drop of FA mounting fluid* and a coverslip, and, after the air bubbles had been removed, it was examined with a Leitz universal microscope.

When pure *M. synoviae* cultures were identified by the FA technique they were cloned and then subcultured on C-agar plates with and without NAD.

The methods described here were applied to 23 trachea specimens, 9 from suspected cases of infectious synovitis, and 14 from cases with air-sacculitis.

RESULTS

Isolation from 9 *synovitis cases*

M. synoviae was isolated from the trachea of cases showing typical synovitis as described by Olson (1972). In every case the hock joints were filled with creamy to caseous pus. Pure cultures of *M. synoviae* were obtained from 7 cases and 1 case revealed *M. synoviae* together with other serotypes (see Table 1).

TABLE 1 Serological classification of the mycoplasmae from 9 typical infectious synovitis cases by the FA technique. All the isolates were from tracheal swabs

No. of specimens	Serotypes isolated				
	A	B	D	E	S
7....................	—	—	—	—	+
1....................	—	+	+	+	+
1....................	—	+	+	+	—

* Difco Laboratories, Detroit, Michigan, U.S.A. 48201

Isolation from 14 *cases with air-sacculitis*

These cases had no history of synovitis but a very high incidence of air-sacculitis. The tracheas of three of these cases revealed pure *M. synoviae* infection, and those of 9 cases pure *M. gallisepticum*. In 2 cases, either *M. synoviae* or *M. gallisepticum* was found together with other serotypes. (See Table 2).

TABLE 2 Serological classification of the mycoplasmae from 14 cases with air-sacculitis by the FA technique. All the isolates were from tracheal swabs

No. of specimens	Serotypes isolated				
	A	B	D	E	S
3....................	—	—	—	—	+
9....................	+	—	—	—	—
1....................	—	—	+	+	+
1....................	+	—	+	+	—

After 19 days incubation *M. synoviae* was only isolated by direct culture on C-agar from 1 trachea specimen. In all the other cases where *M. synoviae* was isolated, growth was first observed in the biphasic medium after 3–7 days as indicated by a drop in pH. Typical *Mycoplasma* colonies developed after 3–5 days subsequent to the biphasic medium being plated on C-agar.

Pure cloned *M. synoviae* cultures, identified by the FA technique, did not grow on C-agar plates without NAD.

DISCUSSION

This publication is the first report of the isolation and identification of *M. synoviae* in the Republic of South Africa. The isolation of *M. synoviae* from the trachea of cases of air-sacculitis supports the conclusions of Kleven *et al.* (1972), Vardaman *et al.* (1973, 1974), Gilchrist & Cottew (1974) and Gillespie (1974), that this organism plays a role in respiratory disease of chickens.

The poor isolation results obtained when the swabs were streaked directly on C-agar might be attributed to the fact that the plates initially were kept for only 14 days. Bradbury & Howell (1974) suggested that plates for *M. synoviae* be kept for 28 days. In 1 instance where this was done, a few atypical colonies as described by them were observed on the 19th day. Contrary to their findings, where atypical colonies were found only in samples taken from the kidney, heart, spleen and bursa of Fabricius, these colonies originated from a sample taken from the trachea.

Du Preez's isolation in 1968 (unpublished observations) of a *Mycoplasma* that could reproduce synovitis in chickens coincided with the first occurrence of clinical infectious synovitis of non-bacterial origin in the Republic of South Africa. He subsequently established the FA technique in this laboratory for identification of 10 avian *Mycoplasma* serotypes. This proved to be a very practical method of identifying isolates of mycoplasmae, especially when several serotypes occurred in the same chicken. In fact, it was possible to identify 4 serotypes in a single specimen.

A combination of solid and biphasic growth media facilitated the isolation of field mycoplasmae.

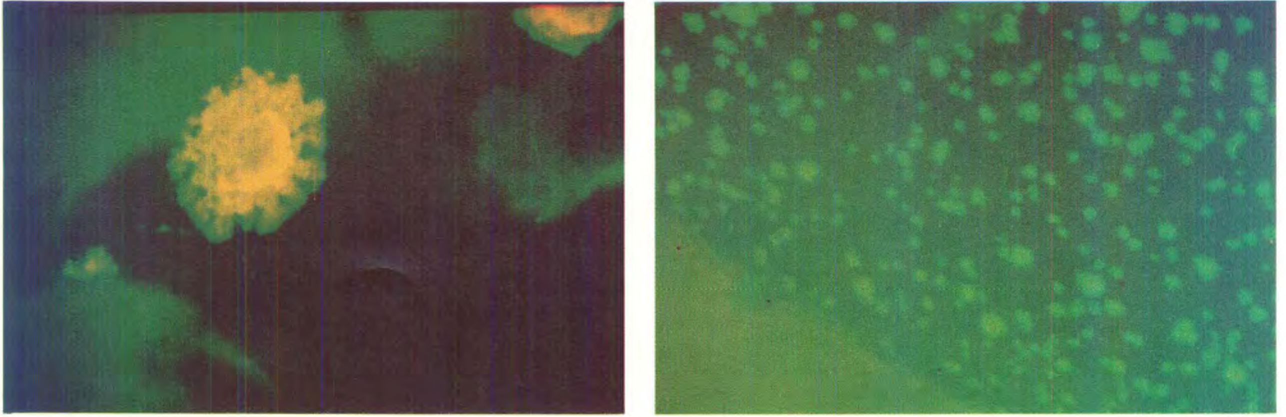

FIG. 1 *Mycoplasma* colonies stained with fluorescent antibodies. (a) A single colony of serotype D. (b) Serotype S colonies. The edge of the agar slice can be seen in the lower right-hand corner. (×128)

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