Characterisation of mycoplasmas isolated from genital tract infections of sheep in Nigeria

J.C. CHIMA *, M.O. OJO **, J.U. MOLOKWU * and P.A. OKEWOLE *

Summary: Four mycoplasma-like organisms isolated from ewes with mucopurulent vaginal discharge and swollen vulva were characterised.

Biochemical tests showed three of the isolates to be negative for glucose fermentation and arginine hydrolysis, while the remaining isolate was negative for glucose fermentation but hydrolysed arginine.

Serological identification using the growth inhibition, growth precipitation and indirect immunofluorescence tests indicated the three similar isolates as Mycoplasma bovigenitalium and the other isolate as Mycoplasma arginini.

There are apparently no previous reports of the isolation of these organisms from the genital tract of sheep in Nigeria.


INTRODUCTION

There are several reports of the isolation of mycoplasmas from genital tract infections of sheep. In Australia, a mycoplasma designated as biotype 2-D was isolated from the prepuce and vagina of sheep with no apparent reproductive problems (5). A mycoplasma identical to biotype 2-D was later isolated, however, from the vagina of Australian ewes with vulvovaginitis (7).

Mycoplasma sp. (2-D) has also been isolated from sheep in Texas (19). Most of the isolates were from apparently-healthy animals, but two were from animals (one ram and one ewe) which had reproductive problems. Some isolates in the same study were identified as Mycoplasma arginini.

In the United Kingdom, an outbreak of vulvovaginitis was reported in a flock of sheep from which M. capricolum and Acholeplasma axanthum were isolated (15). In Egypt, forty strains identified as M. arginini were isolated from cases of granular vulvovaginitis and balanoposthitis in sheep and cattle (3). Varying numbers of A. granularum, A. oculi, A. laidlawii and M. capricolum were isolated from mucosal scrapings of the uterus of apparently-healthy sheep and goats (17).

* Head of the Epidemiology Research Department, National Veterinary Research Institute, Federal Ministry of Agriculture and Natural Resources, Vom, Plateau State, Nigeria.

** Obafemi Awolowo College of Health Sciences, Ogun State University, Ogun State, Nigeria.
In goats, Singh et al. (22) not only isolated *M. agalactiae* from gross lesions of granular vulvovaginitis but were also able to reproduce the gross lesions in 25 of 30 experimentally-infected virgin kids.

Since the first association of ureaplasma with ovine vulvovaginitis (9), other workers (16, 18) have also isolated ureaplasmata from both infected and apparently-healthy sheep.

Genital tract infection of sheep characterised by mucopurulent vaginal discharge and swollen vulva is becoming prevalent in some parts of Nigeria. The purpose of this preliminary report is to describe the characteristics of four mycoplasma-like organisms isolated from ewes with clinical disease. This is apparently the first report of the isolation and identification of mycoplasmas from the genital tract of sheep in Nigeria.

**MATERIALS AND METHODS**

**Mycoplasmas**

The four mycoplasma-like organisms studied were isolated from clinical cases of vulvovaginitis in sheep. These were cloned and designated as AMR-C2327, C2329, C2330 and C2331.

**Media**

The liquid growth medium was composed essentially as described previously (8) and consisted of PPLO (pleuropneumonia-like organism) broth in de-ionised double-distilled water (80% by volume of medium) to which was added 1% (w/v) yeastolate, 0.1% (w/v) glucose, 0.002% (v/v) phenol red, 1,000 international units/ml penicillin G and 20% (v/v) unheated horse serum. The medium was adjusted to pH 7.8. The solid medium was prepared by substituting PPLO agar for PPLO broth. The agar base also contained 0.02% (w/v) thallium acetate but no phenol red, and the pH was not adjusted.

**Biochemical methods**

Tests for fermentation of glucose, hydrolysis of arginine, phosphatase activity, serum digestion, reduction of triphenyl tetrazolium chloride and formation of 'film and spots' on egg yolk medium were performed as described previously (11, 12). Sterol requirement was indirectly assessed by sensitivity to 1.5% digitonin (12).

**Antisera**

Hyperimmune rabbit sera against relevant mycoplasma were prepared as previously described (13). All the sera were kindly supplied by Dr H. Ernø of the Food and Agriculture Organisation of the United Nations (FAO)/World Health Organisation (WHO) Collaborative Centre for Animal Mycoplasma in Aarhus (Denmark).

**Serological methods**

The indirect immunofluorescence (IF) technique (21), agar well modification of the growth inhibition (GI) test (4) and the growth precipitation (GP) test (14) were used for serological identification of the isolates. The isolates which gave negative results for both glucose fermentation and arginine hydrolysis were tested with antisera against *M. bovigenitalium* (PG 11), *M. agalactiae* (PG 2), *M. bovis* (Donetta) and Mycoplasma sp. biotype 2-D, while the isolate which hydrolysed arginine was tested with antisera
against the reference strains of *M. arginini* (AMRC-C22), *M. gallinarum* (PG 16) and strain 145. Normal rabbit serum was included in the tests as a negative control, and PG 11 antigen and homologous antiserum were used as positive controls.

**RESULTS**

**Biochemical characteristics**

Three of the four isolates (C2327, C2330 and C2331) showed similar reactions, being negative for both glucose fermentation and arginine hydrolysis, while the remaining isolate (C2329) did not ferment glucose but hydrolysed arginine (Table I).

All four isolates were sensitive to 1.5% digitonin, with zones of inhibition ranging from 13 mm to 18 mm.

**Serological reactions**

The three isolates showing similar biochemical characteristics gave positive reactions with PG 11 and 2-D strain antisera, and negative reactions with PG 2 and Donetta strain antiserum in the IF test (Table II). A more intense reaction was observed with PG 11 antiserum than with 2-D strain antiserum. In the GI test, PG 11 antiserum gave a total inhibition zone of 2 mm with two of the three isolates, while 2-D strain antiserum gave a partial inhibition zone of 2 mm with only one isolate. All three isolates gave positive reactions with antisera against PG 11 and 2-D strain in the GP test.

The arginine-hydrolysing isolate reacted positively only with antiserum against *M. arginini* in the IF and GI (2 mm) tests, but not in the GP test.

**DISCUSSION**

The IF and GI tests identified three isolates as *M. bovigenitalium* and one as *M. arginini*. The cross-reaction seen in the GP test between PG11 and 2-D strain was not unexpected, as the test detects an antigenic relationship which is group-specific but not species-specific (13).

*M. bovigenitalium* is the species most commonly isolated from clinically-infected or apparently-healthy bovine genital tracts (1, 2, 10). Pathak *et al.* (20) reported the isolation of *M. bovigenitalium* from the genital tract of goats. Thus, the identification in the present report of three of the isolates as *M. bovigenitalium* is not original.

In the present report, *M. arginini* was isolated from an animal with vulvovaginitis. This agrees with the findings of some workers (3), although others (19) failed to specify whether or not the isolate was from clinically-infected ewes.

Many workers (3, 7, 15, 19) have associated mycoplasmas with ovine genital tract infections. Others (5, 17) have also isolated these organisms from the genital tract of apparently-healthy sheep. There are no known reports of isolation of mycoplasmas from genital tract infections of sheep in Nigeria, although a recent study (6) indicated that mycoplasmas could cause serious problems in goats. Opportunities for cross-infection are frequent, due to husbandry practices in Nigeria, where sheep, goats and cattle are usually kept together.
### TABLE I

**Biochemical reactions of mycoplasmas isolated from vaginal swabs of sheep with vulvovaginitis**

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>Digitonin sensitivity (zone in mm)</th>
<th>Glucose fermentation</th>
<th>Arginine hydrolysis</th>
<th>Phosphatase activity</th>
<th>Serum digestion</th>
<th>Tetrazolium reduction *</th>
<th>Formation of 'film and spots' **</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2329</td>
<td>14</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>C2330</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>C2327</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>C2331</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
</tr>
</tbody>
</table>

* aerobic/anaerobic  ** standard/egg medium  + positive  - negative

### TABLE II

**Serological reactions of mycoplasmas isolated from vaginal swabs of sheep with vulvovaginitis**

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>Immunofluorescence</th>
<th>Antiserum</th>
<th>Growth inhibition (zone in mm)</th>
<th>Growth precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PG11 2-D PG-2 Donetta</td>
<td>PG11 2-D PG-2 Donetta</td>
<td>PG11 2-D PG-2 Donetta</td>
<td>PG11 2-D PG-2 Donetta</td>
</tr>
<tr>
<td>C2327</td>
<td>++ + - -</td>
<td>2(T) 2(N) 0 0</td>
<td>+ + ND ND</td>
<td>arginini Strain 145 gallinarum</td>
</tr>
<tr>
<td>C2330</td>
<td>+++ + - -</td>
<td>2(T) 0 0 0 0</td>
<td>+ + ND ND</td>
<td>arginini Strain 145 gallinarum</td>
</tr>
<tr>
<td>C2331</td>
<td>++ + - -</td>
<td>0 0 0 0</td>
<td>+ + ND ND</td>
<td>arginini Strain 145 gallinarum</td>
</tr>
</tbody>
</table>

arminini Strain 145 gallinarum  (T): total inhibition  (NT): partial inhibition  + positive  - negative  ND: no data
Sheep farming is of increasing economic importance in Nigeria, and could be adversely affected by reproductive problems. It is therefore becoming necessary to determine the pathogenic role of various mycoplasmas in genital tract infections of sheep in Nigeria, either alone or in combination with other infectious agents. This line of investigation is currently being pursued.

ACKNOWLEDGEMENTS

The authors wish to thank Dr H. Ernø of the FAO/WHO Collaborative Centre for Animal Mycoplasma, Institute of Medical Microbiology, University of Aarhus (Denmark) for supervising this work. The authors are also grateful to the Director of the National Veterinary Research Institute in Vom (Nigeria) for permission to publish this report.

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Résumé : Le typage porte sur quatre organismes du genre Mycoplasma isolés chez des brebis présentant des pertes vaginales mucopurulentes et une inflammation de la vulve.

Trois isolats ne fermentaient pas la glucose et n'hydrolysaient pas l'arginine alors que le quatrième ne fermentait pas la glucose mais hydrolysait l'arginine.

Les épreuves d'inhibition de croissance, de stimulation de croissance et d'immunofluorescence indirecte réalisées sur le sérum ont révélé que les trois isolats similaires appartenaient à l'espèce Mycoplasma bovigenitalium et le quatrième à Mycoplasma arginini.

Il semble que l'isolement de ces organismes dans l'appareil génital d'ovins au Nigeria n'ait jamais été signalé auparavant.


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Resumen: Se llevó a cabo la caracterización de cuatro organismos de tipo micoplasmático aislados en ovejas que presentaban un cuadro de secreción vaginal mucopurulenta e inflamación de la vulva.

Las pruebas bioquímicas revelaron que tres de los organismos aislados eran negativos para la fermentación de glucosa y la hidrólisis de arginina, mientras que la cepa restante era también negativa para la fermentación de glucosa pero en cambio era capaz de hidrolizar la arginina.
La identificación serológica realizada mediante pruebas de inhibición del crecimiento, de aceleración del crecimiento y de inmunofluorescencia indirecta puso de manifiesto que las tres cepas similares pertenecían a la especie Mycoplasma bovigenitalium, mientras que la cuarta correspondía a Mycoplasma arginini.

Al parecer no existen en Nigeria informes anteriores sobre el aislamiento de estos organismos en el tracto genital de ovejas.


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REFERENCES


