ISOLATION OF *MYCOPLASMA* SP. FROM KERATOCONJUNCTIVITIS OF GOATS

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ABSTRACT

Soeripto and M.B. Poerwadikarta, 1990. Isolation of *Mycoplasma* sp. from keratoconjunctivitis of goats. *Penyakit Hewan* 22 (40): 76-79.

Forty goats were bought from Cisarua Bogor of Indonesia for a pneumonia experiment. One of the goats had clinical conjunctivitis on arrival. All the goats were kept in an enclosed wooden pen in Research Institute for Veterinary Science, Bogor. A week later almost all of the goats had conjunctivitis with whitish lesions in one or both corneas. Swabs were taken from the conjunctiva and cultured onto mycoplasma broth and agar. Both large and small mycoplasma-like organisms were isolated besides *Moraxella ovis* and *Staphylococcus aureus*. The mycoplasma isolates were identified using biochemical tests. The results suggested that 2 isolates resemble to *M. mycoides* subsp *capri*, one isolate to *M. capricolum* and one isolate belong to the *M. mycoides* cluster. The remaining mycoplasma could be *M.* sp. 2D or *M. bovis*, both of which have occurred in goats, although rarely.

Key words: *Mycoplasma* sp., Keratoconjunctivitis and Goats.

ABSTRAK

Soeripto and M.B. Poerwadikarta, 1990. Isolasi *Mycoplasma* sp. dari radang keratoconjunctiva pada kambing. *Penyakit Hewan* 22 (40): 76-79.

Sebanyak 40 ekor kambing yang akan digunakan untuk penelitian radang paru-paru dibeli dari daerah Cisarua, Bogor. Pada mulanya satu ekor dari kambing-kambing tersebut memperlihatkan radang mata pada saat ditempatkan di Balitvet. Kambing-kambing tersebut ditempatkan pada kandang-kandang yang terbuat dari kayu. Seminggu kemudian, hampir semua kambing yang ditempatkan pada kandang tersebut menderita radang conjunctiva dengan lesi-lesi putih pada satu atau ke dua belah corneanya. Hapusan radang dengan kapas lidi yang steril diambil dari mata yang menderita peradangan. Dari hapusan radang tersebut dapat diisolasi kuman menyerupai mikoplasma bentuk besar dan kecil dan kuman *Moraxella ovis* dan *Staphylococcus aureus*. Kuman mikoplasma tersebut kemudian di identifikasi dengan menggunakan uji biokimia. Hasilnya memperlihatkan bahwa 2 isolat menyerupai *M. mycoides* subsp *capri*, satu isolat menyerupai *M. capricolum* dan satu isolat termasuk dalam kelompok *M. mycoides*. Kuman yang kelima kemungkinan adalah *M.* sp. 2D or *M. bovis*, dimana keduanya terjadi pada kambing sekalipun masih jarang.

Kata-kata kunci: *Mikoplasma* sp., radang Keratoconjunctiva, kambing.

INTRODUCTION

Caprine or Ovine mycoplasmas has been reviewed extensively by Cottew (1979) and DaMassa *et al.* (1984). The recognized mycoplasmas in sheep or goats are *M. agalactiae, M. capricolum, M. arginini, M. conjunctivae, M. mycoides* subsp *mycoides* (LC), *M. mycoides* subsp *capri, M. ovipneumoniae, M. putrifaciens, M.*sp. F38, *Acholeplasma granularum, A. laidlawii* and *A. oculi* (Cottew, 1979).

Caprine or Ovine keratoconjuntivitis has been reported overseas for the past 20 years (Surman, 1968; Langford, 1971; McCauley *et al.*, 1971, Barile *et al.*, 1972; Baas *et al.*, 1977 and Egwu *et al.*, 1989), but not in Indonesia. Clinical signs usually are inflammation of corneas and conjunctivae which resulted in lachrymation, blinking or blepharospasm and even blindness (McCauley *et al.*, 1971; Egwu *et al.*, 1989).

Jones *et al.* (1976) reported that *M. conjuntivae* and *M. ovipneumoniae* were isolated from keratoconjunctivitis of lambs and Egwu *et al.* (1989) reported that several microorganisms including *M. conjuntivae* and *M. arginini* are commonly associated with clinically affected and unaffected sheep's eyes. However, *M. conjunctivae* has been reported to produce conjunctivitis in sheep (Jones *et al.*, 1976) and goats (Trotter *et al.*, 1977).

This paper reports the recovery of mycoplasmas from the eyes of goats that were housed in experimental pens and the identification of the isolates.

MATERIALS AND METHODS

Animals

Forty local goats were bought from Cisarua Bogor of Indonesia for a pneumonia experiment. One of the goats had clinical conjunctivitis on arrival. All the goats were kept in an enclosed wooden pen in Research Institute for Veterinary Science, Bogor. A week later almost all of the goats had conjunctivitis with corneal opacity in one or both corneas. Conjunctivitis and lacrymation were the prominent clinical signs. When the corneas were affected, the vision was disturbed and the goats became starved, weak and then died or were killed.

Media

Mycoplasma broth (MB) was based on the formulation of Frey *et al.* 1968). The medium contained mycoplasma broth base (Gibco), cystein HCl (BDH), thallous acetate (BDH), phenol red (Chroma) and distilled water (pH 7.8). The formulation of mycoplasma agar (MA) was similar except that glucose and phenol red were omitted. A Noble agar (Difco) was used for the MA. These media were autoclaved at 121°C for 15 minutes and cooled to room temperature for MB and to 50°C for MA, sterile enrichments were then added. The enrichments contained swine serum (inactivated at 56°C for 30 min), yeast extract (Difco), DNA (Koch-Light), NAD (BDH), actidione (UpJohn) and penicillin G (Hoechst).

Blood agar and sugars were used as described by Cowan and Steel (Cowan, 1977).

Type strains

Several type strains of *M. mycoides* subsp *mycoides* (LC), *M. capricolum, M. mycoides* subsp *capri, M. conjunctivae, M. agalactiae* and *M. arginini* obtained from Adelaide were used for testing homologous antisera.

Antisera

Several antisera of *M. mycoides* subsp *mycoides* (LC), *M. capricolum, M. mycoides* subsp *capri, M. conjunctivae, M. agalactiae* and *M. arginini* obtained from Denmark (NCTC) and/or Adelaide were used for testing homologous type strains.

Collection of samples

Swabs were taken from the conjunctiva using sterile cotton swabs and then cultured onto mycoplasma broth and agar for mycoplasma isolation and blood agar for isolation of other microorganisms. Samplings were taken from 32 affected eyes of goats.

Swabs for mycoplasma isolation were inoculated into MB and incubated at 37°C. Any contaminated cultures were filtered using 0.22 um filters (Millipore) and then re-inoculated into MB. When the phenol red indicator changed from red to pink (approx pH 6.9) the cultures were inoculated onto MA and incubated in a humidified candle jar at 37°C. The plates were observed every day for the presence of mycoplasma colonies. If the indicator did not change but the turbidity was increased then the cultures were also be inoculated onto MA and incubated as described above. Single mycoplasma-like colonies were selected and inoculated into MB and incubated at 37°C. When the indicator changed from red to pink, the cultures were then purified by filtration 3 times before they were stored at -20°C.

For other microorganisms examination, swabs were cultured onto blood agar and then incubated at 37°C for 24 hours. The organisms were then stained with Gram and tested using sugar media and biochemical tests (Cowan, 1977; Krieg and Holt, 1984). The results of these tests indicated the presence of *Moraxella ovis* and *Staphyloccoccus aureus* and this was reported in a separate paper. (Soeripto *et al.*, unpublished data).

Biochemical and serological tests

A modification of the methods described by Aluotto *et al.* (1970) was used for biochemical tests. The tests used were sensitivity to digitonin, utilization of glucose and arginine, phosphatase activity, casein digestion and film and spot formation. The serological test was growth inhibition test followed the method described by Clyde (1983).

RESULTS

Growth inhibition tests showed that antisera against *M. mycoides* subsp *mycoides* (LC) and *M.*

capricolum inhibited the homologous organisms, but that of *M. mycoides* subsp *capri* gave only weak inhibition against the homologous type strain.

Five mycoplasma-like organisms were isolated from eyes of 3 goats out of 32 samples. One isolate with a small colony failed to ferment glucose or hydrolyse arginine. It was isolated from goat no.309. The other 4 isolates had large colonies were glucose fermenters and came from goats nos. 89/659 and 309. They were submitted to biochemical and serological tests.

The results of biochemical and serological tests were shown in Tables 1 and 2. The growth of colonies of all isolates was inhibited by the presence of digitonin. The isolate that had small colonies gave film and spots reaction and produced phosphatase activity. However this organism failed to catabolise glucose and was unable to digest casein. The growth of this organism was not inhibited either by antisera of *M. arginini* or *M. agalactiae.* The other 4 isolates that had large colonies were able to digest casein and produced weak activity in the test for phosphatase, but did not produce film and spots. Of these isolates, one was inhibited by the antiserum of *M. capricolum* and two were inhibited poorly by the antiserum of *M. mycoides* subsp *capri.* The fifth isolate from goat number 309, was not inhibited by either antiserum.

Table 1. Biochemical tests.

Tests	Goat Numbers				
	89/659	90/19	309		
			1	2	3
Digitonin	+	+	+	+	+
Glucose catabolism	+	+	+	—	+
Arginine hydrolysis	—	—	—	—	—
Phosphatase activity	w	w	w	+	w
Film and Spots	—	—	—	+	—
Casein digestion	+	+	+	—	+

Note: w : weak positive reaction.

Table 2. Growth inhibition test.

Antisera against	Goat Numbers				
	89/659	90/19	309		
			1	2	3
M. mycoides subsp *mycoides*	—	—	—	nt	—
M. capricolum	+	—	—	nt	—
M. mycoides subsp *capri*	—	w	—	nt	w
M. conjunctivae	—	—	—	nt	—
M. agalactiae	nt	nt	nt	—	nt
M. arginini	nt	nt	nt	—	nt

Note: w : weak positive reaction
nt : not tested

DISCUSSION

An outbreak of keratoconjunctivitis of goats has been occured recently at RIVS, Bogor. The clinical signs were lachrymation, corneal opacity and inflamation of conjunctiva. The clinical signs were similar to those clinical signs described by McCauley *et al.* (1971) and Egwu *et al.* (1989). *M. conjunctivae* was reported to be the common organisms that can be isolated from keratoconjunctivitis (Jones *et al.*, 1976; Egwu *et al.*, 1989). These organisms were reported to be able to reproduce the disease in sheep (Jones *et al.*, 1976) and goats (Trotter *et al.*, 1977). However, in this outbreak the author failed to isolate *M. conjunctivae.* Instead, other mycoplasmas of at least 2 distinct colony types, *Moraxella ovis* and *Staph. aureus* were able to be isolated from this outbreak of rapidly spreading infectious keratoconjunctivitis.

As all of the isolates on MA were digitonin sensitive they were considered to belong to the genus *Mycoplasma* rather than the genus *Acholeplasma.*

Of the 5 isolates of mycoplasmas, 4 gave similar biochemical reactions and these were characteristic of organisms resembling *M. mycoides* subsp *mycoides* (LC) and others in the *M. mycoides* cluster. Although only weak reactions were obtained in growth inhibition tests, the results suggested that 2 may be *M. mycoides* subsp *capri* and one *M. capricolum,* while the fourth showed no reaction to the 3 appropriate antisera used. The results are rather tentative and will be repeated, together with one of the several supporting serological tests used for identification.

The remaining mycoplasma had the biochemical reactions of *M. agalactiae, Mycoplasma* sp. 2D and *M. bovis.* Growth of this isolate was not inhibited by the antiserum to *M. agalactiae,* suggesting that the organisms could be *M.* sp. 2D or *M. bovis,* both of which have occurred in goats, although rarely. However as the *M. agalactiae* antiserum also failed to inhibit the homologous type strain, the possibility of the isolate being *M. agalactiae* has not been ruled out.

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