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Detection of Antibodies against *Mycoplasma mycoides* subsp. *capri* in Goats with the Complement Fixation Test

José L. Corona-Vargas[1], Myrna A. Vicencio-Mallén[1], Frida Salmerón-Sosa[1], Erika M. Carrillo-Casas[2], Francisco J. Trigo-Tavera[3], Rosa E. Miranda-Morales[1*]

[1]Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, Coyoacán, México
[2]Departamento de Biología Molecular e Histocompatibilidad, Dirección de Investigación, Hospital General Dr. Manuel Gea González, Calz, Tlalpan, México
[3]Departamento de Patología, Facultad de Medicina Veterinaria y Zootecnia,Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, Coyoacán, México
Email: jcorona@biozoo.com.mx, vicencio@yahoo.com.mx, fridass62@yahoo.com.mx, ekarri@gmail.com, Trigo@servidor.unam.mx, *roelmimo@yahoo.com.mx

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Abstract

Mycoplasma mycoides subsp. *capri* is the causative agent of severe and acute respiratory problems in goats, which spreads rapidly and represents high mortality. The serological profile of the goat population, from nine regions in seven states of Mexico, was screened by the Complement Fixation test (CF) in sera from asymptomatic goats and animals with mild respiratory symptoms. Sera and nasal swabs of 827 goats were collected for the isolation of the organism. An antiserum was prepared against a previously isolated field strain of *Mycoplasma mycoides* subsp. *capri*. CF Antibody titers were associated with the results of the isolates to determine the cutoff point. The CF was considered as positive if its result was ≥1/16. The CF registered 251 positive goats (30.35%) and 576 (69.65%) negative; the test showed high sensitivity (93.33%) and specificity (72.27%). In the specific case of diagnosis for mycoplasmosis associated with respiratory problems in goats, the CF proved to be a good diagnosis test, this study determined that 30% of the goat population showed antibody titers against *Mycoplasma mycoides* subsp. *capri* and revealed those animals who have had contact with this microorganism during their lives regardless of the presence or absence of respiratory symptoms.

Keywords

Complement Fixation, Indirect Agglutination, Mycoplasmosis,

Mycoplasma mycoides, *Mycoplasma mycoides* subsp. *capri*

1. Introduction

The caprine respiratory disease is regarded as a multifactorial disease in which virus and bacteria including mycoplasmas are involved. Mycoplasma species are phylogenetically tightly related and have been jointly clustered into the *mycoides* cluster [1]. The most frequently involved mycoplasma is *M. mycoides* subsp. *capri*, and *M. capricolum* subsp. *capripneumoniae* is the etiological agent of the caprine contagious pleuropneumonia (CCPA), which can submit as an acute, subacute or chronic disease. Mortality rate due to CCPA ranges from 60% to 80% among goat herds in Africa [2] [3]. In CCPA free areas, *M. mycoides* subsp. *capri* and *M. capricolum* subsp. *capricolum* turn to be the predominant pathogens involved in caprine diseases [4] [5].

In Mexico, although there is a scanty serological profile of the caprine herd, the *mycoides* cluster has been detected as well as isolations of members of the *mycoides* cluster [6], and *M. mycoides* subsp. *capri* [7] from goat lungs with acute and severe respiratory problems. In this study, the usefulness of the complement fixation assay was assessed to determine the serological status of nine regions.

2. Material and Methods

2.1. Animals and Samples

A sample size of 692 sera samples was estimated with a 0.90 confidence. A total of 827 dairy goats from all ages and breeds from nine regions in the northwest, southeast and central Mexico were aseptically bled (**Table 1**). Sera were conserved at −20˚C until use.

2.2. Antiserum

The immunization methodology described by Tully, *et al*. 1983 [8] was followed to obtain a rabbit antiserum against a strain of *M. mycoides* subsp. *capri* isolated from the lung of a goat with pneumonia. This polyclonal antiserum was evaluated by double immunodiffusion test (DIT) and counter-immune electrophoresis (CIE) against *Mycoplasma bovis*, *Acholeplasma laidlawii* and *Ureaplasma diversum.* Furthermore, to rule out cross reaction during complement fixation (CF), it was also performed with polyclonal serum against *mycoides* subsp. *capri,* and a polyclonal antiserum to *M. bovis* and *M. hyopneumoniae.*

2.3. Complement Fixation Assay (CF)

CF was performed in a 96 wells microplate according to the described by Cunningham, 1986 [9]. Briefly, whole cells of *M. mycoides* subsp. *capri* were used as antigen (Ag). 100% hemolysis was regarded with two units of complement (UC), and two hemolysis units in Triethanolamine Buffer Solution (TBS), pH 7.2. Field samples and control serums (CS); positive and negative, were serially diluted (from 1/2 to 1/256) in a final

Table 1. Geographic location of the herds included in this study.

Zone	Region	State	Sample number	Clinical story
Centre	1	Mexico City	176	Herds with respiratory problems
Centre	2	EdoMex	91	
	3		130	
Centre	4	Guanajuato	1	
	5		73	
	6		33	
Centre	7	Morelos	103	
Southeast	8	Guerrero and Oaxaca	203	
Northwest	9	BCS	17	Herd without respiratory problems

EdoMex: Estado de México; BCS, Baja California Sur.

volume of 25 µl. Twenty-five µl of the *M. mycoides* subsp. *capri* 1/700 were added to each serum dilution, and to each control. Each anticomplement control (AntiC) was graduated to 25 µl with TBS, and 25 µl complement (C) with two UC were added to the serum dilutions, positive and negative controls, antigen complement (Ag-C) and C. The plate was incubated at 37˚C in a laboratory bath for 60 min. The hemolytic system (HS) was incubated at 37˚C for 30 min, then 50 µl were added to each well and again incubated at 37˚C for 30 min. Periodical observations were done since the first 5 min of incubation to determine the serum titers, and were established at the highest dilution in which a positive reaction of sedimentation of the HS was observed.

The threshold for the cut point was established according to the relation between the sample titers from which *Mycoplasma* spp. was isolated, and those from the milk samples and nasal swabs from herds included in the study. Finally, the CF sensitivity and specificity was determined by the Bayes theorem and the positive and negative predictive values were estimated.

3. Results

3.1. Antiserum against *Mycoplasma mycoides* subsp. *capri*

Rabbits were bled to white at day 25 post-inoculation. No cross reaction was detected against *Mycoplasma bovis*, *A. laidlawii,* and *U. diversum* in the CF. To assess the assay homogeneity Xi-square was used to compare whole, sonicated or soluble fraction of *M. mycoides* subsp. *capri* as antigen. No statistical significance ($P = 0.1580$, $P < 0 - 5$) among them was observed, therefore whole antigen was used for further experiments.

3.2. Complement Fixation Assay

The cut off point for CF was established as positive above titers ≥1/16 and negative up to ≤1/8. According to this cut off point 576 (69.65%) samples were negative and 251 (30.35%) were positive (Table 2). Noteworthy, the CF detected around 10% of positive

Table 2. Complement fixation assay results.

Geographic region	Herd's Micoplasmosis background	Negative samples (−)	Negative (%)	Positive samples (+)	Positive (%)
Region 1	Since 2005[b]	170	96.59%	6	3.41%
Region 2	(−)[a]	81	89.01%	10	10.99%
Region 3	Since 2005[a]	98	75.38%	32	24.62%
Region 4	(+)[a]	0	0.00%	1	100%
Region 5	Since 2004	64	87.67%	9	12.33%
Region 6	(+)[a]	0	0.00%	33	100%
Region 7	Since 2005[b]	84	81.55%	19	18.45%
Region 8	(+)[a]	62	30.54%	141	69.46%
Region 9	(−)[a]	17	100%	0	0.00%

[a]Isolation based diagnosis, [b]Histopathology based diagnosis.

sera in regions 9 and 2, in which no previous events of respiratory problems had been reported. In regions 3, 5, 1 and 7, with respiratory problems history the positive percentage was up to 20%. On the other hand, those herds in which the samples were drawn during a respiratory event in region 4, 6 and 8, the positive percentage was over 60% (Table 2). Statistically significant difference was determined to CF ($P < 0.01$) by Xi-square. Furthermore, the sensitivity and specificity of CF were determined by the Bayes Theorem, as shown in Table 3.

4. Discussion

Mexico is not an endemic area; hence the CF threshold is fixed at titers ≥1/16. In contrast, threshold of ≥1/32 is used in Kenia, in which micoplasmosis due to *Mycoplasma capricolum* subsp. *capripneumoniae* is endemic [10] [11]. On the other hand, in Oman a threshold for immunoagglutination has been established at 1/40 for *M. capricolum* subsp. *capripneumoniae* [12]. In these countries, vaccination against mycoplasma is widely accepted. In Mexico, the mycoplasmosis vaccination in goats is not practiced yet; therefore a tighter criterion for results interpretation of the CF was applied. In the mycoplasmosis free herds, ten goats were CF positive with titers of 1/16 - 1/32. These results may be explained due to herd mobilization among adjacent regions. In herds with micoplasmosis history, 66 goats were diagnosed as positive by CF (titers 1/16 - 1/128). These results suggest previous contact with the antigen [13]. According to experimental assays with *M. capricolum* subsp. *capripneumoniae,* CF may detect previous exposure to *Mycoplasma* spp. as long as four months post inoculation [14]. In the herds included in this study, micoplasmosis was diagnosed by CF in 69.46% in region 8 and up to 100% in regions 4 and 6. These results are in accordance with the disease morbidity, which may range from 60% to 100%.

In Mexico, *Mycoplasma* has been reported since the 60's. Solana *et al.* (1966) used the CF and/or Immunoagglutination for *M. mycoides* subsp. *capri* to screen 16 regions

Table 3. Sensitivity and specificity of the CF.

	CF
Sensitivity	93.33%
Specificity	72.27%
Positive predictive value	90.08%
Negative predictive value	80.06%

including regions 4, 5, 6 and 8 [15]. In region 8 they reported 90% of positive sera, and in regions 4, 5 and 6, 50% of positive results. However, no relation among the *Mycoplasma* spp. isolation, the disease symptoms and the CF results was depicted. Besides, *Mycoplasma* spp. has been isolated in the South from pneumonia outbreaks [16].

In this work, we report enhancement of the sensitivity and specificity of the CF by using the whole antigen of *Mycoplasma mycoides* subsp. *capri*. It turns to be quick and reliable for the serological diagnosis of the caprine mycoplasmosis. This assay is able to detect antibodies against *Mycoplasma* spp. up to a two years period post-exposure. We were able to establish that the goat population had been exposed during their productive life to *Mycoplasma mycoides* subsp. *capri* with an overall seroprevalence of 30.35%. Additionally, the serological profile by this sensible and specific serological test allows discerning which animals have been in contact with the *mycoides* cluster members in the short and long term and guides the preventive and control measures for goats' mycoplasmosis.

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