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NOTE

Mycoplasma excretion in reproductive male and female goats

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Abstract

Three experiments were designed in order to study the excretion routes of mycoplasmas involved in caprine contagious agalactia in chronically infected goat populations. The results obtained for external auricular canal (EAC) swabs were compared to other samples: 1) nasal swabs in 95 bucks; 2) conjunctival swabs in 85 bucks; 3) milk and vaginal swabs in 23 goats. Serology to detect specific antibodies against *Mycoplasma agalactiae* (Ma) was also conducted. Out of 1061 samples, the presence of Ma, Mmc and Mcc was detected in 23, 18 and 8 samples, respectively, which belonged to 24 bucks and 7 goats. EAC analyses detected 21 bucks (87.5%) and 4 infected goats (57.1%). Additional sampling (conjunctival, nasal swabs and vaginal swabs in goats) allowed the detection of further infected animals, in comparison to the sole sampling of the EAC.

Key Words: contagious agalactia, control programs, goat, reproduction

Contagious agalactia (CA) is one of the most important diseases affecting dairy small ruminants. This syndrome, which can be caused by four mycoplasma species, *Mycoplasma agalactiae* (Ma), *Mycoplasma mycoides* subsp. *capri* (Mmc), *Mycoplasma capricolum* subsp. *capricolum* (Mcc) and *Mycoplasma putrefaciens* (Mp), is characterized not only by the classical symptomatic triad of mastitis, arthritis and conjunctivitis, but may also be related to reproductive symptoms, such as abortions[2)]. However, the efforts made to control this disease have usually been justified by the loss in milk production and, therefore, its possible repercussions on reproduction have not been previously considered.

Recent studies have addressed the lack of available data on the role of breeding goats in the epidemiology of CA. These works have confirmed the presence of asymptomatic mycoplasma carriers in artificial insemination (AI) centres. Most of these infected goat bucks

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and teaser does carried mycoplasmas latently in their external auricular canal (EAC)[1]. Notwithstanding, the epidemiological role of asymptomatic carriers of mycoplasmas in CA remains unclear, although the movement of these inapparently infected animals between different herds has been associated to the occurrence of clinical disease outbreaks[2]. Hence, as the scientific community accepts the presence of a high number of auricular carriers in chronically infected goat populations[14], these recent data highlight the risk of transmitting pathogenic microorganisms to other animals, and thus, avoiding the presence of these infected individuals in AI centres becomes a need. Moreover, the available studies which describe the anatomic location of mycoplasmas in asymptomatic infected bucks, demonstrate the ability of these pathogens to colonize several anatomic locations such as the respiratory system, reproductive tract or the EAC, showing the sporadic presence of these mycoplasmas in the conjunctiva, the nasal cavity or even in semen. Therefore, infected carriers may be able to transmit pathogenic mycoplasmas via different excretion routes, including a potential risk of venereal transmission[6,7]. In this sense, the presence of pathogenic mycoplasmas in semen and their viability in ready-to-use seminal doses[4,9], as well as the spread of the infection to the rest of the animals of an AI centre population in combination with the presence of Ma and Mcc in semen, highlight the need of further research and solutions.

All in all, these findings demonstrate the sanitary risk associated to asymptomatic animals, and show that the present World Organisation for Animal Health (OIE) recommendations are unsuitable to control the presence of infected animals in these centres[4,5]. Thus, some goat AI centres have adopted new measures to avoid the introduction of bucks infected by mycoplasmas, based on a surveillance protocol consisting of the analysis of EAC swabs and serum samples taken from all candidates before their admission, as a way of controlling the introduction of unchecked reproductive animals[7]. Theoretically, the efficacy of these control measures is arguable, as they could provide false negative results. Consequently, this manuscript includes three experiments designed to achieve a better understanding of the excretion routes of *Mycoplasma* spp. in goat specimens involved in breeding programs.

All experiments were carried out with samples taken from 10 goat populations located in 4 different CA-endemic areas of Spain (Murcia, Andalusia, Castilla-León and the Canary Islands), between 2012 and 2015. Prior to sample collection, animals were clinically examined and any of them showed clinical symptoms of CA.

The efficacy of 2 EAC swabs or serum samples (only for Ma analyses) to detect the presence of CA-causing mycoplasmas carriers was compared to the effectiveness yielded by using other samples: 2 conjunctival swabs taken from 95 goat bucks (experiment 1), 2 nasal swabs collected from 85 goat bucks (experiment 2) or milk, and 2 conjunctival plus 1 vaginal swabs taken from 23 goats (experiment 3). Thus, a total of 406 EAC swabs, 236 conjunctival swabs, 170 nasal swabs, 23 vaginal swabs and 23 milk samples were analysed during this experiment. Serum samples (n = 203) were also collected.

As soon as they arrived at the laboratory, milk samples were inoculated in solid and liquid mycoplasma media[12], and incubated at 37°C in a 5% CO_2 humid atmosphere for 48 hours; the swabs were twirled and left in 1 ml of liquid mycoplasma medium for 30 minutes at room temperature. Subsequently, after discarding the swabs, aliquots of the remaining fluid were cultured in the same conditions as previously described. Isolates from cloned single colonies were used for preliminary identification based on biochemical tests, including fermentation of glucose and mannose, hydrolysis of arginine and urea, tetrazolium reduction and film and spots production[16]. Final identification was performed by PCR[10,13].

Blood samples were subjected to a specific

Table 1. Positive samples to *Mycoplasma agalactiae* (Ma), *Mycoplasma mycoides* subsp. *capri* (Mmc) and *Mycoplasma capricolum* subsp. *capricolum* (Mcc) in each experiment

Exp	Sample	No	Ma	Mmc	Mcc	Ma+Mmc	Ma+Mcc	Mmc+Mcc
1	EAC swab	190	0	14	1	0	0	0
	Conjunctival swab	190	0	3	0	0	0	0
	Serum	95	0	–	–	–	–	–
2	EAC swab	170	8	0	2	1	1	0
	Nasal swab	170	2	0	1	0	0	0
	Serum	85	0	–	–	–	–	–
3	EAC swab	46	2	0	3	0	0	0
	Conjunctival swab	46	1	0	0	0	0	0
	Vaginal swab	23	5	0	0	0	0	0
	Milk	23	3	0	0	0	0	0
	Serum	23	0	–	–	–	–	–
Total		1061	21	17	7	1	1	0

[a]EAC: external auricular canal.

ELISA kit (Pourquier ELISA *M. agalactiae*, Institut Pourquier) to detect antibodies against Ma.

Because of the lack of a gold standard, neither true positive nor true negative animals could be considered. So, to compare the efficacy of different diagnosis strategies concerning the detection of asymptomatic mycoplasma carriers, the percentage of positive animals detected was calculated.

A total of 1061 samples, including 203 serum samples, 23 milk samples and 835 swabs, were analysed in this study to detect the presence of mycoplasmas. Table 1 shows the results obtained for each experiment. In total, 47 of the samples (4.4%) tested positive for mycoplasmas, confirming the presence of Ma, Mmc and Mcc in 23, 18 and 8 samples respectively, as 2 samples presented mixed infections. Mp was not detected in this study.

In general, a positive result was obtained for 32 out of 406 EAC swab samples (7.9%). At this anatomic location, Mmc was the most frequently detected mycoplasma (15 positive samples), followed by Ma (11) and Mcc (7). The presence of *Mycoplasma* spp. was detected in 4 out of 236 (1.7%) and 3 out of 170 (1.8%) conjunctival and nasal swabs, respectively. In the conjunctiva, Mmc was also the most frequently detected species, with 3 positive samples, followed by Ma (1). In the nasal cavity, Ma was detected in 2 samples, followed by Mcc (1). As for the studied goats, Ma was the most isolated species from the EAC, whereas vaginal swabs and milk samples allowed to detect 5 (21,7%) and 3 (14,3%) goats infected by Ma.

Concerning the aim of the present work, Table 2 shows the number of positive animals detected in each experiment. Overall, 31 animals tested positive for the presence of *Mycoplasma* spp. Independently of the mycoplasma species involved, Experiment 1 showed that the analysis of EAC swabs allowed the detection of 10 out of 12 carriers (83.3%), in comparison to the use of conjunctival swabs, though which 3 infected individuals were detected (25%). Similarly, from all the carriers detected in Experiment 2, 11 out of 12 (91.7%) individuals were diagnosed after analysing their EAC swabs, whilst only 25% (3/12) were detected through the sampling of their nasal cavity. Finally, Experiment 3 showed that the analysis of vaginal (5/7, 71.4%) and EAC swabs (4/7, 57.1%) allowed detecting the largest number of infected teaser goats, whereas the

Table 2. Positive animals to *Mycoplasma agalactiae* (Ma), *Mycoplasma mycoides* subsp. *capri* (Mmc) and *Mycoplasma capricolum* subsp. *capricolum* (Mcc) depending on the type of sample used

Exp	No. animals (infected)	EAC swabs	Conjunctival swabs	Nasal swabs	Milk	Mixed
1	95 (12)	9(8Mmc[a],1Mcc)	2(Mmc)	–	–	1[b]
2	85 (12)	9(6Ma[c],2Mcc,1Ma+Mmc)	–	1(Ma)	–	2[d]
3	23 (7)	1(Ma)	1(Ma)	–	0[e]	5[f]

[a]Mmc in both EAC (5)
[b]Mmc in EAC and conjunctiva (1)
[c]Ma in both EAC (1)
[d]Ma+Mcc in EAC and Mcc in nasal (1), Ma in EAC and nasal (1)
[e]No single excretion by milk was detected (see next point).
[f]Mcc in EAC and Ma in vagina (1), Ma in milk and vagina (2) Mcc in both EAC+Ma in vagina (1), and Ma in EAC, vagina and milk (1).

analysis of their milk samples identified only 3 of them (42.9%). Conjunctival swabs only detected 1 infected goat (14.3%).

The results obtained in the studied goat bucks showed that the analysis of swabs taken from the conjunctiva or the nasal cavity were necessary to identify all of the asymptomatic carriers detected in Experiments 1 and 2. In this sense, although respiratory signs caused by Ma are mainly associated to the infection in young animals[2], its effect on the respiratory system of chronically infected adult animals has been proved[8]. Similarly, the presence of mycoplasmas in clinically affected animals is common[6,17], but less information about their presence in asymptomatic carriers is available.[5] Globally, our results are coherent with the systemic infection observed in asymptomatic auricular carriers[8] and the tissue tropism classically assigned to these bacteria[2]. In this context, new control strategies have been effectively adopted by some AI centres of Spain to minimize the presence of individuals infected by CA-causing mycoplasmas. Hence, the analysis of EAC swabs and serum has avoided the admission of carriers of Ma and Mmc in some male goat populations[7], and the results of the present work support the use of EAC swabs as the most appropriate sample to develop these type of control programs.

On the other hand, the use of vaginal swabs allowed the detection of a significant percentage of infected goats. To our knowledge, these results are the first description of Ma and Mcc in the vagina of asymptomatic goats. The capacity of Ma to colonize this anatomic location has been previously demonstrated in experimentally inoculated sheep[3] and Mmc has been isolated from the genital tract of apparently healthy sheep[11] and also associated to clinical outbreaks of abortions in goats[17]. However, this source of excretion has never been considered of concern for a control program of CA. The sole sampling of the EAC was able to detect only a part of the asymptomatically infected goats, taking into account the outcome of Experiment 3. Therefore, these data suggest that checking the EAC is an adequate way to detect the presence of 8–9 out of 10 goat bucks and 5–6 out of 10 goats carrying CA causing mycoplasmas, and support the usefulness of analysing this type of sample as the main measure within caprine CA-control programs, as previously proposed[7]. Nevertheless, this study also highlighted the fact that other samples should be analysed in order to increase the detection of infected animals, especially when checking goats. Hence, the established control protocol could be complemented by collecting samples of vaginal swabs and milk (for goats) or from the nasal cavity and the conjunctiva of goat bucks, prior to the admission of these individuals to an AI centre.

Additionally, the results of this study

confirm that serology is not a valid tool to detect CA caused by Ma in chronically infected animals, which had already been reported, and thus its use should be discarded for this purpose in caprine CA surveillance progams[15]. Finally, we encourage the OIE to review the current recommendations in order to avoid the introduction of CA in caprine AI centres through animal movement, including the detection of mycoplasmas in the EAC, as previously suggested[5].

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

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