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Comparison of productive performance in dairy goats with and without serological evidence of *Mycobacterium avium* subsp. *paratuberculosis* infection

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ABSTRACT

Goat paratuberculosis (PTBC), caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), leads to production losses in clinical cases, but the effects of subclinical infection remain unclear. The aim of this work was to explore the effect of subclinical PTBC (infection on milk production and milk composition in Saanen goats in Argentina. Eighteen lactating multiparous Saanen goats calved during September were selected for the study. They were grouped on the basis of ELISAI, ZN and *Map* culture results in: Negative group (n=8) and Positive group (n=10). Milk production was individually recorded throughout the trial, and milk chemical composition was measured from samples collected 4 times during evening milking. *Map* infection was detected by isolation (from feces, milk or both) in 75% from the positive group of goats. Milk production resulted similar ($p > 0.05$) between negative (927.34 mL/goat) and *Map* serologically positive group (778.9 mL/goat). No significant differences were observed in milk composition or production between groups, indicating no detectable effect of subclinical *Map* infection in goats.

Keywords: goats, paratuberculosis, milk production, milk composition.

RESUMEN

La paratuberculosis caprina (PTBC), causada por *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), provoca pérdidas productivas en los casos clínicos, aunque los efectos de la infección subclínica aún no están bien establecidos. El objetivo del presente trabajo fue evaluar el efecto de la PTBC subclínica sobre la producción y la composición de la leche en cabras Saanen en Argentina. Se seleccionaron para el estudio dieciocho cabras Saanen multíparas en lactancia paridas durante septiembre. Los animales se agruparon según los resultados de ELISAI, ZN y cultivo de *Map* en: Grupo negativo ($n = 8$) y Grupo positivo ($n = 10$). Se registró la producción de leche individualmente durante todo el ensayo, y la composición química de la leche a partir de muestras recolectadas en cuatro ordeños vespertinos. La infección por *Map* se detectó mediante aislamiento (en heces, leche o ambos) en el 75% de las cabras del grupo positivo. La producción de leche fue similar ($p > 0,05$) entre el grupo negativo (927,34 mL/cabra/día) y el grupo positivo serológicamente (778,9 mL/cabra/día). Tampoco se observaron diferencias significativas en la composición de la leche entre ambos grupos, lo que sugiere que la infección subclínica por *Map* no tuvo un efecto detectable sobre la producción ni la composición de la leche en las cabras evaluadas.

Palabras clave: cabras, paratuberculosis, producción láctea, composición de la leche.

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INTRODUCTION

Paratuberculosis (PTBC), caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), is a globally significant granulomatous enteritis in ruminants (Windsor, 2015). In small ruminants, it causes weight loss, emaciation, and occasional diarrhea due to protein-losing enteropathy (Tharwat *et al.*, 2025).

Map is usually introduced to dairy herds through the purchase of infected but clinically normal cattle. The adverse impacts of clinical PTBC in cattle have been well described (Aly *et al.*, 2010). In contrast, specific effects of PTBC during the subclinical stage have been more difficult to determine. Most of the studies have been carried out in cattle, and contradictory results have often been obtained (Johnson *et al.*, 2001; Beaudreau *et al.*, 2007; Gonda *et al.*, 2007; Raizman *et al.*, 2009; Bates *et al.*, 2018).

Map infection can occur during the first months of life from exposure to contaminated feces, colostrum or milk, or even in utero (Benedictus *et al.*, 2008; Rosell *et al.*, 2020). The transmission of the infection is mainly the fecal-oral route either by contact with fecal contaminated teats or by ingestion of infected milk (Benedictus *et al.*, 2008). While animals can develop clinical signs after a long incubation period, *Map* can be shed in feces, colostrum and milk over several months before the onset of clinical signs (Bates *et al.*, 2019).

Ruminants are a major source of MAP environmental contamination, potentially exposing humans regularly (Kuenstner *et al.*, 2020). *Map* has been isolated from a high percentage of Crohn's patients, but whether the association is causal or coincidental is not known (Espescht *et al.*, 2023).

Goat PTBC is distributed worldwide and has been diagnosed in Turkey, France, Norway, Switzerland, Croatia, Canada, the USA, Brazil, Mexico, and Chile (Espescht *et al.*, 2017). In Argentina, it was confirmed in 2012 (Fiorentino *et al.*, 2012), but local prevalence data are not yet available. Argentinian goat production has traditionally utilized extensive/semi-intensive systems with different production purposes (meat, fiber, leather, dairy), with current herds estimated at ~4 million heads (MAGYP, 2025).

Although the disease in goats is of global distribution, the prevalence and economic impact are not yet well recognized in this species, partly due to the low resources available for goat industry research compared to those for sheep (Windsor, 2015). There are no studies on the impact of subclinical infection with *Map* on dairy goat productivity. Therefore, the aim of this work was to explore the effect of subclinical PTBC infection on milk production and composition in Saanen goats in Argentina.

MATERIALS AND METHODS

The study was conducted in December-January on a 203 head Saanen dairy goat herd in Buenos Aires province, Argentina. The herd had a 48% *Map* seroprevalence and was managed under a semi-extensive system, combining daytime pasture grazing with concentrate supplementation. Goats were housed at night with *ad libitum* water access and milked twice daily. Mastitis was monitored throughout the study.

Experimental animals

Eighteen multiparous Saanen goats (3–4 years old) that calved within a 22-day period in September were selected. Only test-positive animals without clinical signs of PTBC were

included, and those that developed clinical disease during the study were excluded from the analysis.

Goats were classified as Negative (n=8) or Positive (n=10) based on ELISAI, Ziehl-Neelsen staining, and *Map* culture. Animals were considered negative if all tests were non-reactive. Goats were considered negative when tested as not positive for any of the techniques.

Serology

To form study groups, from each goat calved in September, blood samples (10 mL) from the jugular vein were taken once and serum was frozen until analyzed by ELISAI using *Map* protoplasmic antigen (PPA-3; Allied Monitor, Fayette, MO, USA) as described by Fiorentino *et al.* (2012).

Fecal smears and culture

Fecal samples (5 g) were collected from each goat on days 0 and 36, stored at –20°C, and processed by Ziehl-Neelsen staining and culture (OIE, 2019). After decontamination with hexadecylpyridinium chloride (Sigma, USA) (final concentration 1%), samples were inoculated on three slants of Herrold's egg yolk medium (pH 7.1–7.4), with and without mycobactin J (HEYM and HEY, respectively) (Allied Monitor Inc., MO, Fayette, USA). The cultures were incubated at 37°C for up to 6 months and examined biweekly for bacterial growth. *Map* colonies were identified based on growth characteristics and confirmed by IS900 PCR and IS1311 PCR-REA (Collins *et al.*, 1993; Marsh *et al.*, 1999).

Milk samples

Milk samples for *Map* culture and quality analysis were collected at various times during the trial. Samples from both teats were taken under strict hygiene, including udder washing, drying, and teat disinfection with 70% ethanol. Milk samples were collected aseptically after discarding the first three jets, kept on ice during transport, and cultured within 8 hours.

Map isolation from milk samples: samples from each goat (80 mL) were collected at days 15 and 34. In order to increase the chances of *Map* isolation, two different decontamination methods were used: oxalic acid (OA) (40 mL/animal) and HPC (40 mL/animal). After centrifugation, samples were processed separately with OA or HPC, incubated or left overnight as appropriate, and the resulting sediment was cultured on HEYM and HEY media.

Milk production and chemical composition

Chemical composition of milk was measured from samples (100 mL) collected 4 times in the evening milking (half milking, HM) on non-consecutive days, at the beginning of the trial (day 0) and then on days 12, 19 and 27. They were analyzed for fat, protein, lactose, and total solid content by mid-infrared spectrophotometry (Milko Scan-Minor, Foss Electric, Hillerød, Denmark).

Milk production was individually recorded 8 times in two weeks throughout the trial in the HM (days 20, 21, 26, 27, 30, 31, 32 and 33).

Statistical analyses

Milk production and composition were analyzed using a completely randomized design with repeated measurements over time using the PROC MIXED program of SAS/STAT® (SAS Institute Inc. 2015) according to the following model:

$$Y_{ijk} = \mu + T_i + A(i)j + D_k + (T \times D)_{ik} + E_{ijk}$$

Where Y_{ijk} = dependent variable; μ = overall mean; T_i = i -th treatment measure effect ((*Map*+); (*Map*-)); $A(i)j$ = random animal effect; D_k = effect of the day; $(T \times D)_{ik}$ = interaction between treatment and day, and E_{ijk} = residual error associated with the i -th experimental unit.

RESULTS

Milk, teats, and udders of all healthy and subclinically infected goats appeared normal. During the trial, two goats from the positive group developed clinical PTBC symptoms and died, with *Map* isolated from their lymph nodes and ileocecal valves.

Map was isolated from feces, milk, or both in 75% of positive goats (50% [4/8] from feces and 37.5% [3/8] from milk). In the seronegative group, *Map* was detected in feces of one animal but not in milk.

In feces, the first *Map* colonies were observed on day 37 of incubation, whereas in milk samples they were observed on day 112. There was no contamination with other bacteria or fungi on the selective media. All suspicious colonies were confirmed as *Map* by PCR.

Results of milk production and milk composition are shown in table 1. Seronegative goats produced, on average, 11.6% more milk (927.34 mL/goat/HM) than *Map*-seropositive goats (778.9 mL/goat/HM), though the difference was not statistically significant ($p > 0.05$) and the variation in this variable over sampling time was independent of the treatment ($p < 0.001$). Average milk protein and lactose concentrations (gr/100 mL) did not differ between groups (figure 1, A and C). However, milk fat showed a significant interaction ($p = 0.0069$) between treatment and day of sampling (figure 1, B).

DISCUSSION

Likewise in cattle dairy, the economic impact of *Map* infection in dairy goat herds with high prevalence is easily observed by farmers who, when they see goats with PTBC, sell them. However, the impact of *Map* infection on milk production in asymptomatic goats is unclear. This study aimed to compare productivity in seropositive or seronegative *Map* dairy goats without clinical symptoms of PTBC.

The fact that two animals with symptoms of PTBC had to be removed from the positive group shows that apparently healthy goats subjected to production pressure can rapidly develop clinical states of the disease with the consequent weight loss, decreased in milk production and eventually slaughter or death. Bush *et al.* (2006) in their study reported that *Map* infection in sheep caused significant economic loss due to deaths, with losses accounting on average for two-thirds of the total estimated financial loss associated with sheep deaths. In addition, in cattle dairy herds the economic impact of PTBC includes production losses due to animal replacement, premature culling, and low beef value, and costs associated with the implementation of control measures (Weber, 2006). These evident losses often prompt farmers to implement control programs.

One of the greatest challenges for management of *Map* disease has been the difficulty of accurately identifying animals with tests of low sensitivity (Whittington *et al.*, 2019). This is compounded by their inability to identify latent and subclinical *Map* infections, particularly as fecal shedding of the organism can be intermittent in these animals and tends to precede serological responses (Magombedze *et al.*, 2016; Mitchell *et al.*, 2015). As in other studies in which the productive effects of subclinical infection by *Map* have been evaluated, we recognize that our interpretation of these results is based on an imperfect testing methodology where tests do not have 100% sensitivity and specificity. In our research we performed ELISAi in serum at the beginning and cultures of fecal matter and milk in two moments of the trial, in order to have a greater chance of identifying animals with subclinical infections. Of the 16 milk samples from the positive group processed in the present investigation, *Map* was detected from 3 milk samples of 3 subclinically infected goats. This incidence (37%) was higher than reported in milk samples of infected goats (Singh and Vihan, 2004) and cows (Giese and Ahrens, 2000). They showed the presence of a low level of cultivable *Map* (<10%) in raw milk samples taken directly from the udder. These differences could be due to the fact that, in our work, the sediment and fat layer were cultivated together and this situation may have increased bacterial recovery.

Regarding the isolation of *Map* in an animal from the negative group (# 29), several authors have reported the passive shedding or pass-through phenomenon, where the animal swallows *Map* organisms but it is not infected (Whitlock *et al.*, 2008). This situation could occur in a heavily contaminated environment when immune adult animals ingest the bacteria with food (Kralik *et al.*, 2014). However, we cannot consider that it was a really infected animal.

In Argentina, goat milk is primarily used for cheesemaking, with cheese yield being the key economic indicator for producers (Pazzola *et al.*, 2019). While PTBC's impact on bovine milk production shows conflicting data, evidence in goats remains limited. Several studies in dairy herds report a reduction in milk production in sub-clinically infected cows (Beaudeau *et al.*, 2007; Gonda *et al.*, 2007; Raizman *et al.*, 2009). Our results agree with those carried out in bovines by Johnson *et al.* (2001), who failed to demonstrate a significant association between reduction in milk production and *Map* infection. Such variability in estimates may be attributable to experimental study design differences, the diagnostic criteria for defining *Map*-infected animals, the selected measure of milk production, and/or the statistical model used to estimate differences in milk production.

Milk composition, particularly fat and protein, impacts producer economics as these parameters are used in cheese yield prediction models (Emmons and Modler, 2010). This study did not identify a statistically significant difference in milk composition (protein, lactose, or fat) between seropositive and seronegative group. Both groups exhibited protein and lactose levels consistent with those reported by Fau *et al.* (2010) for Saanen goats in Argentina, though fat percentages were lower yet still within standard breed parameters.

The lack of effect of subclinical *Map* infection on milk composition in this study aligns with findings in cows (Johnson *et al.*, 2001). In contrast, Pazzola *et al.* (2020) reported higher fat, protein, and casein levels in *Map*-positive but clinically healthy ewes. In cows, the association between production potential and probability of culling due to PTBC was detected when highly pro-

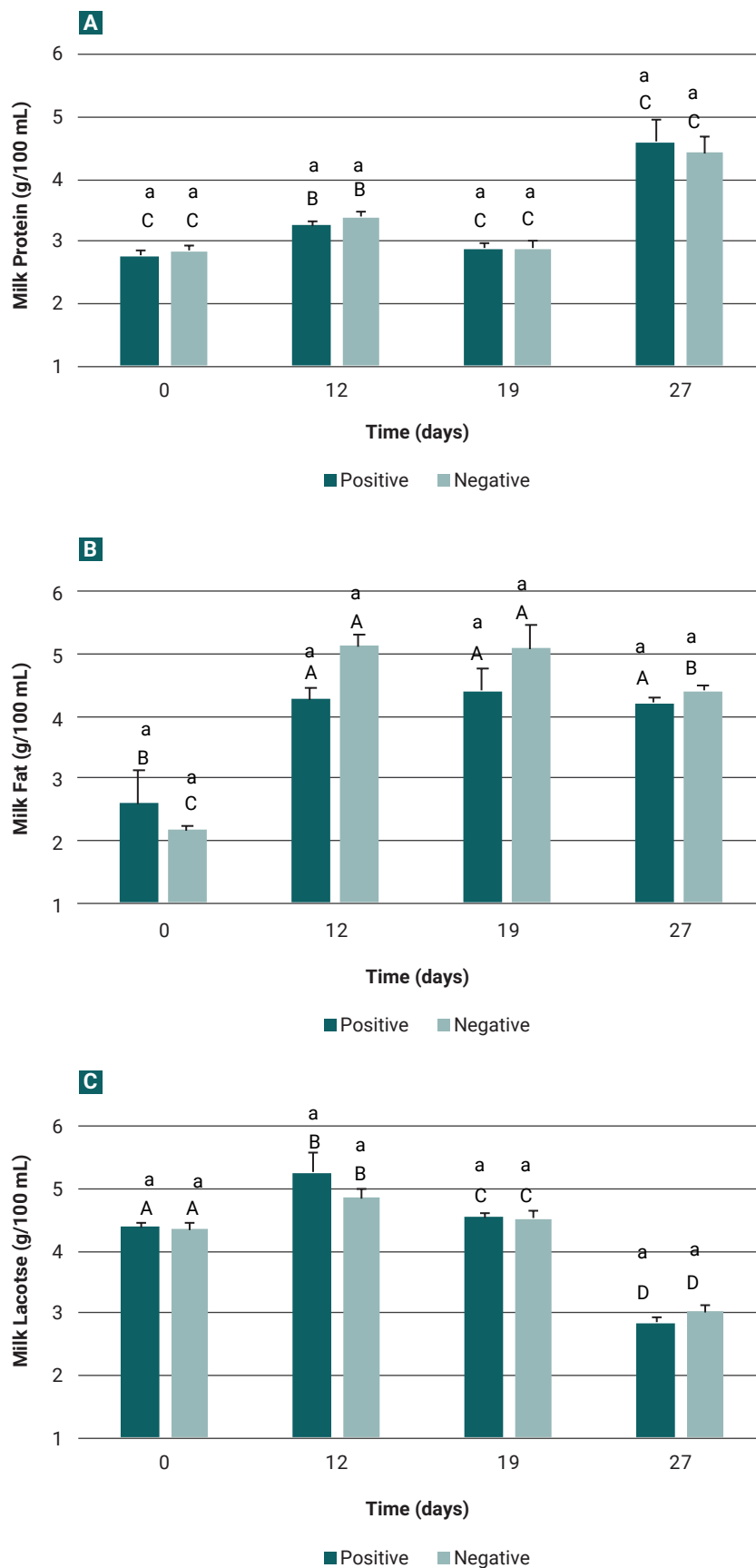


Figure 1. Least squares means $\pm$ SEM of milk composition (protein, fat, lactose) during lactation. Uppercase letters (A, B, C) indicate differences ($p < 0.05$) between timepoints within groups; lowercase letters (a,b,c) denote differences ($p < 0.05$) between groups at each timepoint.

Parameter	Treatment ¹		SEM ²	p-value ³	T*D ⁴
	Positive (n= 10)	Negative (n=8)			
Milk yield (mL/goat/HM)	778.91	927.34	82.06	0.22	ND
Fat (g/100g)	4.03	4.24	0.12	0.51	0.0069
Protein (g/100g)	3.40	3.40	0.10	0.99	ND
Lactose (g/100g)	4.24	4.17	0.11	0.66	ND

¹Least squares means.

²Standard error of least squares means.

³Treatment effect.

⁴Treatment for sampling time interaction.

HM= half milking. ND= No detected

Table 1. Milk production and composition in goats seropositives and seronegatives to *Mycobacterium avium* subsp. *paratuberculosis*.

ductive cows which were infected with *Map* were shown to be more likely to develop clinical disease (Benedictus *et al.*, 1987). In our work, we could not find these differences, maybe due to the genetic homogeneity of the animals in the herd.

CONCLUSION

Although the losses associated with clinical disease have been documented and even shown in the present study, in our study we do not identified a significant effect of subclinical *Map* infection in goats on milk production or milk composition (fat, proteins, and lactose). Although the control group had higher average milk production, the difference was not significant, possibly due to variability and small sample size. The rapid progression from subclinical to clinical disease and *Map* isolation in asymptomatic goats highlight important concerns for farmers. Further studies with more animals and complete lactation records are needed to confirm these trends.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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