DNA presence and viability of *Mycobacterium avium* subsp. *paratuberculosis* in semen of Pelibuey rams during the subclinical stage of paratuberculosis

Velázquez-Morales, José V.[1,2]; Santillán-Flores, Marco A.[3]; Salazar-Ortiz, Juan[4]; Quintero-Elisea, Juan A.[5]; Cortez-Romero, Cesar[6,7*]

[1] Universidad Autónoma Metropolitana, Unidad Iztapalapa. División de Ciencias Biológicas y de la Salud, Departamento de Biología de la Reproducción. Ciudad de México, México. C.P. 09340.
[2] Instituto Mexicano del Seguro Social. Órgano de Operación Administrativa Desconcentrado Estado de México Oriente. Hospital General de Zona No. 57, Cuautitlán, México C.P. 54769.
[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Centro Nacional de Investigación Disciplinaria en Microbiología Animal. Colonia Palo Alto, Alcaldía Cuajimalpa, México. C.P. 05110.
[4] Colegio de Postgraduados, Campus Córdoba. Posgrado de Innovación Agroalimentaria Sustentable. Municipio de Amatlán de los Reyes, Veracruz, México. C.P. 94946.
[5] Universidad Autónoma de Tamaulipas. Unidad Académica Multidisciplinaria Mante. Blvd. Enrique Cárdenas González No. 1201 Pte, Cd. Mante, Tamaulipas, México, C.P. 89840.
[6] Colegio de Postgraduados, Campus Montecillo. Posgrado de Recursos Genéticos y Productividad - Ganadería. Montecillo, Texcoco, México. C.P. 56264.
[7] Colegio de Postgraduados, Campus San Luis Potosí. Posgrado en Innovación en Manejo de Recursos Naturales. Iturbide No. 73, Salinas de Hidalgo, S.L.P., México. CP. 78620.
* Correspondence: ccortez@colpos.mx

Citation: Velázquez-Morales, J. V., Santillán-Flores, M. A., Salazar-Ortiz, J., Quintero-Elisea, J.A., & Cortez-Romero, C. (2025). DNA presence and viability of *Mycobacterium avium* subsp. *paratuberculosis* in semen of Pelibuey rams during the subclinical stage of paratuberculosis. *Agro Productividad*. https://doi.org/10.32854/bnp9at30

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: May 5, 2025.
Accepted: August 12, 2025.
Published on-line: October XX, 2025.

Agro Productividad, 18(9). September. 2025. pp: 207-214.

ABSTRACT

Objective: To determine DNA presence and viability of *Mycobacterium avium* subsp. *paratuberculosis* in semen of Pelibuey rams in the subclinical stage of paratuberculosis.

Design/Methodology/Approach: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiological agent of paratuberculosis (PTB). Reports about the spread of MAP to extraintestinal sites have been confirmed by the presence of MAP-DNA in semen. However, contamination with MAP has not been reported in sheep semen in the subclinical stage of the disease. Such inadvertent contamination would represent a risk factor for reproductive and genetic management, even in the presence of asymptomatic rams. A descriptive, cross-sectional study was carried out with five rams naturally infected with MAP and one PTB-free ram as a negative control. Nested Polymerase Chain Reaction (nested PCR) and MAP culture techniques were used to research the presence of DNA and the viability of *Mycobacterium avium* subsp. *paratuberculosis* in the semen of Pelibuey rams in the subclinical stage of paratuberculosis. The rams came from an infected flock diagnosed with PTB.

Results: The presence of MAP-DNA was detected in the semen of 60% (3 out 5) of the rams in the subclinical stage. However, MAP did not grow in the culture, suggesting that the MAP from semen samples is not viable.

Study Limitations/Implications: The study was carried out with a small number of naturally infected animals in the subclinical stage, taking into consideration animal welfare and care. However, more animals would result in better measurements.

Findings/Conclusions: The presence of MAP-DNA in the semen of rams in the subclinical stage was confirmed. Apparently, the MAP from the semen is not viable for culture.

Keywords: paratuberculosis, MAP culture, clinical signs, nested PCR, ELISA, semen.

INTRODUCTION

Paratuberculosis (PTB) or Johne's disease (JD) is a chronic infectious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It mainly impacts domestic and wild ruminants, but it can also affect non-ruminant animals (camels, wild rabbits, pigs, horses, poultry, and carnivores) (Idris *et al.*, 2022). PTB has a 5-30% prevalence, mainly among sheep, goats, and cattle (Guzmán-Ruiz *et al.*, 2016) and its presence impacts the economy, animal welfare, and public health (Whittington *et al.*, 2019). For each animal diagnosed in its clinical stage, 25 more animals may be infected, although they may not show outward signs (Pritchard *et al.*, 2017). Several clinical signs could help to guess the presence of PTB: intermittent scours and weight loss despite a good appetite (Idris *et al.*, 2022). PTB can be detected with several techniques: acid-resistant bacilli through a stool microscopy, a fecal or tissue culture, a serology test, and molecular methods (Idris *et al.*, 2022). MAP mainly spreads through fecal-oral transmission (Whittington and Windsor 2009). MAP can infect the gastrointestinal tract and the mesenteric lymph nodes. However, some studies have proved that it can also spread to extraintestinal sites of cattle, including the uterus, supramammary lymph nodes, udders, testicles, epididymis, and seminal vesicles (Khol *et al.*, 2010). MAP-DNA presence has been reported in the semen of bulls (Ayele *et al.*, 2004; Münster *et al.*, 2013; Sharifzadeh *et al.*, 2010), as well as in the feces, semen, and blood of a subclinically-infected bull. Therefore, semen would be a transmission path without clinical signs (Khol *et al.*, 2010). In conclusion, using infected rams could be a causal agent for the spreading of PTB (Ayele *et al.*, 2004). Eppleston and Whittington (2001) reported the presence of MPA in the ileum, mesenteric lymph nodes, and semen of sheep in the clinical stage. MAP-DNA has also been identified in the epididymis, Cowper's glands, prostate, and semen through a nested PCR (Velázquez-Morales *et al.*, 2019); however, despite the presence of MAP in semen, no alterations have been detected during semen evaluation (Velázquez-Morales *et al.*, 2022). However, the existence and viability of MAP in semen from rams in the subclinical stage of PTB has not been verified; otherwise, new health management strategies should be applied as part of the genetic improvement programs for sheep. Therefore, the aim of this study was to detect the presence of ADN and the viability of *Mycobacterium avium* subsp. *paratuberculosis* in the semen of Pelibuey rams in the paratuberculosis subclinical stage, using the nested PCR and MAP culture techniques.

MATERIALS AND METHODS

Facilities and Animals

The rams were isolated from the herd in pens established in the Colegio de Postgraduados (COLPOS) – Campus Cordoba, a research unit with prior reports of PTB. Campus Cordoba is located in km 384 of the federal highway Córdoba Veracruz, Congregación Manuel León, municipality of Amatlán de los Reyes, Veracruz (18° 51″ 20" N, 96° 51' 37" W, 720 m.a.s.l.). The climate is subhumid warm, the average temperature is 18 °C, and the annual precipitation reaches 1,807.3 mm (García, 2004). The study involved six Pelibuey rams: five naturally infected with paratuberculosis

(subclininal stage) and a non-infected specimen as control. The diagnosis was based on an Enzyme-Linked Immunosorbet Assay (ELISA test) and a nested PCR using IS900 primers, which were applied 20 days before the start of the test. Herrold's egg yolk medium with mycobactin was used for the confirmation diagnostic test. The animals had an average weight of 55.9±2.15 kg and were 27±0.6 months-old.

Semen Collection

At the start of the semen sample collection, the prepuce was washed with Dermocleen™ antiseptic soap. The semen samples were collected from each ram with the artificial vagina method, using the methodology proposed by Williams *et al.* (2001).

Serology

An ELISA serology was conducted to detect anti-MAP antibodies, considering that >0.196 optical density values are positive to PTB (Martínez-Covarrubias *et al.*, 2012). Blood samples were collected through a puncture in the jugular vein, using a 20G BD Vacutainer™ needles and BD Vacutainer™ collection tubes without anticoagulant. The whole blood was centrifuged at 1,000 x g for 10 minutes to recover the blood serum, which was kept at −18 °C, until it was processed in the CENID–Microbiología lab of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP).

PTB Diagnosis through IS900 Nested PCR

To diagnose the PTB infection in Pelibuey rams, DNA was extracted and an IS900 nested PCR was conducted, following the protocol described by Jaimes *et al.* (2008). The test used DNA from fecal and semen samples from each ram. An IS900 nested PCR was conducted to confirm the MAP-DNA presence in feces and semen, using the primers proposed by Erume *et al.* (2001). In the first cycle and the final reaction, 563 and 210 base pairs (bp) were recorded, respectively. The DNA of a MAP strain (ATCC #700535) was used as positive control. The final product of the nested PCR reaction was observed in a 2% agarose gel, stained with GelRed® (Biotium #41003). The gel was visualized with a Gel Doc™ 2000 UV transilluminator system (Bio Rad®).

MAP Culture

The methodology proposed by Martínez-Covarrubias *et al.* (2012) was used to isolate the bacteria. Initially, the semen samples were washed with the acid-alkali method and placed in Herrold's egg yolk medium with mycobactin (2 mg L^{-1}, Allied Monitor Inc.). The samples were incubated at 37 °C for 14 weeks and were subsequently stained with a Ziehl-Neelsen (ZN) stain to search for acid-fast bacilli (AFB).

Body Condition Estimation

Body condition (BC) was scored following the methodology proposed by Russel *et al.* (1969), using the categories proposed by Guerrero-Cárdenas *et al.* (2020): 1) very poor; 2) bad; 3) moderate; 4) good; and 5) excellent.

Statistical Analysis

The output was generated with the SAS® 9.4 statistical package and presented as frequencies and percentages.

RESULTS AND DISCUSSION

Table 1 shows the result of the PTB diagnosis with the ELISA and nested PCR tests. All (100%, 5 out of 5) Pelibuey rams tested positive with the IS900 nested PCR diagnostic test. In contrast, ELISA only estimated that 40% (2 out 5) rams tested positive to MAP; therefore, this serological test recorded a lower percentage of positive diagnosis than the nested PCR. Each test has a different sensitivity and specificity: ELISA has a 79.3% sensitivity and a 82.2% specificity, while IS900 nested PCR has a 67% sensitivity and a 84% specificity (Martínez-Covarrubias *et al.*, 2012). The positive result of the IS900 nested PCR with ram feces was mainly caused by the great volume of bacilli found in the feces of those animals, since an infected animal can excrete between 1.3×10^5 and 5.9×10^9 microorganisms per gram of fecal matter (Manning *et al.*, 2001). Idris *et al.* (2022) have reported the intermittent elimination of MAP in goat feces in the subclinical stage of the infection. For their part, Münster *et al.* (2013) reported the intermittent excretion of MAP through feces in a 18-month old cow —an unusual event for such a young animal. Therefore, they recommended reconsidering the belief that young livestock is not infectious. Meanwhile, Jaimes *et al.* (2008) pointed out that animals who have tested negative to ELISA sometimes tested positive to PCR or bacterial isolation, because, in the early stages of an infection, the prevailing immune response is cellular and the PCR test has a higher inherent sensitivity. During the gestation, the infection expression among females and the seroconversion of animals infected at an early age are boosted, possibly as a consequence of the immune regulation that prevents the rejection of the embryo (Kostoulas *et al.*, 2006). Likewise, Feola *et al.* (1999) studied prolactin and the bovine growth hormone *in vitro* and reported a reduced capacity of macrophages to hinder the growth of intracellular MAP. Therefore, changes in the circulation levels of these hormones during gestation and lactation force phagocytes to allow the intracellular proliferation of *Mycobacterium*.

Table 1. PTB diagnosis in Pelibuey rams in the subclinical stage, using the ELISA test and nested-PCR technique.

Ram Identification	n-PCR IS900: DNA from feces	ELISA
1	+	+
2	+	−
3	+	+
4	+	−
5	+	−
N	−	−

nPCR=Nested Polymerase Chain Reaction. ELISA=Enzyme-Linked Immunosorbent Assay. "+"=positive result. "−"=negative result. N=negative control.

Table 2 summarizes the body condition (BC) score of the rams, recording a 2.5±0.22 average, with a lack of clinical signs (sticky feces and scours). The BC score indicates that sheep have an adequate nutritional status, without weight loss or the presence of clinical signs, possibly because PTB is recorded in 2-4 years old goats, sheep, and cattle (Idris *et al.*, 2022). The weight loss in sheep changes from one specimen to another; in fact, sticky feces or scours only occur in 20% of the cases, during the final stages of the disease (Idris *et al.*, 2022). However, neither sticky feces or scours were reported in this study for 27±0.6 months old rams, which perhaps accounts for the lack of PTB clinical signs among the males. Ayele *et al.* (2001) reported the appearance of clinical signs related to stress-causing factors, such as malnutrition, since these factors can modify paratuberculosis from the subclinical to the clinical stage. However, as has been previously discussed, rams had a moderate BC.

Regarding the search for MAP in semen, the IS900 nested PCR detected the presence of MAP-DNA in goat semen. These results match the findings of Münster *et al.* (2013), who proved that MAP-DNA can be detected intermittently in the semen of bulls in the subclinical stage, with up to 18-week MAP-free intervals. Likewise, they recorded a significant correlation between the presence of MAP in semen and blood. Consequently, they warned against excluding the risk of MAP transmission, despite the lack of the typical signs of clinical paratuberculosis. The presence of MAP outside the intestine is possibly caused by its spread through blood and the lymphatic system (Buergelt *et al.*, 2004). It is even possible that semen samples had been contaminated during the collection (Eppleston and Whittington, 2001; Khol *et al.*, 2010). Unlike the culture, nested PCR can detect viable and non-viable bacteria, because the former has a 100% specificity, but a <50% sensitivity (Jaimes *et al.*, 2008). Table 3 shows the nested PC and culture results; however, no *Mycobacterium* were observed in the semen culture samples.

Figure 1 shows the MAP-DNA presence in semen with the IS900 nested PCR (60%, 3 out of 5).

Figure 1 shows that MAP did not grow in cultures with semen collected from rams in the subclinical stage, possibly as a result of the low number of viable *Mycobacterium* obtained from the sample. Jaimes *et al.* (2008) reported that at least 10^3 bacilli are required

Table 2. Body condition (BC) and clinical signs of Pelibuey rams naturally infected with PTB.

Ram identification	Body condition (BC, score)	Pasty stools	Scours
1	2	–	–
2	2	–	–
3	3	–	–
4	2	–	–
5	3	–	–
N	3	–	–

"+"=positive result. "–"=negative result. Both results were obtained during the recording of the PTB clinical signs.

Table 3. Presence of *Mycobacterium avium* subsp. *paratuberculosis* in the semen of Pelibuey rams in the subclinical stage.

Ram identification	Semen	
	n-PCR IS900	Culture
1	+	–
2	–	–
3	+	–
4	–	–
5	+	–
N	–	–

"+"=positive result. "–"=negative result. Both results were obtained with the nested PCR and MAP culture tests.

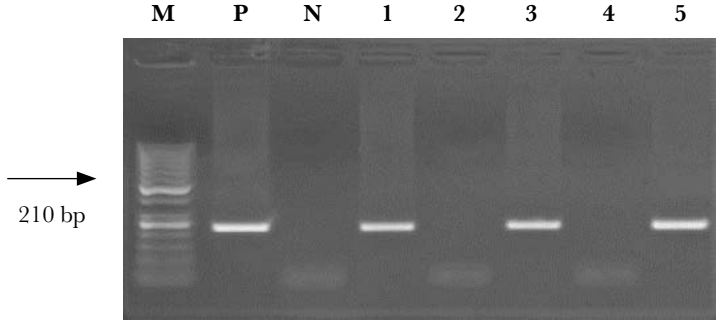

Figure 1. DNA amplification output with IS900 nested PCR in semen samples from Pelibuey rams in the subclinical stage of PTB, using an electrophoresis with 2% agarose gel. Positive fragment of 210 bp. Track M: 50 bp molecular marker. Track P: positive control (MAP-DNA ATCC #700535). Tracks 1, 3, and 5: semen samples with MAP-DNA (positives, 210 bp). Track N: semen samples from the negative control (DNA of a ram without PTB). Tracks 2 and 4: DNA samples with negative response to MAP.

per mL of processed sample to guarantee their growth. For their part, Khol *et al.* (2010) also recorded negative growth results, based on semen samples from PTB-infected cattle, possibly due to the loss of MAP viability during the sample decontamination.

CONCLUSIONS

The presence of *Mycobacterium avium* subsp. *paratuberculosis* DNA was detected with the nested PCR technique in semen from Pelibuey rams in the PTB subclinical stage. Nevertheless, based on the lack of growth of the culture, the MAP from the said samples was shown to be unviable. Therefore, a longitudinal study is recommended to determine if the excretion of MAP through the semen is intermittent and to establish the possibility of its viability.

ACKNOWLEDGMENTS

To Mr. Marco Antonio Santillán Flores (MSc) for the technical training he provided at the Tuberculosis Lab of CENID-Microbiología Animal of INIFAP. The team would also like to thank the Generation and/or

Knowledge Implementation Guidelines (LGAC) of the Colegio de Postgraduados: Sustainable Management of Natural Resources (Campus SLP) and Efficient Cattle Raising, Sustainable Welfare, and Climate Change (PREGEP-Ganadería, Campus Montecillo).

Ethical Approval

The experiment followed the technical specifications of the NOM-062-ZOO-1999 Official Mexican Standard (SAGARPA, 1999) for the production, care, and use of laboratory animals, in accordance with guidelines for the use and care of research animals, approved by the General Academic Council of the Colegio de Postgraduados (ColPos, 2016).

REFERENCES

Ayele, W. Y., Bartos, M., Svastova, P., & Pavlik, I. (2004). Distribution of *Mycobacterium avium* subsp. *paratuberculosis* in organs of naturally infected bull-calves and breeding bulls. *Veterinary Microbiology, 103*(3-4), 209-217. https://doi.org/10.1016/j.vetmic.2004.07.011

Ayele, W. Y., Macháčková, M., & Pavlík, I. (2001). The transmission and impact of paratuberculosis infection in domestic and wild ruminants. In *Vet. Med.* – Czech. (Vol. 46, Issues 7-8, pp. 205-224). https://doi.org/10.17221/7878-VETMED

Buergelt, C. D., Donovan, G. A., & Williams, J. E. (2004). Identification of *Mycobacterium avium* subspecies *paratuberculosis* by Polymeerase Chain Reaction in Blood and Semen of a Bull with Clinical Paratuberculosis. *International Journal of Applied Research in Veterinary Medicine, 2*(2), 130-134.

Colpos. (2016). Reglamento para el uso y cuidado de animales destinados a la investigación en el Colegio de Postgraduados. 1-16. http://www.colpos.mx/wb_pdf/norma_interna/REG_USO_CUIDADODEANIMALES.pdf

Eppleston, J., & Whittington, R. (2001). Isolation of *Mycobacterium avium* subsp *paratuberculosis* from the semen of rams with clinical Johne's disease. *Australian Veterinary Journal, 79*(11), 776-777. https://doi.org/10.1111/j.1751-0813.2001.tb10898.x.

Erume, J., Spergser, J., & Rosengarten, R. (2001). Rapid detection of *Mycobacterium avium* subsp. *paratuberculosis* from cattle and zoo animals by Nested PCR. *African Health Sciences, 1*(12), 83-89.

Feola, R. P., Collins, M. T., & Czuprynski, C. J. (1999). Hormonal modulation of phagocytosis and intracellular growth of *Mycobacterium avium* subsp. *paratuberculosis* in bovine peripheral blood monocytes. *Microbial Pathogenesis, 26*(1), 1-11. https://doi.org/10.1006/mpat.1998.0246

García E. (2004). Modificaciones al Sistema de Clasificación Climática de Köppen (5th ed.). Instituto de Geografía - Universidad Nacional Autónoma de México. https://www.academia.edu/12911044/Modificaciones_al_sistema_de_clasificación_climática_de_Köppen_para_adaptarlo_a_las_condiciones_de_la_República_Mexicana_2004_Enriqueta_García

Guerrero-Cárdenas, I., Alvarez-Cárdenas, S., Gallina, S., Corcuera, P., Romero-Figueroa, G., Lozano-Cavazos, E. A., Tovar-Zamora, I., & Guerrero-Tovar, I. Y. (2020). Estimación de cambios temporales de la body condition del borrego cimarrón (*Ovis canadensis* weemsi) a partir de fotointerpretación. *ACTA ZOOLÓGICA MEXICANA (N.S.), 8445*(36), 1-14. https://doi.org/10.21829/azm.2020.3612235

Guzmán-Ruiz, C. C., Santillán-Flores, M. A., & Córdova-López, D. (2016). Prevalence and possible risk factors for caprine paratuberculosis in intensive dairy production units in Guanajuato, México. *Veterinary Medicine and Animal Health, 8*(11), 156-162. https://doi.org/10.5897/JVMAH2016.0497

Idris, S. M., Eltom, K. H., Okuni, J. B., Ojok, L., Elmagzoub, W. A., Wahed, A. A. El, Eltayeb, E., & Gameel, A. A. (2022). Paratuberculosis: The hidden killer of small ruminants. *Animals* 12(1). https://doi.org/10.3390/ani12010012

Jaimes, N. G., Santillán, M. A., Hernández Cruz, O. A., Córdova López, D., Guzmán Ruiz, C. C., Reynoso, B. A., Aparicio, E. D., Tenorio Gutiérrez, V. R., & Cuéllar, A. (2008). Detección de *Mycobacterium avium* subespecie *paratuberculosis*, por medio de PCR-anidada a partir de muestras de heces de ovinos. *Vet. México, 39*(01), 377-386.

Khol, J. L., Kralik, P., Slana, I., Beran, V., Aurich, C., Baumgartner, W., & Pavlík, I. (2010). Consecutive Excretion of *Mycobacterium avium* Subspecies *paratuberculosis* in Semen of a Breeding Bull Compared to the Distribution in Feces, Tissue and Blood by IS900 and F57 Quantitative Real-Time PCR and Culture Examinations. *Journal of Veterinary Medical Science, 72*(10), 1283-1288. https://doi.org/10.1292/jvms.10-0052

Kostoulas, P., Leontides, L., Billinis, C., Amiridis, G. S., & Florou, M. (2006). The association of sub-clinical paratuberculosis with the fertility of Greek dairy ewes and goats varies with parity. *Preventive Veterinary Medicine, 74*(2-3), 226-238. https://doi.org/10.1016/j.prevetmed.2005.12.001

Manning, E. J., & Collins, M. T. (2001). *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. *Revue Scientifique et Technique (International Office of Epizootics), 20*(1), 133-150. http://europepmc.org/abstract/med/11288509

Martínez-Covarrubias, A. G., Santillán-Flores, M. A., Ruiz-Guzmán, C. C., Favila-Humara, L. del C., Córdova-López, D., Efrén-Díaz, A., Hernández-Andrade, L., & Blanco-Ochoa, M. Á. (2012). Desarrollo de un inmuno-ensayo enzimático (ELISA) para el diagnóstico de paratuberculosis en bovinos. *Revista Mexicana de Ciencias Pecuarias, 3*(1), 1-18.

Münster, P., Völkel, I., Wemheuer, W., Schwarz, D., Döring, S., & Czerny, C. P. (2013). A Longitudinal Study to Characterize the Distribution Patterns of *Mycobacterium avium* ssp. *paratuberculosis* in Semen, Blood and Faeces of a Naturally Infected Bull by IS 900 Semi-Nested and Quantitative Real-Time PCR. *Transboundary and Emerging Diseases, 60*(2), 175-187. https://doi.org/10.1111/j.1865-1682.2012.01336.x

Pritchard, T. C., Coffey, M. P., Bond, K. S., Hutchings, M. R., & Wall, E. (2017). Phenotypic effects of subclinical paratuberculosis (Johne's disease) in dairy cattle. *Journal of Dairy Science, 100*(1), 679-690. https://doi.org/10.3168/jds.2016-11323

Russel, A. J. F., Doney, J. M., & Gunn, R. G. (1969). Subjective assessment of body fat in live sheep. *The Journal of Agricultural Science, 72*(3), 451-454. https://doi.org/10.1017/S0021859600024874

SAGARPA. (1999). Norma Oficial Mexicana (NOM-062-ZOO-1999), especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. http://sagarpa.gob.mx

Sharifzadeh, A., Doosti, A., Fazeli, M. H., & Adavoudi, I. (2010). Nested PCR on semen samples for the detection of *Mycobacterium avium* subsp *paratuberculosis*. *African Journal of Microbiology Research, 4*(24), 2787-2789. https://www.researchgate.net/profile/Abbas-Doosti/publication/264860175_Nested_PCR_on_semen_samples_for_the_detection_of_Mycobacterium_avium_subsp_paratuberculosis/links/53f382450cf2155be34e3f59/Nested-PCR-on-semen-samples-for-the-detection-of-Mycobacteri

Velázquez-Morales, J. V., Santillán-Flores, M. A., Navarro-Maldonado, M. C., Salazar-Ortiz, J., Ambríz-García, D. A., Palacio-Núnez, J., Ponce-Covarrubias, J. L., & Cortez-Romero, C. (2022). Reproductive and seminal characteristics of Pelibuey rams infected with *Mycobacterium avium* subsp. *paratuberculosis* in the subclinical stage. *Agro Productividad, 15*(12), 3-10. https://doi.org/10.32854/agrop.v15i12.2270

Velázquez-Morales, J. V., Santillán-Flores, M. A., Gallegos-Sánchez, J., Cuca-García, J. M., Navarro-Maldonado, M. del C., Rojas-Martínez, R. I., & Cortez-Romero, C. (2019). Detection of *Mycobacterium avium* subsp. *paratuberculosis* in reproductive tissue and semen of naturally infected rams. *Animal Reproduction, 16*(4), 930-937. https://doi.org/10.21451/1984-3143-AR2018-0147

Whittington, R., Donat, K., Weber, M. F., Kelton, D., Nielsen, S. S., Eisenberg, S., Arrigoni, N., Juste, R., Sáez, J. L., Dhand, N., Santi, A., Michel, A., Barkema, H., Kralik, P., Kostoulas, P., Citer, L., Griffin, F., Barwell, R., Moreira, M. A. S., … De Waard, J. H. (2019). Control of paratuberculosis: Who, why and how. A review of 48 countries. *BMC Veterinary Research, 15*(1). https://doi.org/10.1186/S12917-019-1943-4

Whittington, R. J., & Windsor, P. A. (2009). In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis. *Veterinary Journal, 179*(1), 60-69. https://doi.org/10.1016/j.tvjl.2007.08.023

Williams, M. A., Stellflug, J. N., & Lewis, G. S. (2001). Technical note: Artificial vagina vs a vaginal collection vial for collecting semen from rams. *Journal of Animal Science*, 79, 2964-2967.