Reception: **August 23, 2021** | Acceptance: **March 10, 2022**| Publication: **October 1, 2022**

Epidemiological situation of bovine paratuberculosis in three economic regions of the state of Chiapas-Mexico

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To quote this article:

Méndez Morales, E. G., León Velasco, H., Gutiérrez Hernández, J. L., Díaz Aparicio, E., & León Velasco, O. (2022). Situación epidemiológica de la paratuberculosis bovina en tres regiones económicas del estado de Chiapas-México. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a01

— Abstract—

The objective of this study was to know the epidemiological situation of bovine paratuberculosis and determine the management practices that favor the presence of this disease in three economic regions of major livestock importance in the state of Chiapas. An n = 460 animals from 92 livestock production units (LU) was obtained. Samples were taken from five females and one stallion older than two years by LU. The serological diagnosis was made with a commercial ELISA IDEXX paratuberculosis Screening kit. In addition, a questionnaire was applied to the producers to detect some possible management practices in the transmission of the disease. The Relative Risk (RR) was calculated with a 95% confidence interval (CI). Based on the analysis of the management practices of this study, a RR of 0.19 to 0.30 was found for these factors ($P < 0.05$); this implies there is a significant probability of manifesting the disease if these factors are present in the livestock production unit. The seroprevalence was calculated with the Epi Info 7. ® program, and the association between variables by the odds ratio (RR) with the Win Episcope Ver. 2.0® program. General seroprevalence was 15% (95% CI: 12.03 - 18.55%) for the Frailesca region was 12% (95% CI: 7.58 - 17.76%), while in the Istmo-Costa region, it was 17.89% (95% CI 12.72 - 24.1%) and in the Zoque Valley of 14.74% (95% CI: 8.3 - 23.49%). Poor management practices were identified such as dirty drinkers and feeders, pens with dirt floors, cleaning pens, age at weaning of calves, and stallion management. It is concluded that bovine paratuberculosis is present in cattle in the state of Chiapas and it is advisable to establish strategies that favor its control and prevention, such as the detection and segregation of positive animals, as well as the reduction of factors that allow the transmission in livestock farming.

Keywords:

Test diagnostic; Paratuberculosis; Disease.

Bovine paratuberculosis (PTB) is a chronic infectious disease caused by *Mycobacterium avium subspecies paratuberculosis* (MAP). The main organ affected by the disease is the small intestine, which causes enteritis that generates clinical manifestations such as progressive thinning, diarrhea, and, finally, death in affected animals. It is a disease of worldwide distribution and economic importance, with a prevalence ranging from 5 to 30%. Young animals (less than six months of age) are infected by the ingestion of bacteria, mainly through food, water, and nipples contaminated with feces (Castellanos, *et al*., 2010).

PTB disease may be evident after a stress period, such as childbirth or lactation. Economic losses from this disease are mainly due to premature disposal and replacement cost, decrease in milk production, low feed efficiency, infertility, decreased carcass quality, and susceptibility to other diseases.

One report estimated that the prevalence of PTB in Latin America and the Caribbean at the herd level ranges from 17 to 76% (Correa, *et al*., 2015). In Mexico, PTB in cattle is not monitored regularly, so the information about its prevalence is almost zero. In Chiapas, the study and information on PTB are of utmost importance because they are the livestock regions with the highest productivity, ignorance of this disease generates uncertainty about the sanitary status of the cattle herds of the state of Chiapas, due to the lack of productive, reproductive, and sanitary records. Undoubtedly, a disease such as paratuberculosis, with its chronic nature, could impact the herds of the state. Therefore, the objective of this study is to know the epidemiological situation of bovine paratuberculosis and determine the management practices that favor the presence of this disease, in three economic regions of the state of Chiapas.

MATERIALS AND METHODS

Study area

The state of Chiapas is in southeastern Mexico, bordered to the north by the state of Tabasco, to the west by Veracruz and Oaxaca, to the south by the Pacific Ocean, and the east by the Republic of Guatemala. North 17°59', south 14°32' from the north latitude; east 90°22', west 94°14' from the west longitude (INEGI, 2013).

The research was carried out in three economic regions of the state of Chiapas: Frailesca, Istmo-Costa, and Valle Zoque, comprising the municipalities of Villaflores, Villacorzo, La Concordia, Arriaga, Tonalá, Pijijiapan, Mapastepec, Jiquipilas, Cintalapa and Ocozocoautla (Figure 1).

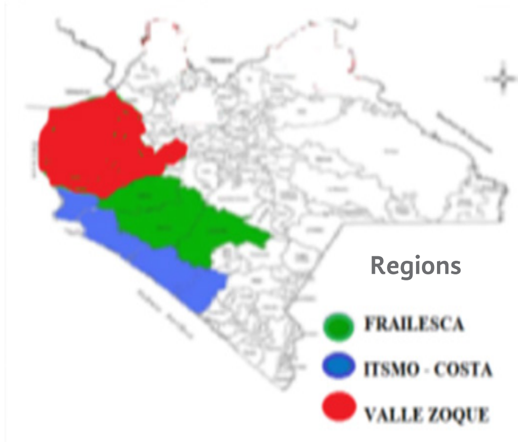

Figure 1. Main cattle regions of the state of Chiapas. Source: Own elaboration

Sample size determination

The minimum sample size was determined with the following formula (Pérez-Rivero, *et al*., 2017):

$$n = \frac{\frac{Z^2}{E^2}\,\frac{q}{p}}{1 + \frac{1}{N}\left[\frac{Z^2}{E^2}\frac{q}{p} - 1\right]}$$

Where:
Z = 1.96
E = 10 %
p = 80 %
q = 20 % (1-p)
N = 14680
n = 92

A proportion (p) of paratuberculosis of 80% was considered with a confidence (z^2) of 95% and an accuracy of 10%, the study universe (N) was 14,680 primary producers of the Local Livestock Association of each of the municipalities studied in the state of Chiapas. Resulting in 92 producers for the study, with a total of 460 samples (Table 1).

Table 1
Distribution of livestock production units and animals sampled by economic region

Regions	Municipality	LU*	Male	Female
Frailesca	Villaflores	10	10	40
	Villa Corzo	14	14	56
	La Concordia	11	11	44
Istmo- Costa	Arriaga	7	7	28
	Pijijiapan	11	11	44
	Tonalá	13	13	52
	Mapastepec	7	7	28
Valle Zoque	Cintalapa	6	6	24
	Jiquipilas	5	5	20
	Ocozocoautla	8	8	32
Total		**92**	**92**	**368**

*Livestock Units (LU)

Source: Own elaboration

The inclusion-exclusion criteria that were considered for the producer to participate in the study were as follows:

- **Included**
  Adult animals (over six months of age), bellies, and active stallions of each productive unit (LU).
  Active stallions (older than six months) that were owned or lent by another producer.
  Producers belonging to the Local Livestock Association of each municipality.

- **Excluded**
  Animals of producers who do not belong to the Local Livestock Association of the municipality.

Serological sampling

The selection of the ten municipalities was made based on their livestock activity within the state, then, a database of the producers of each region was used, who were invited to participate in the study, and finally, the selection of the animals was made for convenience, following the inclusion and exclusion criteria.

Samples were taken for serological study by puncture of the coccygeal vein. The sample was taken in vacutainer tubes with serum separation gel (7ml). Blood samples were stored in refrigeration at 4°C. Once the samples were obtained, they were centrifuged at 3000 rpm for 3 min. Subsequently, the serum was taken in aliquots of 2-3 ml, which were frozen and kept at -20° C until processing.

Serological diagnosis

The detection of antibodies contained in blood serum against MAP was performed in the Laboratorio de Pequeños Rumiantes CENID-Microbiología, INIFAP, where the commercial ELISA technique IDEXX Paratuberculosis Screening (IDEXX, 2017) was used, which can detect antibodies against MAP in serum and milk from cattle, with a sensitivity of 60-80% and a specificity of 90-99% (IDEXX, 2017). This way, it will be ensured that when an animal is seropositive to a specific antigen, this response is due to field agent exposure.

The microplates are upholstered with MAP antigen. The analyzed samples were first diluted and incubated with *Mycobacterium phlei* to neutralize possible cross-reactions with any mycobacteria in the microplate wells by predilution. After incubation with *Mycobacterium phlei*, samples are deposited on the upholstered microplate. Any antibody present in the specific sample against MAP forms an antigen-antibody complex on the surface of the well. After washing, an anti-ruminant antibody attached to an enzyme is incubated in the wells. The conjugate binds to antigen-antibody complexes. After another wash, the substrate enzyme (TMB) is added to the wells. In the presence of the enzyme, the substrate oxidizes generating a blue coloration, which turns yellow when the braking solution is added. The color intensity is proportional to the concentration of specific anti-MAP antibodies present in the sample. The result is obtained by comparing the optical density (DO) of the sample with the mean of the positive control.

Epidemiological questionnaires

For the descriptive study, a survey of 15 questions was carried out simultaneously with serological sampling in each bovine production unit with questions related to sanitary problems, zootechnical management, and productive characteristics. It is important to mention that the sampling and survey were carried out by Veterinarian Zootechnicians who knew the region and the animals management extensively, and were previously trained to avoid bias in the research.

Statistical analysis

A database was developed with the spreadsheet program Microsoft Excel 2016, and data analysis with the Epi info™ version 7 program, calculating the frequencies and, where appropriate, the respective 95% confidence interval (95% CI).

To assess the strength with which the disease is associated with a certain factor (which is presumed to be its cause), relative risk (RR) was used as an indicator. Likewise, to evaluate statistical significance, the Chi-Square test was used to determine whether the proportions of the disease were homogeneous (Jaramillo & Martínez, 2010).

RESULTS AND DISCUSSION

Serological outcomes in the three economic regions: Frailesca, Istmo – Costa and Valle Zoque

Based on the results of the sampling of 460 cattle from 92 livestock production units (LU), a frequency of seropositivity of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP) of 15% (69/460) was obtained, while 85% (391/460) were negative for this disease (Table 2). In this sense, Ocepek, *et al*. (2002), who obtained a prevalence of 0.59%, analyzed serum from cattle between 6 and 24 months of age, through the indirect ELISA technique. For their part, Guamán (2017), determined the prevalence of the disease per animal at 1.72% (87/5074) in cattle between 12 and 24 months of age in four regions of Ecuador. However, Crossley, *et al*. (2005) point out that larger herds would have a higher prevalence due to population density and therefore greater environmental pollution with MAP which would promote exposure to the agent at an early age. This is consistent with Seville (2007) who cites that paratuberculosis is a disease of global distribution with variability in prevalence data in cattle.

Although in the present study the proportion found of seropositive animals is low, comparing it with seronegative animals, it does not mean that the disease is of little importance in the herd, since it must be considered that the clinical disease in addition to presenting in animals older than 24 months, there may also be young animals that carry MAP without presenting a sign that helps differentiate them from healthy animals.

Table 2

General serological MAP diagnosis using ELISE IDEXX Screening paratuberculosis

Diagnostic	Frequency	(%)	IC 95%
Negative	391	85.00	81.45- 87.97
Positive	69	15.00	12.03 - 18.55
Total	**460**	**100.00%**	

CI= Confidence Interval

Source: Own elaboration

According to the serological results by economic regions, in the Frailesca region, 175 animals were sampled; there were 154 negative cases corresponding to 88% and 21 positives corresponding to 12%. While the Istmo-Costa region sampled 190 cattle, of which 156 were negative (82.1%) and 34 cattle as positive, which corresponds to 17.9%. Finally, for the Valle Zoque region, 95 cattle were sampled, of which 85.3 % (81/95) were negative and 14.7% (14/85) were positive (Table 3). For its part, Guamán (2017), determined the frequency of four regions of Ecuador, where the highest number of positive animals was in the Costa region with 2.05% (49/2391), followed by the Sierra region with 1.64% (28/1703), the Insular region with 1.32% (2/132) and finally the East with 1.00% (8/801), concluding that the regions with the largest bovine inventory manifest more presence of antibodies to this disease. Similarly, Milian, *et al.* (2015) mention that the disease presents with a high prevalence in areas of the country that covered a higher bovine population density per km^2 of territorial extension. In this sense, the largest number of animals is found in the Istmo-Costa region, which is characterized by being one of the regions with the largest livestock inventory in the state of Chiapas as reported by the Servicio de Información Agroalimentaria y Pesquera (SIAP, 2017); the Istmo–Costa region (Arriaga, Tonalá, Pijijiapan, and Mapastepec) has 304,429 heads of cattle, unlike the Frailesca region (Villaflores, Villa Corzo and La Concordia) which has a total of 276,600 heads, and the Valle Zoque region (Ocozocoautla, Jiquipilas, and Cintalapa) has an inventory of 73,232 heads of cattle.

Table 3
Serological MAP diagnosis per region using ELISA IDEXX Screening paratuberculosis

Regions	Diagnosis	Frequency	%	CI 95%
Frailesca	Negative	154	88	82.24 - 92.42
	Positive	21	12	7.58 - 17.76
	Total	175	100%	
Istmo - Costa	Negative	156	82.11	75.9 - 87.28
	Positive	34	17.89	12.72 - 24.1
	Total	190	100%	
Valle Zoque	Negative	81	85.26	76.51 - 91.7
	Positive	14	14.74	8.3 - 23.49
	Total	95	100%	

CI= Confidence Interval

Source: Own elaboration

As for the results per livestock production unit (LU), 40 positive and 52 negative were found, which corresponds to 43.48 and 56.52% respectively. A positive LSU was considered when there was at least one positive animal (Figure 2). For its part, Guamán (2017) obtained a prevalence of LU disease of 9.5% (68/716). However, when comparing the results of the present study with those studies that report a result of seroprevalence by the herd, the prevalence result of the present study is higher than that observed by Waldner, *et al*. (2002), who found within-herd prevalence between 2 and 12.8% while Roussel (2011), observed within-herd prevalence between 2 and 12%. Another study by Roussel, *et al*. (2007) detected within-herd prevalence in most of the LU analyzed between 23 and 75%. Concerning the prevalence per herd found in the present study and in others that used ELISA as a diagnostic test, a variability in prevalence can be observed, perhaps due to the different values of sensitivity and specificity that ELISA kits have. It is also important to mention that, due to the characteristics of the state, the results obtained only show a proportion of individuals who present the disease in a certain place and time.

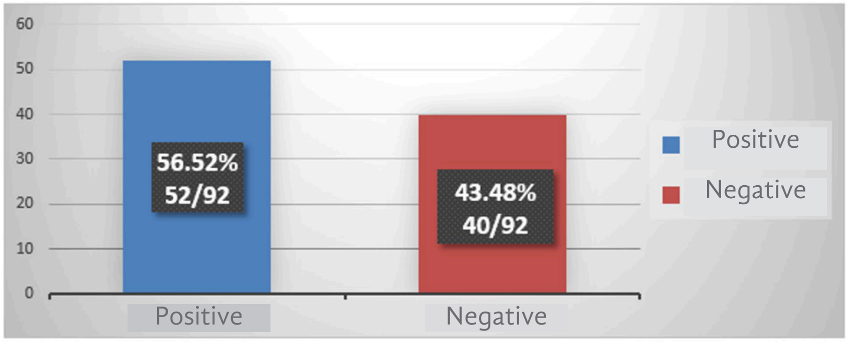

Figure 2. Results at a productive unit level against MAP. Source: Own elaboration

Regarding the results by sex per animal, 368 cows were sampled, of which 55 were positive (14.9%) and 313 negative (85.1%) for this disease. While of the 92 males sampled, 14 were positive (15.2%), and 78 were negative (84.8%) (Figure 3). Regarding the sex of the animals, a greater number of positive female animals was observed than males. These data agree with the studies conducted by Fadhel, *et al.* (2010) and Vélez, *et al.* (2016) who observed a higher percentage of seropositivity in females. The authors indicate that the difference between females and males could be due to a smaller number of males sampled. It should be noted that the system with the highest proportion in tropical regions is the dual-purpose system with 45% of the national bovine inventory and according to its production characteristics this system has two fundamental objectives: milk production and meat rearing calves at weaning, which are sold at 6-8 months of age and the disposal of cattle for the supply of meat, therefore has a higher number of bellies than stallions (González, 2018).

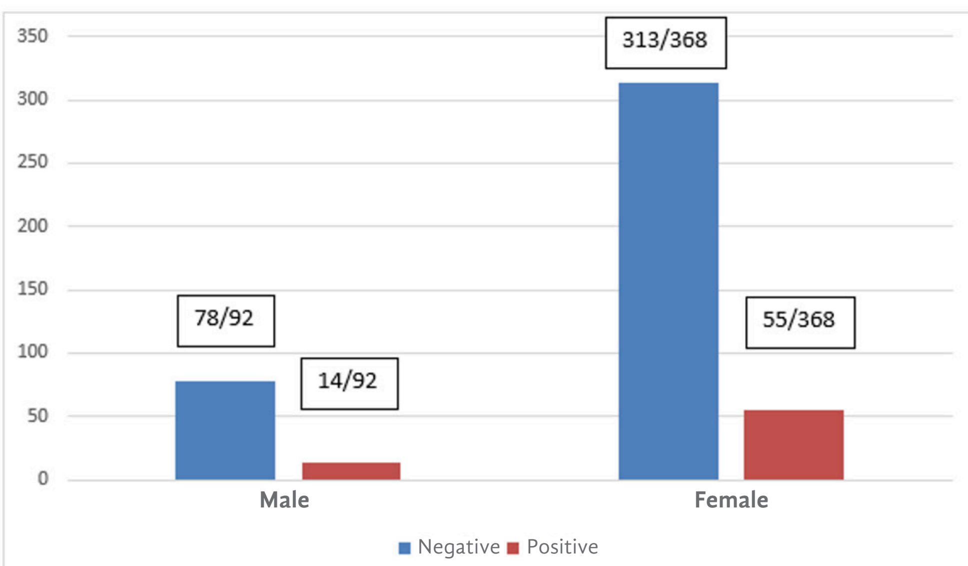

Figure 3. Paratuberculosis diagnosis results by sex. Source: Own elaboration

The ELISA technique (IDEXX, 2017), which was used in the present work, reports a sensitivity of 60-80% and a specificity of 90-99% by the manufacturer, these data coincide with the work of Martínez, *et al.* (2012), who obtained a sensitivity of 79.31% and a specificity of 82.25% in the standardization and development of an ELISA with protoplasmic antigen obtained from the MAP 3065 strain. ELISA tests are less expensive and can be applied to blood or milk samples. The ability to perform ELISA testing on collective milk samples has led to the availability of large numbers of test records in the dairy data registry.

Tobergte and Curtis (2013) mention that the indirect ELISA technique is considered the gold standard for determining the prevalence of the disease, due to the sensitivity and specificity to detect serum antibodies against the agent. However, Sánchez, *et al.* (2009) mention that this is considered a screening technique and is useful for the establishment of surveillance and control measures within the affected herds.

Therefore, it should be considered that a diagnostic test that is intended to be used as the gold standard, should have a high sensitivity, which is the ability of a test to detect a true positive case, if this test has low sensitivity, it will yield a false positive more frequently. Therefore, the ELISA-IDEXX 2017 test, which was used in the present work, should be used as a surveillance and control test for herds, because it has a sensitivity of 60-80%.

Survey results

Based on the data obtained from the 92 production units surveyed, from the three economic regions, Frailesca, Istmo-Costa, and Valle Zoque, it was observed that the main zootechnical purpose of the LU is the dual-purpose system: milk and meat production representing 92.4% (85/92), while 7.6% of producers are engaged in milk production. In addition, the production system identified in all the LU surveyed was semi-intensive with food supplementation and a basic preventive medicine program. It was also identified that 100% of the production units use water from wells for feeding livestock, cleaning facilities, and equipment. In this sense, Vilaboa, *et al.* (2009) in the state of Veracruz indicate that the dual-purpose system represents more than 77% of the production units studied. Similarly, González (2012) found that in the exploitation system in the Istmo-Costa region in Chiapas, 88% of cattle were of dual purpose. As a result, there is ample evidence that the dual-purpose cattle farming system usually predominates in the tropics.

According to the cattle breeds predominant in the animals, it was observed that the cattle Swiss-Brahman is the most predominant, followed by Brahman, Holstein, Gyr-Holstein, and in smaller numbers, cows of the Swiss American breed (Table 4). Regarding the breed of cattle, Benavides, *et al*. (2016) found that in dairy herds, the Jersey breed presented higher seropositivity; however, the Holstein breed was the one with a greater proportion from the study population, finding no correlation of the sick animals with the breed variable. On the other hand, Vir Singh, *et al*. (2013) mention that breed differences play a fundamental role in the genetics of disease resistance. In this regard, it is important to mention that racial susceptibility to the disease can be confused with the popularity of the breed. On the other hand, the selection of a breed with greater susceptibility to diseases in the tropics can be a fundamental tool for the control of paratuberculosis in the state.

Table 4

Bovine breeds distribution by economic region

Regions	Breed	Frequency	(%)	CI 95%
Frailesca	Brahman	7	36.84	16.29 - 61.64
	Gyr-Holstein	1	5.26	0.13 - 26.03
	Swiss	1	5.26	0.13 - 26.03
	Swiss-Brahman	10	52.63	28.86 - 75.5
	TOTAL	19	100%	
Istmo - Costa	Brahman	17	50.00	32.43 - 67.57
	Gyr-Holstein	1	2.94	0.07 - 15.33
	Swiss	3	8.82	1.86 - 23.68
	Swiss-Brahman	13	38.2	22.17 - 56.44
	TOTAL	34	100%	
Valle Zoque	Brahman	5	38.46	13.86 - 68.42
	Swiss-Brahman	8	61.54	31.58 - 86.14
	TOTAL	13	100%	

CI= Confidence Interval

Source: Own elaboration

One of the important features for MAP transmission to susceptible animals is the accumulation of feces on the floor of the handling pen. In this sense, it was observed that in most of the management pens, the floor was 83.7% soil (77/92) and only 16.3% (15/92) concrete (firm floor).

In the Frailesca region, 20% of LU were found with concrete floor pens and 80% with soil. For the Istmo-Coast region, 10.5% of pens with concrete floors were identified, and the remaining percentage corresponds to soil. Finally, for the Valle Zoque region, 5.3% of concrete pens and 94.7% of soil pens were identified. As can be seen, the floor that has a greater frequency among the three economic regions corresponds to soil (Table 5).

Table 5

Types of handling pens in production units in the state of Chiapas

Regions	Concrete or Soil Pens	Frequency	(%)	CI 95%
Frailesca	Concrete	7	20	8.44 - 36.94
	Soil	28	80	63.06 - 91.56
	TOTAL	35	100 %	
Itsmo - Costa	Concrete	4	10.53	2.94 - 24.8
	Soil	34	89.47	75.2 - 97.06
	TOTAL	38	100 %	
Valle Zoque	Concrete	1	5.2	0.13 - 26.03
	Soil	18	94.74	73.97 - 99.87
	TOTAL	19	100 %	

CI= Confidence Interval

Source: Own elaboration

Another important item to mention is the cleaning and disinfection of pens, where 85.7% (30/35) of the producers of the Frailesca region do not clean the pens and 14.3% (5/35) do clean the waste of the animals. For the Istmo-Coast region, 100% (38/38) do not clean the pens. Finally, for the Valle Zoque region, 73.7% (14/19) do not resort to cleaning their pens, and 26.3% (5/19) clean the handling pens (Table 6).

Table 6

Pens cleaning in the three regions of the state of Chiapas

Regions	Pen Cleaning	Frequency	(%)	CI 95%
Frailesca	No	30	85.71	69.74 - 95.19
	Yes	5	14.29	4.81 - 30.26
	TOTAL	35	100%	
Itsmo - Costa	No	38	100	90.75 – 100
	TOTAL	38	100%	
Valle Zoque	No	14	73.68	48.8 - 90.85
	Yes	5	26.32	9.15 - 51.2
	TOTAL	19	100%	

CI= Confidence Interval

Source: Own elaboration

On the other hand, one of the practices that must be done in all production units is the feeders and drinkers cleaning, which is an important factor so that MAP does not spread. In the present study, it was observed that the percentage that do not clean the feeders and drinkers in the Frailesca, Istmo-Costa, and Valle Zoque was 62.8, 63.2 and 52.6%, respectively. Undoubtedly, this is a risk factor for the spread of the disease (see Table 7).

Table 7
Feeders and drinkers cleaning

Regions	Feeders and Drinkers Cleaning	Frequency	(%)	CI 95%
Frailesca	No	22	62.86	44.92 - 78.53
	Yes	13	37.14	21.47 - 55.08
	TOTAL	35	100 %	
Itsmo - Costa	No	24	63.16	45.99 - 78.19
	Yes	14	36.84	21.81 - 54.01
	TOTAL	38	100 %	
Valle Zoque	No	10	52.63	28.86 - 75.55
	Yes	9	47.37	24.45 - 71.14
	TOTAL	19	100 %	

CI= Confidence Interval

Source: Own elaboration

In this sense, it has been described that MAP transmission has as its main route the fecal-oral route (Yayo, *et al.*, 2001), so the sanitary conditions that are in a productive unit are of great interest for the disease to be established or not. Therefore, the results shown about the sanitary management of the pens (cleaning in pens and the type of floor) in this study, are important, because MAP can be present in the drinkers and feeders that are not given adequate cleaning, not to mention that the pens, by having a soil floor, are more at risk, since MAP tends to stay longer on the ground due to humidity and temperature conditions. Therefore, MAP can persist in soil and water contaminated with the feces of infected animals. Some studies mention that this mycobacterium can survive in the feces of infected cattle for 152-246 days and that the survival period of MAP depends on environmental conditions such as freezing, drought, exposure to sunlight, changes in environmental temperature, and rainfall. On the other hand, Whittington, *et al.* (2000) have described that MAP can survive up to 280 days in puddles. So, it can remain viable in the environment for several months, which is undoubtedly a determining factor for the spread of infection.

Another important point is the number of viable bacilli eliminated in the feces of infected animals. Whitlock, *et al.* (2000) mention that the eliminated dose of MAP is 10^6–10^8 CFU / g, and it has been established that the infective dose is approximately 10^3 bacilli. According to this, minimal fecal contamination of the environment is sufficient to produce infection of susceptible animals.

Regarding the epidemiology of the disease, Greig, *et al.* (1999), have pointed out that some of these wild hosts, such as rabbits, could play an important role in the transmission of infection to domestic ruminants in some regions since they can release millions of MAP/hectares into their feces and domestic ruminants become infected when grazing or eating food contaminated with MAP. In addition, another possible route of infection that has been considered is predation in the case of carnivores. Greig, *et al.* (1997 and 1999) describe that the percentage of MAP isolation in predators is 62%, compared to 10% isolation of M. avium paratuberculosis in prey. Also, Stevenson, *et al.* (2009) carried out through molecular techniques the identification of the same MAP genotypes between the different wild and domestic hosts that lived in the same habitat, supporting the theory of interspecies transmission. This means that wild animals play an important role in the epidemiology of bovine paratuberculosis.

Regarding the above, it can be said that the construction of a pen with a concrete floor is of higher cost for producers, so they choose to stick to soil. However, the socioeconomic level of a LU should not justify little or no cleaning and disinfection of a pen with soil or concrete floor, as well as drinking troughs and feeders, since the frequent route of transmission for MAP in bovine animals is fecal-oral.

Regarding the internal deworming of animals, it was observed that 100% of producers deworm all their animals (Table 8). Proper deworming consists of two applications per year, i.e., every six months at least. Since the producers' conditions and economy vary every six months and every year, it was found that 71.4% (25/35) of the Frailesca region internally deworms animals each year and 28.6% (10/35) every six months. For the Istmo-Costa region, it was observed that 76.32% (29/38) do so every six months and 23.7% (9/38) deworm every year. Finally, in the Valle Zoque region, twelve producers (63.2%) deworm their animals every six months, and seven producers (36.8%) deworm them every year. It is important to mention that one of the common prophylactic practices that are done in any bovine production unit is internal deworming. However, the clinical signs of PTB are very similar to those of parasitosis, so the producer or veterinarian can give an erroneous treatment without first having the proper diagnosis.

Table 8

Deworming of animals in the three regions of the state of Chiapas

Regions	Deworming	Frequency	(%)	CI 95%
Frailesca, Itsmo - Costa y Valle Zoque	Yes	92	100	90 – 100
How Often they Deworm Them		**Frequency**	**(%)**	**CI 95%**
Frailesca	Every Year	25	71.43	53.7 - 85.36
	Every Six Months	10	28.57	14.64 - 46.3
	TOTAL	35	100%	
Itsmo - Costa	Every Year	9	23.68	11.44 - 40.24
	Every Six Months	29	76.32	59.76 - 88.56
	TOTAL	38	100%	
Valle Zoque	Every Year	7	36.84	16.29 - 61.64
	Every Six Months	12	63.16	38.36 - 83.71
	TOTAL	19	100%	

CI= Confidence Interval

Source: Own elaboration

Characteristics related to the presence of MAP in production units and its possible transmission

A series of questions were asked to livestock producers to know the presence of signs suggestive of MAP within their production units. Regarding signs in cattle, 16.7% of producers reported having had problems with submandibular swelling and decay in adult cows, and 56.5% of animals suffering from diarrhea that is treated with deworming or antibiotics and do not recover within their LU; 36.8% of producers reported having had progressive weight loss in sick cattle and 6.5% of producers had cows with hirsute hair and weakness (Table 9).

In this sense, Martinez, *et al.* (2012) describe that the signs observed are diarrhea at first intermittent and later permanent, loss of body condition, although appetite is maintained, decreased milk production, and submandibular and ventral edema caused by hypoproteinemia. Likewise, Whitlock and Buergelt (1996) mention that the clinical signs of the disease are not very evident, sometimes variable, and are mainly associated with the different stages of the disease. In this case, the clinical signs that the producers mentioned having presented in this investigation correspond to an advanced clinical phase. In this phase, the animals are slaughtered due to the reduction in milk production and loss of live weight, although sometimes some animals die from severe dehydration and cachexia. It is estimated that for each clinical case, there are 25 subclinical cases; hence the importance of having diagnostic tests that can detect infected animals in time to avoid economic losses within the LU.

Table 9

Presence of clinical signs in herds observed by producers within livestock production units

Cattle with submandibular swelling and sadness	Frequency	(%)
Submandibular Swelling and Sadness	(11/92)	16.68
Nothing	(81/92)	83.33
Cattle suffering from diarrhea, treated with deworming or antibiotics and do not recover		
Treated diarrhea	(52/92)	56.52
Nothing	(40/92)	43.48
Progressive weight loss in sick cattle		
Yes	(37/92)	36.80
No	(55/92)	59.78
Cattle with hirsute hair and weakness		
Yes	(25/92)	27.17
No	(67/92)	72.82

Source: Own elaboration

On the other hand, 84% of producers indicate that the cattle they own were bought and born in their livestock production unit. 43.5% of producers mainly introduce stallions, either purchased or borrowed from neighboring LU, and to a lesser extent (18.5%) replace females. Of the introduced cattle, 13% of producers acquire cattle within the same community, 21.7% from the same municipality, 58.7% from a different municipality, but in the same state (Table 10), and only 6.5% of producers outside the state of Chiapas.

Therefore, the purpose of introducing males and females into a dual-purpose system is done for reproductive purposes, as they are used as replacements for stallions and bellies. This agrees with González (2018), who describes that one of the characteristics of the dual-purpose production system is the rearing of calves at weaning and the disposal of cattle for the supply of meat, some calves are selected for future belly replacements, and very few bovine females are purchased externally. In this case, the future stallion is obtained outside the LU to avoid consanguinity within the herd.

Table 10

Origin of cattle from livestock production units

Characteristics		
Cattle Origin	**Frequency**	**%**
Born at the ranch	(15/92)	16.31
Bought or came from somewhere else	(0/92)	0
Born at the ranch and bought	(77/92)	83.69
Types of introduced cattle		
Stallions	(40/92)	43.48
Females	(17/92)	18.48
Stallions and Females	(35/92)	38.04
Where they bought it or Origin		
Same community	(12/92)	13.04
Same municipality	(20/92)	21.74
Another municipality of the State	(54/92)	58.69
Another State	(06/92)	6.52

Source: Own elaboration

Regarding the disposal of the stallion in productive units, 73.9% of producers declared that the stallion changes every three years, 9.8% every five years, and 16.3% have never changed it. 76% of producers lend or borrow the stallion (Table 11). Old stallions can be a risk factor for many infectious diseases within the herd. One of the ways in which PTB can be transmitted is by borrowing infected stallions used for natural riding, which represents a very common practice within dual-purpose LU, as this animal may be removing the micro bacteria through feces during the time it was lent and also consider the sanitary conditions of the facilities.

Furthermore, Roussel (2011) points out that the handling practices of calves are the biggest difference between beef and dairy cattle when considering transmission and control of paratuberculosis. The calf of milk is separated from the mother within the first 24 h. of birth, while the beef calves stay with mothers from five to six months of age. For his part, González (2018) mentions that the dual-purpose system is by breeding calves at weaning sold at 6-8 months of age. This means that the exposure of calves to adult animal manure is much higher in meat and dual-purpose farms than in milk farms, due to the close coexistence between young and adult calves.

Table 11
Characteristics that favor MAP transmission

Characteristics		
At what age weans calves from mothers	**Frequency**	**(%)**
At seven months	(54/92)	58.69
One year	(27/92)	29.34
They're not separated	(11/92)	11.95
How often they change stallion		
Every three years	(68/92)	73.91
Every five years	(09/92)	9.78
They never change it	(15/92)	16.31
Borrows or lends a stallion		
Yes	(70/92)	76.08
No	(10/92)	10.86
Another municipality of the State	(12/92)	13.04

Source: Own elaboration

Based on the analysis of management practices in this study, a relative risk (RR) of 0.19 to 0.30 was found for these factors ($P<0.05$). This implies that there is a significant probability of disease if these factors are present in the livestock production unit. Therefore, Nuñez, *et al*. (2006) detected that the management practices with the greatest impact were poor micro-environmental conditions, care of newborn and growing calves, handling of pregnant animals, management practices, and manure disposal. In this work, it was detected that the greatest risk factor is the cleaning of dirty drinkers and feeders, soil pens, cleaning of pens, age of weaning of calves, how long it takes to change stallions, and stallion loan. Over all, a source of permanent contagion and 90% of producers clean pens twice a year and 86% of them have soil facilities (Table 12) (Pérez-Rivero, *et al*., 2017). However, Table 13 shows the results by economic regions of the relative risks for MAP disease. In this respect, a relative risk of 0.20, 0.23, and 0.27 was found, while the calculated values of the confidence interval (95%) were 0.13 - 0.30, 0.14 - 0.39 and 0.10 - 0.37 for the regions Frailesca, Valle Zoque and Istmo-Costa respectively.

Table 12
Calculation of relative risk

	Positive	Negative	
Exposed	a	b	a+b
Non-exposed	c	d	c+d
	a+c	b+d	a+b+c+d

$$\text{RELATIVE RISK} = \frac{\text{Incidence of those exposed}}{\text{Incidence of those not exposed}} = \frac{\frac{a}{a+b}}{\frac{c}{c+d}}$$

Source: Own elaboration

Where ***a***= exposed to the risk factor and they became ill during follow-up; ***b***= exposed to the risk factor and did not become ill during follow-up; ***c***= not exposed to the risk factor and became ill during follow-up; ***d***= not exposed to the risk factor and did not become ill during follow-up; ***a***+***c***= total subjects who became ill during follow-up (cases); ***a***+***b***= total subjects exposed to the risk factor; ***c***+***d***= total subjects not exposed to the risk factor.

To evaluate statistical significance, the Chi-Square test was used to determine whether the proportions of the disease are homogeneous. Therefore, the calculated value for Chi-Square in the present study was 33.96, there is a significant difference ($P<0.05$) in the proportion of disease to exposure to the risk factor.

Table 13
Calculation of risk and MAP confidence interval of the three regions

Regions	(RR)	CI=95%	X^2
Frailesca	0.20	0.13 - 0.30	5.67
Valle Zoque	0.23	0.14 - 0.39	1.61
Istmo-Costa	0.27	0.19 - 0.37	10.37

CI= Confidence Interval, X^2 = Chi-square, RR= Relative Risk

Source: Own elaboration

CONCLUSIONS

MAP infection in cattle is confirmed in the three economic regions of the state of Chiapas: Frailesca, Istmo – Costa and Valle Zoque the most impactful management practices were found to be cleaning dirty drinkers and feeders, soil pens, pens cleaning, age of weaning calves, stallion change time and stallion loan.

The ELISA technique (IDEXX, 2017) that was used in the present work, presented a sensitivity of 60-80% and a specificity of 90-99%. These types of tests are less expensive and can be applied to blood or milk samples. This technique is ideal for the identification of animals that are in a subclinical state of the disease and is considered a screening technique and is useful for the establishment of surveillance and control measures within the affected herds.

Finally, derived from the survey of producers, it was possible to identify risk behaviors that allow establishing hypotheses that in subsequent studies should be explored in depth in multidisciplinary studies to establish prevention and control strategies for this disease.

It is important to take biosecurity measures against this disease at a national and international level, which is why the creation of the national campaign against paratuberculosis is urgent. In this sense, sensitive and specific diagnostic tests are required at a good price to achieve the detection and subsequent elimination of clinical and subclinical cases of LU, so that, in this way, the exposure of young animals that are the most susceptible to infection to MAP is reduced.

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