Vol. 13(3), pp. 114-120, July-September 2021
DOI: 10.5897/JVMAH2020.0885
Article Number: 8D7A5D367293
ISSN: ISSN: 2141-2529

http://www.academicjournals.org/JVMAH

ACADEMIC JOURNALS
expand your knowledge

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Paratuberculosis epidemiological study (risk factors and prevalence) in ovine livestock production units in the State of Guanajuato, Mexico

Marco Antonio Santillán-Flores[1*], Dionisio Cordova-López[1] and Claudia Celic Guzmán-Ruiz[2]

[1]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarías, CENID Microbiología Carretera México -Toluca Km. 15.5, Colonia Palo Alto, CP. 04110. Delegación Cuajimalpa. Ciudad de México.
[2]Universidad de Guanajuato, Division Ciencias de la Vida, Campus Irapuato –Salamanca. Ex hacienda; El Copal Km 9, Carretera Irapuato-Silao, CP 36820. Irapuato, Guanajuato México.

Received 11 October, 2020; Accepted 28 June, 2021

In order to determine the prevalence and risk factors associated with paratuberculosis in ovine livestock production units in Guanajuato, Mexico; a cross-sectional epidemiological study was carried out for one year long. Serum samples (n = 1387) were analyzed with an agar gel immunodiffusion test. Bacteriological culture and IS900 nested PCR were done from the fecal samples (n=640). For epidemiological data, two questionnaires (individual animal and LPU) were filled out and statistical analysis was done with STATA 7 ® software. Ovine paratuberculosis prevalence in the studied population was 4.54%; in ovine older than 11 to 24 months age was 3.52%; females with more than three parturitions showed 6.5% prevalence; and in the livestock production units that carried mucking out and elimination of feces only once a month the prevalence was 50%. Odds-ratio analysis (OR) showed that the presence of thin females after parturition had an OR of 3, females with more than 3 parturitions had an OR of 4.74, while livestock production units that mucked out and eliminated feces once a month had OR of 1.41. Results in this study showed the degree of spread of paratuberculosis among sheep throughout various zones in the State of Guanajuato. Comprehensive ovine paratuberculosis epidemiological studies shall be a tangible asset that will allow the design of appropriate control programs for the elimination of the disease in livestock production units.

Key words: Ovines, paratuberculosis, risk factors, prevalence, epidemiology

INTRODUCTION

Paratuberculosis, of which the etiological agent is *Mycobacterium avium* subspecies *paratuberculosis* (*Map*), is a chronic infectious disease that affects domestic and wild ruminants (García and Shalloo, 2013). Paratuberculosis causes inflammation and disorders of the intestinal tract presenting macroscopic lesions in which the intestinal walls are thickened and edematous. Most of the lesions are present in the jejunum, ilium,

*Corresponding author. Email: mayco1768@gmail.com; santillan.marco@inifap.gob.mx.

cecum fold and cecum. Mesenteric lymph nodes can be inflamed and edematous. Intestinal lesions cause nutrient malabsorption which causes the individual to lose body condition with consequent reduction in production capacity (García and Shalloo, 2013; Ventura-Angulo, 2010). The prevalence of the disease in sheep herds ranges from 2 to 25%. Even though the death of animals is not high, the slow dispersal of the disease and its chronic presentation cause periodical economic loss. Monetary compensation value of clinically infected animals can be insignificant due to the high degree of under nourishment (Bedolla et al., 2011; Ventura-Angulo, 2010). Diverse studies on risk factors associated with paratuberculosis have determined that the most common factors by which animals become infected are, the constant exposure of individuals to fecal matter contaminated with *Map*; close contact with infected adult animals; feeding of colostrum from females positive to paratuberculosis; number of parturitions, multiparous females; fecal consistency, and size of the herd (Guzmán et al., 2016; García and Shalloo, 2013). Paratuberculosis is one of the main problems that affect the sheep production system in Mexico: It is associated with the sanitary conditions in which production is carried out, and in consequence, herds are susceptible to developing the disease, which becomes more frequent as time goes by.

The purpose of the study was to determine the prevalence and risk factors that are associated with paratuberculosis in ovine livestock production units, in the State of Guanajuato, Mexico.

MATERIALS AND METHODS

Study population and sample size estimation

A year-long cross-sectional epidemiological study was carried out on 18 municipalities within five zones of the State of Guanajuato. The study population consists of ovine livestock production units under the Validation and Technology Transfer Livestock Producers Group (GGAVATT). Sample size was estimated from the ovine census of the State of Guanajuato by applying Levy's proportional formula (Levy and Lemeshow, 1980) using a 10% prevalence of paratuberculosis, a 1% error and a 95% confidence level. Number of animals sampled in each farm was estimated using Cannon and Roe's formula (1982) that has a 95% confidence of including at least one positive animal if the disease is present under the estimated prevalence. Inclusion criteria were male or female sheep older than six months, and any animal with deficient body condition when compared to the total general status of the herd.

Epidemiological information

Two questionnaires were filled out, one for each animal selected and one for the livestock production unit (LPU). Variables for the LPU and the individual were encoded to ensure confidentiality of the cooperating producers. At the LPU level, the recorded variables were location, experience as sheep producer, herd size, origin of the sheep, and handling practices. From everyone the following were obtained, identification, age, gender, status, breed, body condition, type of animal, origin. Use of these questionnaires allowed us to record the variables that could be regarded as possible risk factors for the disease. All information collected and the results of diagnostic tests were entered into two electronic databases (one with LPU data and the other with individual data) for the epidemiological analysis using the STATA 7® software. Descriptive statistics were calculated and the odds ratio under logistic regression models was used to estimate the relative risk of infection.

Blood and fecal samples

Blood samples were taken from the jugular vein using vacutainer tubes without anticoagulant. Samples were centrifuged at 3500 rpm, serum transferred to 1.5 ml tubes and stored at -20°C until used. Fecal samples were collected directly from the rectum of each ovine, using a polyethylene bag, and stored at -20°C.

Serological test

Agar gel immunodiffusion tests (AGIT) were done on the serum samples following the procedure outlined by Hernández (2007). The protoplasmic antigen of the 3065 strain of *Mycobacterium avium* subspecies *paratuberculosis* was used and the results were used as the dichotomous variable positive/negative for paratuberculosis infection.

Polymerase chain reaction test (IS900 Nested PCR)

The nested PCR and bacteriological culture were done using samples that had a positive AGIT test and negative random samples selected from each LPU, reaching a total of 640 fecal samples. DNA was extracted from each sample using the methods described by Garrido et al. (2000). The following primers specific to *Map* were used for amplification of the insertion sequence 900 (IS900) Erume et al. (2001): ptb1 (5' TGA TCT GGA CAA TGA CGG TTA CGG A 3') and ptb4 (5'CGC GGC ACG GCT CTT GTT 3'). These primers amplify a 563-base pair (bp) product that is used in the nested PCR using the primers ptb2 (5' GCC GCG CTG CTG GAG TTA A 3') and ptb3 (5' AGC GTC TTT GGC GTC GGT CTT G 3'). The latter amplifies a 210 bp product. Amplicons were visualized in 2% agarose gels stained with ethidium bromide.

Bacteriological culture

Bacteriological culture was carried out using the protocol described by Payuer et al. (1993). Samples were seeded in plates containing Herrold's egg yolk agar with mycobactin (2 mg/L) in duplicate. Plates were incubated at 37°C for 20 weeks. To confirm the presence of acid-fast bacilli, Ziehl-Neelsen (ZN) stain of prepared slides was carried out.

RESULTS

Serological tests

Using the AGIT test, the presence of anti-*Map* antibodies was detected in 63 of 1387 individuals sampled.

Polymerase chain reaction test (IS900 Nested PCR)

A total of 640 samples were tested with IS900 PCR of

Table 1. Ovine paratuberculosis, risk by variable and category zones and municipalities in the State of Guanajuato, Mexico.

Variable	Category	Prevalence %	p-value	Odds ratio	IC 95%
State sample	--	4.54 (63/1387)	-	--	1-8
Zone	1 (Dolores, San Diego de la Union)	1.48 (3/203)	0.003	1.0	--
	2 (San Jose de Iturbide)	7.51 (13/173)	-	5.41	1-19
	3 (Manuel Doblado, Guanajuato, Leon, Silao)	4.8 (6/123)	-	3.41	0.8-14
	4 (Celaya, Coroneo, Apaseo el Grande)	8.07 (18/223)	-	5.85	1.7-20
	5 (Salamanca, Cueramaro, Irapuato; Jaral del Progreso, Abasolo, Valle de Santiago, Huanimaro, Penjamo)	3.46 (23/665)	-	2.38	0.7-8
Municipality	Apaseo el Grande	16.4(14/85)	0.000	9.07	2.5-33
	Coroneo	4.6 (4/87)	-	2.21	0.5-10
	Dolores Hidalgo	2.13 (3/14)	-	1.0	
	Irapuato	3.37 (6/178)	-	1.6	0.4-6.5
	Penjamo	5.76 (8/139)	-	2.82	0.7-31
	Salamanca	4.26 (6/141	-	2.0	0.5-8.3
	San Jose Iturbide	7.51(13/173)	-	3.73	1-13.3
	Silao	15.6(5/32)	-	8.51	1.9-38

which 52 were positive due to the presence of the 210 bp amplicon specific to *Map*. Of the 52 samples that were positive via PCR, 48 had been positive to the AGIT test.

Bacteriological culture

A total of 27 isolates were obtained from the 640 fecal samples collected. Bacterial colonies were seen from the sixth week of incubation. Bacteriological smears stained with ZN revealed the presence of acid-fast bacilli that together with the speed of bacterial growth and the development of mycobactin classified the bacteria as *Map.*

Descriptive analysis of ovine paratuberculosis: Population level

Prevalence reached 4.5% using the AGIT test. Estimated prevalence, ranging from 1.48 to 8.07%, indicate the degree of spread of paratuberculosis among sheep throughout various regions in the State of Guanajuato. The municipality with the highest prevalence was Apaseo el Grande with 16.4%.

Odds ratio estimation showed that sheep within zone four had an OR of 5.85, while those in zone two had an OR of 5.41; a higher risk of positive paratuberculosis was present when compared to zone one. When compared to the LPU of the municipality of Dolores, the OR in sheep in Apaseo el Grande reached OR of 9.07, those of Jose Iturbide resulted in an OR of 3.73 and sheep in Silao had an OR of 8.51 (Table 1).

Paratuberculosis was detected in sheep since 11 months of age on. The prevalence of herds with animals between 11 and 24 months of age reached 3.52%, while herds with animals between 37 to 48 months of age increased to 6.77%.

Females that had two to three parturitions and those that had more than three parturitions had a prevalence of 6.0 and 6.5%, respectively. In terms of breeds, Dorper sheep had 15.79% prevalence while Pelibuey crosses had up to 14.0%.

Animals with bad body condition had prevalence of 5.87% while those with regular body condition had 3.76%. Sheep purchased in other municipalities had a prevalence of 9.8%.

Odds ratio results indicate that herds that have animals between the ages of 37 to 48 months had an OR of 1.28, while females that had more than three parturitions had an OR of 4.74. Dorper breed had an OR of 3.6 and Pelibuey crosses had an OR of 3.13. Interestingly, sheep that had been bought in market had an OR of 1.75 for becoming infected when compared to those that had been born in the LPU (Table 2).

Descriptive analysis of ovine paratuberculosis: Livestock production unit level

The used 66 LPU are distributed throughout five production zones, in the State of Guanajuato. Mean paratuberculosis prevalence in LPUs was 40.91%.

With the highest odds ratio in zone five reaching up to 1.87, in terms of municipalities, the highest odds ratio was found in Penjamo with an odds ratio of 2.8. Apaseo el Grande had 2.33 and Salamanca 1.9 (Table 3).

Prevalence of paratuberculosis regarding time of experience of the ovine livestock producers ranged between 14.2 and 52.9%. Animals that were purchased

Table 2. Ovine paratuberculosis, prevalence, risks by variables and categories of population level.

Variable	Category	Prevalence %	p-value	Odds ratio	IC 95%
Gender	Male	1.2 (2/167)	0.027	1.0	
	Female	5.0 (61/1220)	--	4.34	1-18
Ovine group	Future studs <2 years	1.45 (2/138)	0.031	1.0	
	Studs >3 years	3.64 (2/55)	--	2.56	0.3-18
	Females in development	0.0 (0/42)	--	--	--
	Yearlings	2.34 (4/171)	--	1.52	0.2-9
	Females 2-3 parturitions	6 (24/400)	--	4.34	1- 19
	Females >3 parturitions	6.52 (29/445)	--	4.74	1-12
Age (months)	6 to 10	0 (0/44)	--	--	--
	11 to 24	3.52 (22/625)	0.1504	0.71	0.3-1
	25 to 36	4.83 (19/393)	--	0.69	0.2-1
	37 to 48	6.77 (13/192)	--	1,28	0.4-4
	>49	5.33 (4/75)	--	0.77	0.2-2
Body condition	Bad	5.87 (40/682)	0.028	2.36	0.9- 6
	Regular	3.76 (177/452)	--	1.48	0.5-4
	Good	2.56 (5/195)	--	1.0	--
Breed	Pelibuey	4.95 (36/728)	0.000	1.0	--
	Dorper	15.79 (9/57)	--	3.6	1.6-8
	Katadhyn	6.0 (2/33)	--	1.24	0.3-5
	Rambouillet	0.56 (1/180)	--	0.1	0.1-7
	Blackbelly	6.38 (3/47)	--	1.31	0.3-4
	Pelibuey cross	14.0 (8/57)	--	3.23	1-7
Origin of the ovine	Born within the LPU	4.95 (27/545)	0.001	1.0	--
	Purchased	8.38 (29/345)	--	1.75	1-3
	Without known data	1.61 (8/496)	--	0.28	0.1-1
Place of purchase of the ovine	Same municipality	5.26 (4/76)	0.028	1.0	--
	Another municipality	9.8 (10/102)	--	1.95	0.6-7
	Another State	2.78 (2/72)	--	0.51	0.09-3
	Without known data	3.43 (20/583)	--	0.63	0.2-2

had a prevalence of 27.2% while those that were born within the LPU had a prevalence of 33.3%. In terms of herd size, those farms that had between 101 and 200 animals had a prevalence of 40%, while those that had more than 300 animals had a prevalence of up to 42.1%. Not surprisingly, farms that mucked out animal pens every 2 to 12 months had a prevalence that reached 45%. The presence of thin females was indicative of the presence of paratuberculosis since its prevalence reached 50%.

Odds ratio analysis found that LPUs where livestock producers had between 49 to 96 months of experience had odds ratio of 1.76. Other notable results include LPUs that purchased animals and kept those born in the LPU showing an OR of 1.87 as well as presence of thin females after parturition resulting to an OR of 3.0, while those LPUs that had more than 300 individuals had an OR of 3.25 (Table 4).

DISCUSSION

The results obtained from this study indicate that paratuberculosis has a prevalence of 4.54% in ovine LPUs that participated in this study in the State of Guanajuato. It should be noted that there could be more animals infected with paratuberculosis in the herd since this disease is a group problem and the detection of a

Table 3. Ovine paratuberculosis, prevalence, risks by variables and categories livestock production unit level.

Variable	Category	Prevalence %	p-value	Odds ratio	IC 95%
Ovine LPUs	--	40.9 (27/66)	--	--	--
Zone	1 (Dolores, San Diego de la Union)	25. (2/8)	0.755	1.0	--
	2 (San Jose de Iturbide)	45.4 (5/11)	--	1.81	0.02-12
	3 (Manuel Doblado, Guanajuato, Leon, Silao)	40 (2/5)	--	1.6	0.2-15
	4 (Celaya, Coroneo, Apaseo el Grande)	30 (3/10)	--	1.2	0.15-9
	5 (Salamanca, Cueramaro, Irapuato; Jaral del Progreso, Abasolo, Valle de Santiago, Huanimaro, Penjamo)	46.8 (15/32)	--	1.87	0.3-9.9
Municipality	Abasolo	0 (0/1)	0.457	NC	
	Apaseo el Grande	56.6 (2/3)	--	2.33	0.21-25
	Celaya	0 (0/2)	--	NC	
	Manuel Doblado	0 (0/1)	--	NC	
	Coroneo	20 (1/5)	--	NC	
	Cueramaro	0 (0/1)	--	NC	
	Dolores Hidalgo	28.5 (2/7)	--	NC	
	Guanajuato	0(0/1)	--	NC	
	Huanimaro	16.6 (1/6)	--	NC	
	Irapuato	37.5(3/8)	--	1.31	0.16-10
	Jaral del Progreso	100 (1/1)	--	NC	
	Leon	50 (1/2)	--	NC	
	Penjamo	80 (4/5)	--	2.8	0.36-22
	Salamanca	55.5 (5/9)	--	1.9	0.3-13
	San Diego de la Union	0 (0/1)	--	NC	
	San Jose Iturbide	45.4 (5/11)	--	1.59	0.23-11
	Silao	100% (1/1)	--	NC	
	Valle de Santiago	100% (1/1)	--	NC	

NC: Not calculated.

Table 4. Ovine paratuberculosis, prevalence, risks by variables and categories of livestock production unit level.

Variable	Category	Prevalence %	p-value	Odds ratio	IC 95%
Experience in ovine LPUs producers (months)	<12	14.2 (1/7)	0.409	0.47	0.04-5
	13-24	44.4 (4/9)	-	1.48	0.25-8
	25-48	40.9 (10/22)	-	1.51	0.34-7
	49-96	52.9 (9/17)	-	1.76	0.38-8
	>97	30 (3/10)	-	1.0	
Herd size	< 50	7.69 (1/3)	0.414	0.75	0.5-10
	51 - 100	40 (6/15)	-	1.33	0.9-18
	101 - 200	42.1 (8/19)	-	1.09	0.2-4
	>300	68.4 (13/19)	-	3.25	0.7-13
	Occasionally	62.5 (5/8)	-	1.52	0.43-5
Origin	Purchased	27.2 (3/11)	0.004	0.81	0.15-6
	Born in the LPU	33.3(2/6)	-	1.0	-
	Both origins	62.5(20/32)	-	1.87	0.3-10
Type of management	Pens	45.7 (16/35)	0.493	1.0	0.3-2.6
	Grazing	45.5 (5/11)	-	1.0	-
	Both	30 (6/20)	-	0.66	0.16-3

Table 4. Cont'd

Graze together with ovine, caprine, and bovine individuals of other LPUs	Yes	33.3 (3/9)	0.608	0.77	0.2-3
	No	44.8 (24/56)	--	1.0	--
Frequency of mucking out pens	Weekly	35.2 (6/17)	0.649	1.0	--
	Every two weeks	28.5 (4/14)	--	0.8	0.2-3.4
	Monthly	50 (6/12)	--	1.41	0.4-5.4
	Between 2 and 12 months	45 (9/20)	--	1.27	0.4-4.3
There are females after parturition always thin	Yes	50 (21/42)	0.049	3.0	0.8-11
	No	16.65 (3/18)	--	1.0	--

single positive animal implies that there could be up to 25% of infected animals in various clinical stages (García and Shalloo, 2013). Prevalence observed in this study ranges from 1.48 to 8.07%, which is similar to studies done by Maresca et al. (1999) and Atilli et al. (2011) who carried out epidemiological studies on paratuberculosis within the Umbria and La Marche regions in Italy and found a seroprevalence between 4.8 to 6.29%. Sergeant and Baldock (2002) from work done in Australia reported a prevalence of paratuberculosis in sheep herds between 2.4 and 4.4%.

Several factors influence prevalence, detection, and the effect of paratuberculosis in herds. It has been previously described that the age of animals is an important factor for detecting animals that are positive to paratuberculosis (Kostoulas et al., 2013). It is thought that most of the animals that are positive to paratuberculosis have between 3 and 4 years of age. In this study, positive animals were detected from 11 to 24 months of age. Being able to detect animals that are positive before two years of age, could be related to the fact that ovine LPUs have up to 40.91% prevalence. Likewise, feces handling practices can determine prevalence from 28 to 50%, since animals are constantly exposed to *Map*. We also observed that the highest prevalence by age group was 6.77% in those with 37 to 48 months of age, coinciding with the mean time for presentation of the clinical phase of the disease (Morón-Cedillo et al., 2015; García and Shalloo, 2013; Kostoulas et al., 2013; Atilli et al., 2011). The number of parturitions is considered a risk factor mainly due to the stress that is caused by gestation, parturition, and lactation, which triggers the manifestation of paratuberculosis clinical signs (Sergeant and Baldock, 2002) and even concurrent infections may also prompt clinical infections as in tuberculosis (Gupta et al., 2009). Results in this study indicate that females with more than three parturitions had 4.74 times greater risk of being infected with paratuberculosis. It is important to consider this fact to establish control measures specific for the group of females with more than three parturitions and their recently born lambs, including reduced contact with feces and possible transmission of *Map* through the consumption of colostrum from these females.

It is thought that all ovine breeds are susceptible to becoming infected with paratuberculosis. Results from this work indicated that Dorperbreed and Pelibuey crosses have a higher risk of becoming infected (3.6 and 3.13 times, respectively). Morón-Cedillo et al. (2015) when carrying out an epidemiological study on paratuberculosis detected that the highest number of seropositive animals was in Rambouillet breed when compared to local breeds or crosses and relates this to susceptibility-resistance to infection characteristics between breeds. They also noted that purebreds that are purchased for genetic improvement of the herd and have an intensive management system are possibly already infected with paratuberculosis before they are brought into the herd. Some ovine breeds included in this study did not have positive animals, probably because the sample number was relatively low, so another study should be carried out with a greater number of samples from those breeds.

The number of individuals within the herd has been previously identified as a risk factor for the spread of this disease. Herds with more than 100 individuals and LPUs that have purchased new individuals have a higher risk of having animals that are positive to paratuberculosis (Sayers and Cook, 2009; Barrett et al., 2011). The odd ratio results of this study indicate that sheep herds that have more than 300 animals present the risk of becoming infected with paratuberculosis up to 3.25 times. Also, animals that were purchased for inclusion into the herd without having the knowledge of their health situation represent a focus of infection for the rest of the herd, hence should have better control over the number of animals in the herd and that the sheep purchased come from herds free of paratuberculosis. These results seem to suggest that as the herds become larger, seroprevalence is greater. The more intensive the management is in the LPU the higher the risk of animals becoming infected with paratuberculosis.

Guzmán et al. (2016) found that dairy goats that had an emaciated body condition had a higher seroprevalence of paratuberculosis. Results in this study agree with those of Guzmán et al. (2016) since sheep with bad body condition had an odds ratio of 2.36, and females that

were thin after parturition had an odds ratio of 3.0. It is thought that these types of animals could be in the clinical phase of the disease and are therefore the main source of infection for the rest of the herd.

Villalobos (2011) and Guzmán et al. (2016) found a risk of 3.6 and 2.10, respectively, for the presence of paratuberculosis in herds in which the mucking out of pens and elimination of feces was irregular. The results in this study obtained from LPUs where pens were mucked out every month had an odds ratio of 1.41. It is important to note that *Map*can survive in fecal matter and stagnant water for up to 48 to 55 weeks (García and Shalloo, 2013). Thus, it is important to have a program in the LPU that includes periodic cleaning and removal of feces to decrease a risk of infection by *Map* due to contamination of water and food by the accumulation of feces.

Sensitivity and specificity of various diagnostic tests for detecting paratuberculosis can be highly variable, which is one of the greatest challenges for the control of the disease. In this ovine paratuberculosis epidemiological study, the use of bacteriological culture and nested PCR as confirmatory tests allowed the validation of the results obtained with AGIT testing. Bacteriological culture sensitivity for *Map* is less than 50%, so having the alternative that provides greater sensitivity, such as the nested PCR that has the advantage of detecting *Map* DNA in fecal samples, allows the detection of low levels of *Map* bacilli DNA in animals that are within an early disease stage and have a low-level humoral response. It is recommended that for integral diagnosis of paratuberculosis, a serological test be carried out, together with AGIT or ELISA testing, and confirmatory tests be applied, such as bacteriological culture or PCR. The diagnosis of paratuberculosis should be considered before the mobilization or entry of sheep to LPUs, as well as carrying out constant monitoring, at least every 6 to 12 months.

Conclusion

The epidemiology of paratuberculosis in ovine herds is complex, and the clinical manifestations and economic impact of the disease on livestock are highly variable. An integral understanding of the prevalence studies and the epidemiological aspects in sheep herds are of great tangible value to facilitate the design of prevention and control programs intended for the reduction and, preferably, the elimination of paratuberculosis in ovine LPUs.

CONFLICT OF INTERESTS

The authors declared have not any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to INIFAP for the financial resources provided (SIGI 12484419581).

REFERENCES

Atilli AR, NguNgwa V.Preziuso S. Pacifici LDomesi A.Cuteri V (2011). Ovine paratuberculosis: A seroprevalence study in dairy flocks reared in the Marche Region, Italy Veterinary Medicine Internship 11(1):1-10

Barrett DJ, Mee JF, Mullowney P, Good M, McGrath G, Clegg T, More SJ (2011). Risk factors associated with Johne's disease test status in dairy herds in Ireland. Veterinary Record 168:410.

Bedolla CC, Castañeda VH, Castañeda AM, Walter W, Alvarez M (2011). Paratuberculosisovina, scientia-*CUCBA* 13(1-2):73-88. https://www.researchgate.net/publication/271517590

Cannon RM. Roe RT (1982). Livestock disease surveys: a field manual for veterinarians. Australian Government Publishing Service.

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(2):83-89.

García AB, Shalloo L (2013). Invited Review: The economic impact and control of paratuberculosis in cattle. Journal Dairy Science 98:5019-5039.

Garrido J, Cortabarria N, Oguiza J, Juste R (2000). Use of a PCR method on fecal samples for diagnosis of sheep paratuberculosis. Veterinary Microbiology 77(3-4):379-386.

Gupta MP, Kumar H, Singla LD **(**2009). Trypanosomosis concurrent to tuberculosis in black bucks. Indian Veterinary Journal 86:727-728.

Guzmán-Ruiz CC, Santillán-Flores MA, Cordova-López D (2016). Prevalence and possible risk factors for caprine paratuberculosis in intensive dairy production units in Guanajuato, Mexico. Journal of. Veterinary Medicine and Animal Health 8(11):156-162.

Hernández COA. (2007). Obtención de un antigenoprotoplasmático de Mycobacterium aviumsubespecieparatuberculosispara el diagnóstico de la enfermedad de Johne en ovinos (tesis de Licenciatura). CuautitlanIzcalli, Edo. de Mex: UNAM.

Kostoulas PW, Browne J, Nielsen SS, Leontides L (2013). Bayesian mixture models for partially verified data: Age- and stage-specific discriminatory power of an antibody ELISA for paratuberculosis. Preventive Veterinary Medicine 111:200-205.

Levy PS, Lemeshow S (1980). Sampling for Health Professionals Lifetime Learning Publications. Belmont, California.

Maresca C, Giorgi G, F. Feliziani F (1999). Indagineepidemiologicasulla paratubercolosis ovina nelterritorio di una A.S.L. dellaregione Umbria. Large Animal Review 5(4):71-74.

Morón-Cedillo FJ; Cortez-Romero C; Santillán-Flores MA; Figueroa-Sandoval B, Gallegos-Sánchez J (2015). Prácticas de manejo asociadas con la seroepidemiologia de paratuberculosis ovina en San Luis Potosí. Agroproductividad 8(6):30-36.

Payuer JB, Jarnagin JL, Marquardt JG, Schaper LA, Martin BM (1993). Laboratory Methods in Veterinary Mycobacteriology for the Isolation and Identification of Mycobacteria. Iowa, USA: United States Department of agriculture animal and plant health inspection service. National Veterinary Service Laboratories, pp. 39-40.

Sayers R, Cook AJC (2009). Farm characteristics and their association with participating in the Johne's disease survey and with Johne's disease status. Pages 113–133 in An Integrated Strategy to Determine the Herd Prevalence of Johne's Disease in the UK Dairy Herd. Defra, London, UK. Accessed Aug. 16,2020. www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/documents/johnes-report0911.pdf.

Sergeant ESG, Baldock FC (2002). The estimated prevalenceof Johne's disease infected sheep flocks in Australia. Australian Veterinary Journal. 80(12):762-768.

Ventura-Angulo JR (2010). Evaluación de trestécnicas de diagnóstico en la detección de Mycobacterium aviumsubespecieparatuberculosis en ovinos. Tesis de Maestría en Ciencias. PosgradoInterinstitucional en CienciasPecuarias. Universidad de Guadalajara. Guadalajara, México.

Villalobos SI (2011). EstudioEpidemiológico de brucellosis y paratuberculosiscaprina en el Estado de Guerrero. (Tesis de Licenciatura) México DF, Universidad NacionalAutónoma de México.