Animal Husbandry, Dairy and Veterinary Science

Research Article ISSN: 2513-9304

Bull and cow-level prevalence of paratuberculosis in Lidia's cattle breed from the Azores

Pacheco-Lima J, Faria N, Silva H, Valadão L and Moreira da Silva JF*

Department of Animal Reproduction, University of the Azores - Faculty of Agrarian Sciences and Environment Animal, IITAA, 9701-851 Angra do Heroísmo, Portugal

Abstract

Mycobacterium avium subspecies paratuberculosis (MAP) causes paratuberculosis, also known as Johne's disease, in domestic ruminants and wild species, resulting in economic losses among farmed ruminants. In Portugal, the true herd-level prevalence of paratuberculosis is unknown and the disease is considered under diagnosed. Particularly in the Azores only a very few studies were developed reporting this infection considering the potential loss in milk production. The aim of the present study is to evaluate the incidence of bovine paratuberculosis in a Lidia bovine population called "Brava dos Acores", destined to the typical Azorean street bullfighting as well as the traditional equestrian bullfight (in the ring). For such purpose, 68 animals belonging to that bullfight population mainly females (n= 55 vs 13 males) aged between 7 to 223 months were submitted to a blood collection, via the coccygeal vein, for diagnostic of paratuberculosis through the Elisa diagnostic test. Results showed that, from the 68 studied animals, 63 were negative for the presence of Paratuberculosis, 4 were positive and 1 was doubtful. The percentage of infected animals with positive and doubtful animals was 7.4%.

In addition to the new knowledge obtained, it was concluded that further studies on this breed will be essential to expand the existing information.

Introduction

Lidia Cattle is an autochthonous Iberian Breed whose production has great economic and social importance in Spain, Portugal and particularly in The Azores. In most cases, this type of ranches preserving much of the traditional methods of animal management and the fighting bull breed in Terceira-Azores, called "Brava dos Açores", presents self-characteristics ideal for Rope Bullfight, an ethnographic entertainment common in this Island. This style involves a group holding fast to a long stout rope tied around the bull's neck, called "tourada à corda" (bull-on-a-rope). The breeding pattern of the Brava cattle breed is extensive and many of these animal's graze in high and difficult access areas, where they spend severe winters and with little pasture available, since this bovine seems to adapt more easily to adverse realities [1]. One of the diseases that affects most individuals of these animals in the Azores is paratuberculosis. This disease, also known as Johne's disease, is an infectious caused by a specific mycobacterium: Mycobacterium avium subs. Paratuberculosis (MAP), which in addition to the Azores wild cattle affects in general all domestic ruminants [2], impacting animal welfare and arouses public health. It is characterized by a long incubation period, for months or years, during which the infection is inapparent. The clinical signs are manifested in most cattle between the ages of two to seven years, and when animals are in an environment of strong pressure of infection early cases can be observed, in younger animals [3].

The first descriptions of this debilitating disease were first noticed a little over 100 years ago in Europe and North America but is likely to be much more widespread today. The risk of infection is not only an economic one for farmers but also a human health concern because there is evidence that MAP is also pathogenic for people [4]. In 1894 Heinrich Albert Johne (hence the name Johne's disease) and Langdon Frothingham evaluated the intestines, stomach, and omentum of a cow that neither produced milk nor gained weight and had been negative to the intradermal tuberculinization test [5]. These two scientists noted the thickening of the mucosa and the infiltration of the intestinal wall by leukocytes and epithelioid cells. Through resistant acid-alcohol staining they observed bacteria stained red, which were like those that caused tuberculosis. After inoculating the infected tissues in rats without causing the disease, they concluded that the etiologic agent was the same that caused tuberculosis in birds [6]. The name paratuberculosis comes from the initial description of the bacterium that seemed very similar to the bacterium that causes bovine tuberculosis [7]. This pathogen can remain viable in the environment for up to 1 year. On natural conditions the incubation period is at least 2 years and it can be extended up to 10 years [8]. The course of the disease depends on age, immune status, and resistance of the affected individual [9]. Persistent infections occur when there is contact with the agent in the first weeks or months of life, especially in the first sucked milk [10]. Normally, the infection begins orally, immediately after the agent is taken up by M cells in Peyer's patches, which appears to be accelerated by colostrum antibodies, present in the intestinal lumen [11]. The macrophages take up bacteria released by M cells and a dissemination, clinically inapparent. After that lesions in the distal jejunum and ileum takes place and chronic enteritis originates with specific granulation tissue [12]. The clinical signs appear mainly after a situation of stress.

Correspondence to: Moreira da Silva J.F, Department of Animal Reproduction, University of the Azores - Faculty of Agrarian Sciences and Environment Animal, IITAA, 9701-851 Angra do Heroísmo, Portugal, Tel: 919219251, E-mail: Joaquim.fm.silva@uac.pt

Key words: *lidia cattle; paratuberculosis; seroprevalence*

Received: May 14, 2021; **Accepted:** May 24, 2021; **Published:** May 31, 2021

Observing intermittent diarrhea at first, to later become in permanent and in some cases advanced submandibular edema develops [13]. The transmission of paratuberculosis can occur intrauterine, in the postnatal period through colostrum and, mainly, through the feces of contaminated animals [14]. Although there is no definitive cure for this disease, several agents can be administered to reduce or relieve clinical signs and prolong the animal's life [15]. To detect clinical and subclinical paratuberculosis, tests measuring humoral and cellular immune reactions, are normally employed:

- Complement Fixation: has an intermediate sensitivity, however it is one of the most effective in animals with clinical signs. The disadvantage that it presents is cross reactions with another Mycobacterium [16].
- Immunodiffusion in Gel Agar (IDGA): it has a low sensitivity in cases subclinical [17].
- Enzyme Immunoassay (ELISA) test: has the best balance between sensitivity and specificity, and detects the largest number of animals with subclinical infection. The disadvantage of this test is that it presents cross reactions with another Mycobacterium or with Nocardia [18].
- Hypersensitivity test or Introdermoreaction: these are the older tests as they are not very specific, since they present cross reactions with other mycobacteria [8].
- Interferon Gamma (IFN-gamma): useful for the detection of young animals that are in the early stages of infection. The downside is its low specificity and high cost [19].

Bacteriological diagnosis is carried out by means of ileum smears and/or fecal cultures, which are 2-3 times more expensive than the ELISA test. Otherwise, results cannot be obtained before two to four months. The advantage of this diagnostic method is, however, its high specificity. Post-mortem diagnosis is based on gross lesions and histopathological with the identification of the bacteria in organs, using the immunohistological staining Ziehl-Neelsen, to detect the Mycobacterium tuberculosis organisms and their precise localization in the tissues [20].

Concerning control and prevention there is no effective treatment for paratuberculosis and trying to treat an infected animal, can favour the infection of other animals through its stay on the farm [21]. The prevention of this infection is, this way, the most effective approach to control paratuberculosis in a herd. Therefore, there is a need to establish a control program for paratuberculosis, which includes several challenges, such as the fact that it is a disease with a subclinical phase and a slow development, making difficult to recognize and control the level of infection of the herd [3]. As to eliminate paratuberculosis from a farm, it would have to be completely depopulated and reintroduced new non-infected animals, the best option is to keep the prevalence of the disease very low. Therefore, the control of paratuberculosis should be based on two principles: the identification of infected animals through diagnostic tests and the prevention of new infections involving the isolation and elimination of infected animals, good hygiene practices and preventing the entry of animals affected in the farm [21,22].

Nowadays in most countries, the implementation of control measures is still entirely dependent on the owners, such as management, work, and financial resources [23], as in the European Union, there is no paratuberculosis control program [6]. When implementing a control plan for paratuberculosis, it is necessary to consider that there are no static plans, and therefore this control must consider the particularities of the farms. One of the main factors is the availability of resources present on the farm, since this control is based on preventive management and good management practices. The financial power of farms is another factor to consider because it influences the investment in diagnostic tests and the number of positive animals to be suppressed.

A major infection control point is the general hygiene of the farm, keeping feeders and drinkers at a high level of cleanliness, considering that the fallow of contaminated pastures should not be less than three years [23]. Besides vaccination is a good practice to reduce the number of animals presenting the clinical form of the disease, vaccinated animals cannot be considered free of the disease [24]. As known several species of animals are at risk of being infected by MAP, so it is suspicious that the infection may also occur in humans [3]. This infection has been detected through CRP in humans who have Crohn's disease, which is identical to paratuberculosis, both epidemiologically and pathologically [25,26]. Crohn's disease is characterized by a chronic inflammatory process that essentially affects the gastrointestinal tract with similarities with paratuberculosis [3]. The possibility of MAP causing Crohn's disease arose from a series of studies in 1984, in which microorganisms were isolated in a patient with this disease [27]. These two affect adults and young people, and are characterized by chronic granulomatous lesions, usually concentrated in the terminal ileum, and provide the appearance of clinical signs of diarrhoea and weight loss [28]. Crohn's disease has been treated effectively and, in some cases, even cured by long-acting antibiotics. However, it was necessary for laboratories specialized in MAP cultures to take samples from patients with Crohn's disease to prove that MAP is one of the causes of human disease [26].

Most MAP infected animals are farm animals, so the contamination of food produced by them must be weighed before slaughter for consumption, considering that MAP can be isolated in muscles, internal organs, and milk. Contamination can happen ante-mortem, in animals already infected or during the procedures of the slaughter line for faecal contamination. In the case of milk, it is the same situation, it can come from animals already infected or by faecal contamination [29].

Cheeses with high humidity and pH and made from raw milk have a high probability of contamination by MAP [26]. Water for domestic consumption, should also be considered as a source of contamination for humans, as for example rainwater that falls in an infected livestock production area, may contain organisms for more than one year and mycobacteria, MAP, are resistant to chlorine levels used in water disinfection [26].

According to the World Organization for Animal Health, further studies must be carried out to determine the real risk to public health from the consumption of food contaminated by MAP. Much remains to be investigated on how to mitigate human exposure to this potentially zoonotic pathogen.

The problems related to infectious and contagious diseases represent for the extensive cattle, and in a unique way for Lidia Cattle, the main source of risk of potential economic losses [30,31]. Among all of them they highlight the pathogenic agents that have a manifest tropism for the reproductive, respiratory, and digestive systems. Therefore, changes in the potential of reproduction, as well as respiratory digestive disorders are the main problems, we confront in breeding process [32]. The paratuberculosis is present in most of the Lidia Cattle herds and poses difficulties in the control strategies to be implemented because it has a very long incubation period and subclinical infection in most

animals. In the Azores, in Terceira Island in particular, this pathology spread with the importation of cattle, from the Iberian Peninsula in the last decades of the 20th century and the beginning of the 21st century [33]. In addition, as these animals circulate on all the islands, this fact contributes to the spread of the disease in the archipelago [33].

Materials and methods

For this study, a group of 68 animals (55 females and 13 males) registered in the breed book of "Brava dos Açores" with an average age of 83.07 ± 7.3 months were used. All of them were grazing in a high (Summer/Autumn) and middle altitude (Winter/Spring) of the Terceira island, Azores. Blood collection (10 ml) was performed from the coccygeal (tail) vein with the animal restrained in a crush and carried to the Regional Veterinary Laboratory within 24 h of bleeding, serum or plasma samples were separated and stored at −20°C until they were assayed. Mycobacterium avium subspecies paratuberculosis antibodies were determined using an Indirect Absorbed ELISA (enzyme-linked immunosorbent assay), detecting paratuberculosis antibodies in the plasma according to [34]. Briefly, soluble antigens of Mycobacterium phlei were used to remove nonspecific antibodies in the bovine serum. Before transferring serum samples of cattle to the plate that coated with (MAP) antigens, serum samples of cattle were diluted and pre-incubated with a dilution buffer containing M. phlei antigens. Anti-ruminant immunoglobulin G and Horse Radish Peroxidase (HRP) were added to all microwells. After washing, the substrate solution tetra-methyl benzidine (TMB) was added to eliminate the excess conjugate. The microplate was read using a spectrophotometer (ELISA reader, Bio Rad-Model 620) at 450nm in the Regional Veterinary Laboratory -Azores Portugal. All ELISA-positive samples were cultured in media tubes (3 tubes of Herrold's egg with Mycobactin and 1 tube of Herrold's egg without Mycobactin for every sample).

Statistical analysis was performed by simple ANOVA using the SPSS Statistics software and the differences P <0.05 were considered significant. MAP prevalence was calculated in relation to animal's age as well as studied animals were males or females.

Results

In Table 1 the frequency and the percentage of animals subjected to the diagnosis of paratuberculosis are presented. As it can be observed, from the 68 animals evaluated, 63 of them (92.6%) were considered negative, 4 (5.9%) were considered positive and 1 of them (1.5%) resulted in a doubtful outcome.

The prevalence values between males and females did not demonstrate different responses to the pathogen M. avium subsp. paratuberculosis. Of the 55 females under study, only 4 were positive to the paratuberculosis, while of the 13 males only 1 was positive, which represents, respectively 72.7% and 76.9%.

In relation to age, the lowest seroprevalence value (0.0%) was found in animals over 10 years old, and the highest (4.7%) in animals 2-4 years old, and these differences were statistically significant (p<0.05).

Table 1. Frequency and percentage of animals subject to analysis in relation to paratuberculosis

Paratuberculosis	Frequency	Percentage
Doubtful	1	1.5
Negative	63	92.6
Positive	4	5.9
Total	68	100

Discussion

Mycobacterium avium subsp. paratuberculosis is the etiologic agent of a severe gastroenteritis in ruminants known as Johne's disease. It is considered a chronic progressive enteritis of ruminants, worldwide spread causing important economic losses and affecting the productivity of infected cows [35]. In the present study analyses were carried out on 68 animals belonging to a bull fight breed called "Brava dos Açores", from which 13 were males and 55 females. Results showed that from our sample, 63 animals were considered negative for the presence of Paratuberculosis, 4 were positive and 1 was doubtful. Besides no statistical differences were observed among prevalence on females or males the age was considered a factor influenced the prevalence. As known usually, ruminants are infected at an early age of life by ingestion of Map-contaminated milk or by contact with faeces from infected herds [36]. Pregnant cows with advanced disease may be able to transmit it to the fetus [37]. Besides animals are generally thought to become infected within the first few months of life [38], clinical signs are usually seen only in adults due to the long incubation period of the disease [28].

Regarding the ages it has been observed that prevalence in youngest animals were higher than in the oldest. In the same way, the male considered doubtful was also a young, with 23 months old. The reason for the differences in susceptibility observed between adult animals and calves are not completely elucidated. The abundance of gd T lymphocytes in cattle at a time when they are most susceptible to MAP and their peculiar location in mucocutaneous tissues, which also represent the preferred point of entry for mycobacterial pathogens, is supposed to be related with the maintenance of the infection [28]. Other species like sheep and deer can become infected at any age and present clinical signs of the disease [39].

In wild cattle breeding, females are used as breeders and can be kept in a cattle farm for several years to generate offspring. The new females, after going through several selection criteria according to the objectives of the rancher, serve to replace the older females. Males are bred up to 3 years of age in order to be run in bullfights. Then, they can live for several years according to their characteristics and their behaviour in bullfighting, and they can even become reproducers.

The percentage of infected animals with positive and doubtful animals is 7.4%. As previously mentioned, paratuberculosis is a contagious disease and there is no effective treatment for definitive elimination of MAP. Therefore, the 4 females that obtained positive results in the tests must be identified and separated as soon as possible, to be slaughtered to avoid future contamination of other animals, which can lead to an increase in the percentage of contamination on the farm, generating great economic losses. Offspring of these 4 females should also be selected for analysis because paratuberculosis can be transmitted through the presence of mycobacteria in colostrum and milk or via the transplacental route. Moreover, the majority of infections occur shortly after birth when the animals are most susceptible. After 6 months of age, cattle become more resistant to infection, and a much higher dose is needed to cause infection. At least 25% of heavily infected cows spread the disease transplacentally to their foetuses and shed organisms in their milk. Infected bulls shed organisms in their semen, but sexual transmission has not been proven. For that reason, the male considered doubtful should also be separated from the rest, to carry out another test, until confirmation whether it is positive or negative for the presence of paratuberculosis. If the future result is positive, the best solution will be slaughtered to avoid contamination of the remaining animals, but

as these are almost two years old (23 months) this one could be breed separately from the rest to avoid contamination, before being used as bullfight at 3 years old, even because when it is lost to slaughter, which can bring prejudice to the exploitation.

In the future we would like to develop a control plan, having two principles: identify the sources of infection and prevent new infections.

To identify the sources of infection, all animals on the farm may be analysed using diagnostic tests to identify infected animals. As the soil can also be a source of infection, it would be of great interest to observe the levels of contamination, since the organism survived for up to 24 weeks on grass that germinated through infected faecal material applied to the soil surface in completely shaded boxes and for up to 9 weeks on grass in 70% shade [40]. The introduction of new animals can also be a source of infection, as in a livestock farm, with the aim of improving the breed, sometimes breeding stock is used from other livestock farms, diagnostic tests must be carried out on these animals to make sure that these are not a source of infection [41].

The prevention of new sources of infection would involve the isolation and refuse of animals considered as a source of infection such feeders are practically at floor level, so building new ones at a higher level was an asset to avoid food contamination. At the soil level, if they are contaminated, the pastures must be set aside for at least three years. Finally, pay attention to the introduction of new animals.

Further research on Paratuberculosis in the wild race would be essential to expand the existing information. Much of the current information relates to milk and meat breeds as they have a very different behaviour and management from Lidia Cattle Breed.

Acknowledgment

This project was financed in 85% by FEDER and in 15% with regional funds through the "Programa Operacional Açores 2020 (Operational Program Azores 2020), in scope of the project Cryotec-ACORES-01-0145-FEDER-000092".

Funding

IITAA (University of the Azores) is fully acknowledged. Project was financed in 85% by FEDER and in 15% by Regional Funds through the Operational Program of Açores 2020 under the scope of the project CRYOTEC-AÇORES-01-0145-FEDER-000092.

References

1. Pacheco-Lima J, Fernandes da Silva D, Campillo Beneitez J, Moreira da Silva J, Silva H (2019) Effect of vaccination against Ibr/Bvd on the reproductive performances of brava dos açores - A bovine lidia breed. *Am J Biomed Sci Res* 6: 266-272.

2. Adhikari N (2020) An overview on resistivity, diagnostic challenges and zoonotic significance of: Mycobacterium avium ssp. paratuberculosis (MAP). *The Open Microbiol J* 14: 157-163.

3. Cruz LC (2015) Paratuberculose: Associação dos dados clínicos com os dados de rejeição de carcaças de bovino no matadouro por caquexia." Msc Thesis, University of Evora. pp: 128.

4. Tood ECD (2014) Bacteria: mycobacterium avium ssp. paratuberculosis, encyclopedia of food safety. Academic Press, Editor Yasmine Motarjemi. pp: 462-467.

5. Chiodini RJ, Buergelt CD (1993) Susceptibility of BALB/c, C57/B6 and C57/B10 mice to infection with Mycobacterium paratuberculosis. *J Comp Pathol* 109: 309-319. [Crossref]

6. Behrand MA, Collins DM (2010) Paratuberculosis: Organism, disease, control. (1st end), *CAB International*. pp: 375.

7. OIE (2021) Manual of diagnostic tests and vaccines for terrestrial animals, paratuberculosis general disease information sheets what is paratuberculosis? general disease information sheets where is the disease found.

8. Whittington R, Donat K, Weber MF, Kelton D, Nielsen SS (2019) Control of paratuberculosis: Who, why and how. A review of 48 countries. *BMC Vet Res* 15: 198. [Crossref]

9. Roller M, Hansen S, Knauf-Witzens T, Oelemann W, Czerny CP (2020) Mycobacterium avium subspecies paratuberculosis infection in zoo animals: A review of susceptibility and disease process. *Front Vet Sci* 7: 572724. [Crossref]

10. Eisenberg SW, Nielen M, Koets AP (2012) Within-farm transmission of bovine paratuberculosis: Recent developments. *Vet Q* 32:31-35. [Crossref]

11. Fecteau ME (2018) Paratuberculosis in cattle. *Vet Clin North Am Food Anim Pract* 34: 209-222.

12. Criado M, Benavides J, Vallejo R, Arteche N, Gutiérrez D (2020) Local assessment of WC1+ γδ T lymphocyte subset in the different types of lesions associated with bovine paratuberculosis. *Comp Immunol Microbiol Infect Dis* 69: 101422. [Crossref]

13. Ludwig L, Egan R, Baquero M, Mansz M, Plattne BL (2019) WC1(+) and WC1(neg) gammadelta T lymphocytes in intestinal mucosa of healthy and Mycobacterium avium subspecies paratuberculosis-infected calves. *Vet Immunol Immunopathol* 216: 109919. [Crossref]

14. Sweeney RW (2011) Pathogenesis of paratuberculosis. *Vet Clin North Am Food Anim Pract* 27: 537-546. [Crossref]

15. Fecteau ME, Whitlock RH (2011) Treatment and chemoprophylaxis for paratuberculosis. *Vet Clin North Am Food Anim Pract* 27: 547-557. [Crossref]

16. Sherman DM, Gay JM, Bouley DS, Nelson GS (1990) Comparison of the complement-fixation and agar gel immunodiffusion tests for diagnosis of subclinical bovine paratuberculosis. *Am J Vet Res* 51: 461-465. [Crossref]

17. Hilbink F, West DM, de Lisle GW, Kittelberger R, Hosie BD (1994) Comparison of a complement fixation test, a gel diffusion test and two absorbed and unabsorbed ELISAs for the diagnosis of paratuberculosis in sheep. *Vet Microbiol* 41: 107-116. [Crossref]

18. Hemati Z, Haghkhah M, Derakhshandeh A, Chaubey KK, Singh SV (2020) Novel recombinant Mce-truncated protein-based ELISA for the diagnosis of Mycobacterium avium subsp. paratuberculosis infection in domestic livestock. *PLOS ONE* 15: 2020. [Crossref]

19. Hughes V, McNair J, Strain S, Barry C, McLuckie J (2017) Gamma interferon responses to proteome-determined specific recombinant proteins in cattle experimentally- and naturally-infected with paratuberculosis. Res Vet Sci 114: 244-253. [Crossref]

20. Fodstad FH, Gunnarsson E (1979) Post-mortem examination in the diagnosis of Johne's disease in goats. *Acta Vet Scand* 20: 157-167. [Crossref]

21. Teixeira P, Simões J (2008) A paratuberculose bovina em explorações leiteiras de pequena e média dimensão: que opções para o veterinário assistente? *PUBVET* 2: 1-33.

22. Radostits E, Gay C, Inchcliff K (2006) Veterinary medicine textbook of the disease of cattle, sheep, pigs, goats, and horses. (9th edn), Sounders Company Press, NY, USA. pp: 867-882.

23. Rodrigues A (2005) Paratuberculose em bovinos: Analises anatomoclínica, bacteriológica, imunoistoquímica e pela reação em cadeia da polimerase. PhD Thesis, Universidade Estadual do Norte Fluminense Darcy Ribeiro. pp: 109.

24. Patton EA (2011) Paratuberculosis vaccination. *Vet Clin North Am Food Anim Pract* 27: 573-580. [Crossref]

25. Collins MT, Lisby G, Moser C, Chicks D, Christensen S (2000) Results of multiple diagnostic tests for Mycobacterium avium subsp. paratuberculosis in patients with inflammatory bowel disease and in controls. *J Clin Microbiol* 38: 4373-4381. [Crossref]

26. Chiodini RJ, Rossiter CA (1996) Paratuberculosis: A potential zoonosis. *Vet Clin North Am Food Anim Pract* 12: 457-467. [Crossref]

27. Clarke CJ (1997) The pathology and pathogenesis of paratuberculosis in ruminants and other species. *J Comp Pathol* 116: 217-261. [Crossref]

28. Grant IR, Hywel J, Rowe MT (2002) Incidence of Mycobacterium paratuberculosis in bulk raw and commercially pasteurised cows' milk from approved dairy processing establishments in the United Kingdom. *Appl Environ Microbiol* 68: 2428-2435. [Crossref]

29. Eusebi PG, Cortés S. Dunner O, J Cañón (2017) Genomic diversity and population structure of Mexican and Spanish bovine Lidia breed. *Anim Genet* 48: 682-685. [Crossref]

30. Pelayo R, Valera M, Molina A, Royo LJ (2015) Contribution of Lidia cattle breed historical castes to the paternal genetic stock of Spain. *Anim Genet* 46: 312-315.

31. Méndez A, Hervás J, Gómez-Villamandos JC, Fernández A, Sierra MA (1995) Ostertagiasis in bulls used for bullfights. *Zentralbl Veterinarmed B* 42: 551-556. [Crossref]

32. Pacheco-Lima J, Moreira da Silva F (2019) Reproductive characterization of the bovine lidia called "Brava Dos Açores". *Biomed J Sci Tech Res* 15: 11014-11019.

33. Teymouri H, Mosavari N, Poor Taghi H (2016) Detection of Mycobacterium avium subsp. Paratuberculosis in cattle by using indirect absorbed ELISA (enzyme-linked immunosorbent assay) system and culture in Alborz Province, Iran. *Intern J Mycobac* 5: 220-221. [Crossref]

34. Lilenbaum W, Marassi CD, Oelemann WMR (2007) Paratuberculosis: An update. *Br J Microbiol* 38: 580-590.

35. Olsen I, Reitan LJ, Holstad G, Wilker HG (2001) Alkyl hydroperoxide reductases C and D are major antigens constitutively expresses by Mycobacterium avium subsp. Paratuberculosis. *Infect Immun* 68: 801-808. [Crossref]

36. Bech-Nielsen S, Jorgensens JB, Ahrens P, Feld NC (1992) Diagnostic accuracy of M. phlei absorved serum ELISA for diagnosis of bovine paratuberculosis in dairy cows. *J Clin Microb* 30: 613-618. [Crossref]

37. Sweeney Transmission of paratuberculosis. Vet Clin North Am Food Anim Pract 12: 305-312. [Crossref]

38. Collins CT (1996) Diagnosis of paratuberculosis. Vet Clin North Am Food Anim Pract 12: 357-371.

39. Whittington RJ, Marshall DJ, Nicholls PJ, Marsh IB, Reddacliff LA (2004) Survival and dormancy of Mycobacterium avium subsp. paratuberculosis in the environment. *Appl Environ Microbiol* 70: 2989-3004. [Crossref]

40. Stevenson K (2015) Genetic diversity of mycobacterium avium subspecies paratuberculosis and the influence of strain type on infection and pathogenesis: A review. *Vet Res* 46: 64-78. [Crossref]