academicJournals

Vol. 7(10), pp. 302-307, October 2015
DOI: 10.5897/JVMAH2015.0402
Article Number: 2178CF955217
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Prevalence and associated risk factors for *Mycobacterium avium subsp.* paratuberculosis in dairy cattle in Mexico

Feliciano Milián-Suazo[1*] Marco A. Santillán-Flores[2], Horacio Zendejas-Martínez[1], Leticia García-Casanova[2], Laura Hernández-Andrade[2] and Germinal J. Cantó-Alarcón[1]

[1]Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro. Avenida de las Ciencias S/N, Juriquilla. Delegación Santa Rosa Jáuregui, Querétaro, C.P. 76230. México.
[2]Centro Nacional de Investigación Disciplinaria en Microbiología Animal. Km. 15.5 Carretera México-Toluca. Colonia Palo Alto. Cuajimalpa, Distrito Federal. C.P. 05110. México.

Received 3 July, 2015; Accepted 26 August, 2015

The purpose of this study is to determine the seroprevalence and the associated risk factors to *Mycobacterium avium* subspecies paratuberculosis. A total of 4,487 serum samples were collected from cattle in 173 farms from different parts of Mexico. Information about potential risk factors and spatial location of the farms was obtained through a questionnaire to farm´ owners and by global positioning system (GPS) apparatus respectively. An enzyme-linked immunosorbant assay (ELISA) kit was used to detect the presence of antibodies against *paratuberculosis* (MAP). Maps showing areas of high risk of MAP and maps showing areas with environmental conditions for the presence of paratuberculosis were elaborated with Maxent. The overall prevalence of the disease was 5%. The higher seroprevalence was in family run systems (11.3%). The spatial analysis showed higher prevalence (6%) in the South Pacific region. Three factors had a significant relationship with prevalence of the disease: history of tuberculosis, grazing in open fields and belonging to the family run system.

Key words: *Mycobacterium*, paratuberculosis, dairy cattle, seroprevalence, Mexico.

INTRODUCTION

Paratuberculosis (PTB), or Johne's disease (JD), is a chronic, progressive, infectious granulomatous enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), which affects ruminants, especially dairy cattle, and a variety of domestic species (Manning and Collins, 2001; Kudahl et al., 2007). Characteristic symptoms include: diarrhea, progressive weight loss and death in adult animals. MAP has been reported to survive milk pasteurization (Grant et al., 2002), and has been related to Crohn's disease in humans (Timms et al., 2012). The main route of infection with MAP is the fecal-oral route, but it can also be transmitted through colostrum (Streeter et al., 1995), and milk from subclinical or clinical infected cows (Streeter et al., 1995; Sweeney et al., 1992; Taylor et al., 1981). In utero, infection has also been reported (Whittington and

*Corresponding author. E-mail: feliciano.milian@uaq.mx.

Windsor, 2009).

Clinical paratuberculosis has an important economic impact on the dairy industry; losses due to this disease in the dairy industry in the US have been estimated in 1.5 billion dollars annually (Merkal et al., 1987). No data about the economic impact of the disease is available in Mexico. The reasons for such losses are: low milk production (Benedictus et al., 1987), large calving intervals (Abbas et al., 1983), low slaughter weights (Whitlock et al., 1985), shorter life expectancy, loss of potential breeding value, infertility and increased incidence of mastitis (Buergelt and Duncan, 1978). Efforts to treat the disease or to develop a vaccine have not been successful. Until an effective cure or prevention is found, early diagnosis and scientific management practices alone will help in protecting the animals against this disease.

Johne's disease has a worldwide distribution. The prevalence in some countries is as high as 40%, as is the case for the United States of America (Sánchez-Villalobos et al., 2009), similar rates are reported in Canada (Sorensen et al., 2003). In cattle, it is endemic in The Netherlands, Austria, and Belgium, where the prevalence rate is 54, 7, and 41% respectively. In Europe, Sweden is the only country with a MAP-free status (Singh et al., 2008). In Australia, infection rates fluctuate between 9 and 22% (Sánchez-Villalobos et al., 2009) respectively. In the south west of England, the mean seroprevalence is 7.1% (Woodbine et al., 2009).

Latin America is not the exception; Argentina reports prevalence rates of 18.8% in dairy farms and 6.8% in beef farms. In Rio de Janeiro, Brazil, the prevalence is 33%. In Venezuela 72% of the herds is infected (Sánchez-Villalobos et al., 2009). In Mexico, reports from different studies, usually involving small sample sizes, show a prevalence of about 30%, especially in dairy cattle.

Therefore, the main objective of this study was to determine the seroprevalence of *M. avium* subspecies paratuberculosis in dairy cattle and the associated risk factors.

MATERIALS AND METHODS

The data

Data came from a large cross-sectional study in 173 farms conducted between January, 2010 and December, 2012. The study involved farms from different parts of Mexico which are under three main systems of milk production prevalent in the country: intensive, family-run and double-purpose farms. Intensive and family-run farms have Holstein-Friesian cattle, double-purpose farms have mainly *Bos indicus* breeds, which are primarily used for calving and, as a secondary purpose, milk production. The estimated sample size was about 3,500 animals, using a 10% hypothetical prevalence for brucellosis, 1% error and 95% confidence level. Even though the sample size estimated was 3, 500 animals, the final number of samples collected was 4,487. A stratified multistage sampling design was used. Considering that the population of dairy cattle is located in specific regions, each region was considered as a stratum in the first stage. In the second stage, states were selected within each stratum, and counties selected within each state. Counties were not randomly selected; instead, they were selected from a list of milk producing counties. Finally, due to the lack of a good sampling frame, convenience sampling was used to select herds and animals within herds. All sampling personnel were advised to select herds from different areas of each county which will form a representative sample. To reduce the total variance of sampling, the sampling fraction by stratum (region) was determined dividing the total number of samples by the total population (3,500/2,000 000=0.0019). Subsequently, to determine the number of animals sampled per stratum, the sample fraction was multiplied by the size of the population in each stratum.

Blood samples

Ten milliliters of blood were collected from each animal from the middle coccygeal vein with a 20-gauge, 1-in needle in a 10 ml serum-separator Vacutainer tube **(**Becton Dickinson and Company, Becton Dickinson Vacutainer Systems, Franklin lakes, NJ. 07417 to 1885. USA). Antibodies against PTB were determined by Enzyme-link-immunosorbent-assay (ELISA) kit **(**IDEXX Laboratories, Inc., Westbrook, ME, USA), using the protoplasmatic strain 3065 MAP (Martinez et al., 2012).

Epidemiological information

In order to collect epidemiological information, a questionnaire was administered to the owners of the herds to identify farm management practices and herd performance. The questionnaire included open items (any answer possible) and closed items (possible answers provided in the questionnaire) related to general characteristics of farms, such as size, breed and production, as well as target questions referring to potential risk factors for MAP transmission.

Statistical analysis

The statistical analysis was carried out in three phases. First, a univariate descriptive analysis was performed throughout frequencies and descriptive statistics, followed by a bivariate analysis to identify those variables potentially associated with MAP prevalence. Finally, all variables with a p value ≤ 0.20 were considered for a multivariate logistic regression model to obtain adjusted odds ratios. Analysis was performed with Epi info tm7.1.0.6. (Centers for Disease Control and Prevention) and SPSS (SPSS Inc.233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412 EE.UU).

Spatial information

All farms were spatially located using a spatial location apparatus (GPS). This information was used to estimate risk areas of the disease throughout geostatistical modeling (kriging). These analyses were performed with ArcView from ArcGis 10 (ESRI, Redlands, CA).

Ecological niche modeling

In order to determine a relationship between environmental variables from BIOCLIM (http://www.worldclim.org) and the presence of MAP, an ecological niche modeling with maxent was performed. Maps showing predicted relative suitability for the

Table 1. Prevalence of paratuberculosis in cattle in Mexican dairy farms, by system of production.

System of production	Positive animals	Total	Prevalence (%)
Intensive	105	2303	4.6
Dual-purpose	63	1617	3.9
Family-run	64	565	11.3
Total	232	4485	5.2

Table 2. Prevalence of paratuberculosis in cattle in Mexican dairy farms by state.

State	Positive animals	Total	Average prevalence (%)	Number of herds	Prevalence range (%)
Intensive					
Aguascalientes	13	147	9	7	0-29
Chihuahua	13	714	2	15	0-7
Coahuila	11	202	5	6	0-12
Durango	3	222	1	7	0-10
Guanajuato	14	147	10	4	2-22
Hidalgo	36	379	9	16	0-50
Querétaro	15	492	3	17	0-17
Family-run					
Jalisco	64	565	11	24	0-42
Dual-purpose					
Chiapas	6	509	1	20	0-14
Sinaloa	18	319	6	10	0-18
Veracruz	39	791	5	47	0-25
Total	232	4487	5	173	0-50

presence of cases were elaborated. Twenty-five percent of the herds were randomly selected to test the model accuracy. Environmental data used by maxent were: temperature and precipitation, and the 19 environmental variables from BIOCLIM with 2.5 min of resolution, converted to a common projection.

RESULTS

The overall herd seroprevalence *of M. paratuberculosis* in dairy cattle in Mexico was 48%, and the animal seroprevalence was 5% (range 0 to 50%). The highest animal prevalence was observed in the family-run system (11.3%), compared to the intensive (4.6%) and the double-purpose (3.9%) systems (Table 1). Results show that prevalence varies from state to state, from 1 to 11%, and in herds within a state, from 0 to 50% (Table 2). The highest prevalence was found in some herds in the State of Hidalgo (50%) which has a high density of dairy cattle (25000 dairy cattle) in 102 herds in the production complex "Tizayuca". This complex is characterized by the closeness of the farms, origin of replacement stock, national and international. According to herd size, prevalence was higher in herds of 200 to 300 cows (9.5%) and in farms using raw milk to feed calves (7.7%).

In order to identify risk factors associated to prevalence, a logistic regression analysis was performed. Very few factors showed significant correlation ($P<0.05$); animals with positive serology to tuberculosis, OR=2.9 ($CI_{95\%}$ 1.9 to 4.2), animals grazing in open fields, OR=2.1 ($CI_{95\%}$ 1.1 to 4.2), and the production system: OR=2.4 ($CI_{95\%}$ 1.2 to 4.8) for dual-purpose and OR=4.5 ($CI_{95\%}$ 3.1 to 6.7) in family-run systems (Table 3).

The spatial analysis to establish risk areas of paratuberculosis in dairy cattle showed that the highest risk is in the South Pacific region (over 6%), where the family-run system is located. In the Gulf of Mexico and central north regions, the prevalence is rather low, 0 to 6% (Figure 1). It is worth mentioning that the Northwest and Southeast areas of the country were not sampled. Therefore, prediction does not cover the whole country.

Figure 2, shows the ecological niche modeling (maxent) map with predicted relative habitat suitability for the presence of MAP. Habitat suitability increases from blue to yellow to red. The climatic variable with the highest prediction value in the model was seasonal temperature. The zones with favorable climatic conditions

Table 3. Adjusted odds ratio for risk factors associated with the prevalence of dairy cattle paratuberculosis in Mexican dairy herds.

Risk factor	Category	P	OR	95% CI	
				Lower	Upper
History of tuberculosis	No	-	-	-	-
	Yes	0.000	2.9	1.9	4.2
Feeding	Concentrates	-	-	-	-
	Open field Grazing	0.026	2.1	1.1	4.2
Production system	Intensive	-	-	-	-
	Double purpose	0.017	2.4	1.2	4.8
	Family run	0.000	4.5	3.1	6.7

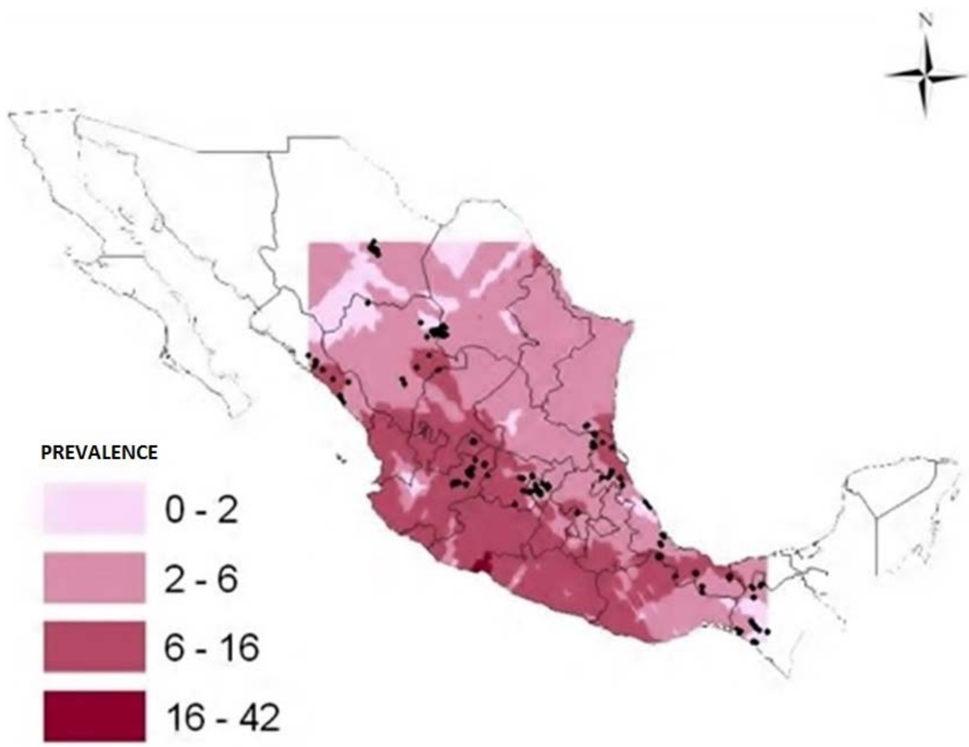

Figure 1. Predicted risk of paratuberculosis in dairy cattle in Mexico.

such as temperature and humidity for the survival of the organisms show highest incidence. These areas include Sinaloa, a region formed by parts of Jalisco, Guanajuato and Aguascalientes, and La Laguna, which includes parts of Coahuila and Durango, and some parts of Chiapas.

DISCUSSION

The average prevalence rate of paratuberculosis in cattle used for milk production in Mexico was 5%. This prevalence rate is similar to that obtained in some other parts of the world; 5% in the State of New York in the US (Obasanjo et al., 1997), 5.8% in England (Cetinkaya et al., 1997), but much lower than that reported in Michigan, also in the US (66%) (Johnson-Ifearulundu and Kaneene, 1998) and Alberta, Canada, 9.1% (Scott et al., 2006). In Latin American and Caribbean countries, the prevalence rate ranges from 16% in cattle to 4.3 in sheep and goats (Fernández-Silva et al., 2014).

Earlier reports of the prevalence of paratuberculosis varies: Salman et al. (1990) reported a prevalence of

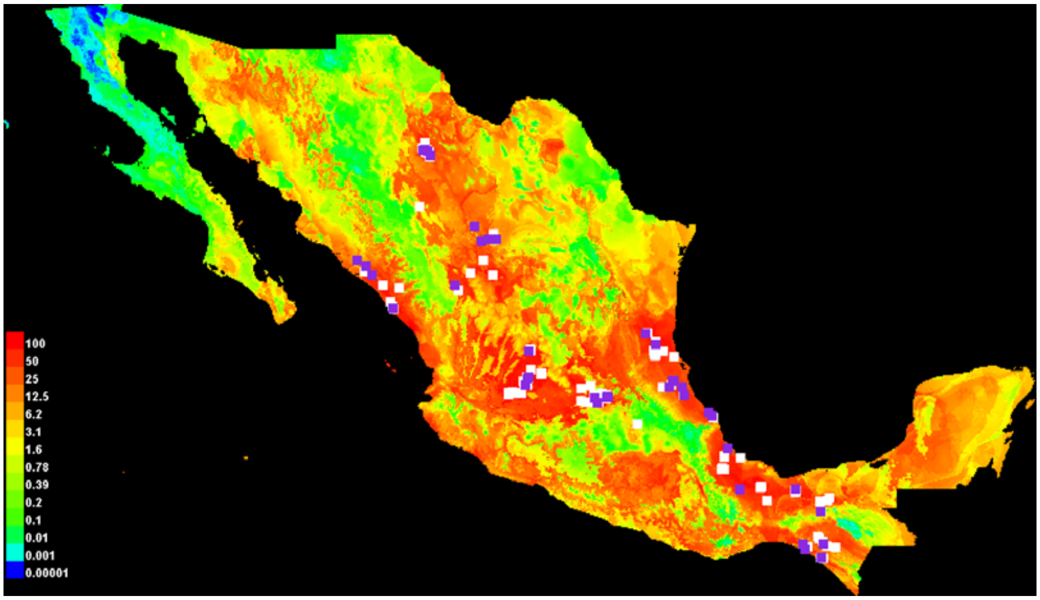

Figure 2. Predicted potential suitable areas for the presence of paratuberculosis in dairy cattle in Mexico. Areas in red indicate favorable conditions.

46% in 8,100 dairy cattle from 110 premises in Baja California. Chávez et al. (2004) reported a low prevalence of 13% in fighting bulls in Veracruz (Morales, 1994) while prevalence rates in dairy cattle and dual purpose cattle in Guanajuato were 30 and 25%, respectively (Santillán et al., 2003). An earlier report by Miranda (2005) in the Tizayuca dairy complex in the state of Hidalgo, indicated a prevalence rate of 8.8%, which is much lower than that shown in this study. The variation in the prevalence rate could be attributed to the low sample size in the earlier study as compared to this study survey.

In general, the main differences between this results and those from other studies are the sample size and the number of herds included in the study. Most studies in Mexico are based on low sample sizes and smaller geographic areas, whereas this study involved 173 herds from 11 states, including cattle from three different milk production systems. The study did not randomize the sample, and included a more representative population in comparison with earlier studies.

In this study, the highest prevalence was found in family-run systems (11.3%). This could be a consequence of the minimal use of technical services, such as veterinary support and technology. In addition, in this system cows are kept in production for longer periods of time (4.5 to 7 years) as opposed to the intensive system; therefore, there is a higher chance for developing this chronic disease.

The low prevalence (4.6%) in the intensive production system could be the result of the high replacement rate in this population, the average life span of a cow in this system is about 3.5 years. Cattle in the dual purpose system had a prevalence of 3.9%. The low prevalence rate in this area could be attributed to the low density of cattle per Km and also the tropical conditions prevailing in this area may be detrimental to the survival of the organism.

Results of the maxent model are shown in Figure 2. Habitat suitability for presence of *M. paratuberculosis* is observed in wide areas of the national territory, especially in the tropical, central and central north areas. However, maxent is a model that assumes random distribution of species, and in the present study the dairy herds are not randomly distributed. Therefore, this map should be taken with caution. The highest predicted habitat suitability in our study could be more the result of the location of human populations than environmental variables *per se*.

The ability to rapidly diagnose and identify the causative agent are critical for combating diseases and stopping epidemics (Wadhwa et al., 2012; Kaur et al., 2013). Recent technological developments have led to the proliferation of new and improved diagnostic tests that hold promise for a better management and control of infectious diseases (Wadhwa et al., 2014). New technologies such as microfluidics (Wadhwa et al., 2012) and “Lab-on- Chip” (Liu et al., 2011) are examples of promising new technologies with the potential to be used as a laboratory-free diagnostic devices for infectious diseases in animal husbandry.

This study indicated that the prevalence of MAP in dairy cattle in Mexico is low compared to previous reports either from Mexico or from our neighboring and European countries. Therefore, a practical recommendation for national authorities in animal health is to take actions now to reduce, and eventually eliminate, this disease from dairy cattle population before it spreads and prevalence increase.

Conflicts of interest

The authors have none to declare.

REFERENCES

Abbas B, Riemann HP, Hird DW (1983). Diagnosis of Johne's disease (paratuberculosis) in northern California cattle and a note on its economic significance. California Vet. 8:20-24.

Benedictus G, Dijkhuizen AA, Stelwagen J (1987). Economic losses due to paratuberculosis in dairy cattle. Vet. Rec. 121(7):142-146.

Buergelt CD, Duncan JR (1978). Age and milk production data of cattle culled from a dairy herd with paratuberculosis. J. Am. Vet. Med. Assoc. 173(5):478-480.

Cetinkaya B, Erdogan HM, Morgan KL (1997). Relationships between the presence of Johne's disease and farm and management factors in dairy cattle in England. Prev. Vet. Med. *32* (3): 253-266.

Chávez GG, Trigo FJ, Svastova TP, Pavlik EI (2004). Identificación del polimorfismo genético de aislamientos de *Mycobacterium avium* subsp. paratuberculosis de caprinos del centro de México. Vet. México 35:75-82.

Fernández-Silva JA, Correa-Valencia NM, Ramírez NF (2014). Systematic review of the prevalence of paratuberculosis in cattle, sheep, and goats in Latin America and the Caribbean. Trop. Anim. Health Prod. 46(8):1321-1340.

Grant IR, Ball HJ, Rowe MT (2002). Incidence of *Mycobacterium paratuberculosis* in bulk raw and commercially pasteurized cows' milk from approved dairy processing establishments in the United Kingdom. Appl. Environ. Microbiol. 68(5):2428-2435.

Johnson-Ifearulundu YJ, Kaneene JB (1998). Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA, dairy herds. Prev. Vet. Med. 37(1):41-54.

Kaur S, Singla LD, Hassan SS and Juyal PD (2013) Application of indirect plate ELISA in early diagnosis of paramphistomosis using purified polypeptides of somatic antigen of *Paramphistomum epiclatum.* Trends Parasitol. Res. 2(1):9-15.

Kudahl AB, Østergaard S, Sørensen JT, Nielsen SS (2007). A stochastic model simulating paratuberculosis in a dairy herd. Prev. Vet. Med. 78(2):97-117.

Liu X, Yang K, Wadhwa A, Eda S, Li S. Wu J. (2011). Development of an AC electrokinetics-based immunoassay system for on-site serodiagnosis of infectious diseases. Sens. Actuators A Phys. 171: 406-413.

Manning EJ, Collins MT (2001). *Mycobacterium avium* subsp. paratuberculosis: pathogen, pathogenesis and diagnosis. Rev. Sci. Tech. OIE 20(1):133-150.

Merkal RS, Whipple DL, Sacks JM, Snyder GR (1987). Prevalence of Mycobacterium paratuberculosis in ileocecal lymph nodes of cattle culled in the United States. J. Am. Vet. Med. Assoc. 190(6):676-680.

Miranda BMV (2005). Evaluación del impacto económico de la Paratuberculosis en ganado bovino lechero (sistema intensivo), en el Complejo Agropecuario Industrial Tizayuca, Hidalgo, México (Doctoral dissertation, Tesis de Maestría), México DF: Facultad de Medicina Veterinaria y Zootecnia, UNAM).

Morales RH (1994). Frecuencia de Paratuberculosis en ganado de lidia, Tlaxcala, (Tesis de Licenciatura), México DF. Facultad de Medicina Veterinaria y Zootecnia, UNAM 1994.

Obasanjo IO, Gröhn Y, Mohammed HO (1997). Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. Prev. Vet. Med. 32(3):243-251.

Salman MD, Hernandez JA, Braun I (1990). A seroepidemiological study of five bovine diseases in dairy farms of the coastal region of Baja California, Mexico. Prev. Vet. Med. 9(2):143-153.

Sánchez-Villalobos A, Arráiz-Rodríguez N, Becerra-Ramírez L, Faria N, Montero-Urdaneta M, Oviedo-Bustos A, Pino-Ramírez D (2009). Infección por *Mycobacterium avium* subsp. Paratuberculosis en un rebaño criollo limonero. Rev. Cient. 19(6):555-565.

Santillán FMA, Córdoba LD, Gúzman RCC (2003). Situación Epidemiológica de la Paratuberculosis en ganado bovino del estado de Guanajuato, México (Datos preliminares). Memorias de la XXXIX Reunión Nacional de la Investigación Pecuaria. (2003 octubre) 27-31. México, DF.

Scott HM, Sorensen O, Wu JT, Chow EY, Manninen K, VanLeeuwen, JA (2006). Seroprevalence of *Mycobacterium avium* subspecies paratuberculosis, *Neospora caninum*, Bovine leukemia virus, and Bovine viral diarrhea virus infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity. Can. Vet. J. 47(10):981.

Singh SV, Singh AV, Singh R, Sharma S, Shukla N, Misra S, Sandhu KS (2008). Sero-prevalence of Bovine Johne's disease in buffaloes and cattle population of North India using indigenous ELISA kit based on native *Mycobacterium avium* subspecies paratuberculosis 'Bison type'genotype of goat origin. Comp. Immunol. Microbiol. 31(5):419-433.

Sorensen O, Rawluk S, Wu J, Manninen K, Ollis G (2003). *Mycobacterium paratuberculosis* in dairy herds in Alberta. Can. Vet. J. 44(3):221.

Streeter RN, Hoffsis GF, Bech-Nielsen S, Shulaw WP, Rings DM (1995). Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am. J. Vet. Res. 56(10): 1322-1324.

Sweeney RW, Whitlock RH, Rosenberger AE (1992). *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. J. Clin. Microbiol. 30(1):166-171.

Taylor TK, Wilks CR, McQueen DS (1981). Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. Vet. Rec. 109(24):532-533.

Timms VJ, Gehringer MM, Mitchell HM, Daskalopoulos G, Neilan BA, Nielsen SS (2012). Isolation of *Mycobacterium spp*. from patients with inflammatory bowel disease. In Proceedings of the 11th International Colloquium on Paratuberculosis, Sydney, Australia, 5-10 February 2012 pp. 400-401.

Wadhwa A, Foote RS, Shaw RW, Eda S (2012b). Bead-based microfluidic immunoassay for diagnosis of Johne's disease. J. Immunol. Methods 382(1):196-202.

Wadhwa A, Hickling GJ, Eda, S. (2012b) Opportunities for improved serodiagnosis of human tuberculosis, bovine tuberculosis and paratuberculosis. Vet. Med Int. 2012(2012):1-13.

Wadhwa A, Rachel EJ, Eda K, Waters WR, Palmer MV, Bannantine JP, Eda S (2014). Evaluation of ethanol vortex ELISA for detection of bovine tuberculosis in cattle and deer. BMC. Vet. Res. 10(1):147.

Whitlock RH, Hutchison LT, Merkal RS, Glickman LT, Rossiter C, Harmon S, Spencer P, Fetrow J, Bruce JL, Benson CE, Dick J (1985). Prevalence and economic consideration of Johne's disease in the northeastern US. Proc. Ann. Meeting. US. Anim. Health Assoc. 89:484-490.

Whittington RJ, Windsor PA (2009). In utero infection of cattle with Mycobacterium avium subsp. paratuberculosis: a critical review and meta-analysis. Vet. J. 179(1):60-69.

Woodbine KA, Schukken YH, Green LE, Ramirez-Villaescusa A, Mason S, Moore SJ, Medley GF (2009). Seroprevalence and epidemiological characteristics of *Mycobacterium avium* subsp. paratuberculosis on 114 cattle farms in south west England. Prev. Vet. Med. 89(1):102-109.