J Parasit Dis (Apr-June 2021) 45(2):359–365
https://doi.org/10.1007/s12639-020-01312-7

ORIGINAL ARTICLE

Prevalence of antibodies to *Anaplasma* in cattle and buffaloes of different organized herds in India

Laxmi Narayan Sarangi[1] · Samir Kumar Rana[2] · Amitesh Prasad[1] · Nadikerianda Muthappa Ponnanna[1] · Girish Kumar Sharma[2]

Received: 14 May 2020 / Accepted: 3 November 2020 / Published online: 12 November 2020

Abstract Bovine anaplasmosis is one of the most important tick borne disease in ruminants causing huge economic loss to the dairy industry. A cross-sectional study was carried out to detect serum antibodies to *Anaplasma* infection in cattle and buffaloes housed in 14 organized herds located at various climatic zones spreading over 9 different states in India. A total of 911 serum samples, collected from 667 cattle and 244 buffaloes, were subjected to a competitive enzyme linked immune-sorbent assay detecting an epitope of major surface protein 5 (MSP5) of *Anaplasma*. The overall true prevalence was 48.72% (95% CI 45.13–52.32%). The prevalence rate was higher in cattle (51.58%) than buffaloes (40.89%) and the difference was statistically significant ($p < 0.05$). Indigenous cattle (59.30%) showed higher seropositivity than crossbreed (57.16%) and exotic cattle breeds (42.28%). Although statistically not significant, female (52.37%) showed higher seropositivity than male (46.43%). Similarly, significant difference in prevalence ($p < 0.05$) was observed for animals reared in different climatic zones with highest prevalence recorded in arid zone (90.49%) and lowest in semi-arid zone (29.83%). Very wide variation in prevalence (9.95–100%) was recorded between farms. The present study indicates endemicity of *Anaplasma* in India, similar to other tropical and sub-tropical countries of the world. Endemic instability was recorded in some of the studied farms suggesting possibility of outbreak of new clinical cases resulting in economic loss. Therefore, suitable policies and procedures for prevention and control of *Anaplasma* infection should be adopted in these farms.

Keywords Seroprevalence · *Anaplasma marginale* · India · Cattle · Buffaloes · ELISA · MSP-5

Introduction

Bovine anaplasmosis, primarily caused by *Anaplasma marginale*, is considered as one of the most important tick-borne disease in ruminants, especially in tropical and sub-tropical regions (Kocan et al. 2003; M'ghirbi et al. 2016; Maharana et al. 2016a). The organism is an Gram negative obligate intraerythrocytic rickettsial pathogen causing fever, anaemia, jaundice, anorexia, depression, weight loss, reduction in milk production, sporadic abortion and sometimes death during acute infection (Aubry and Geale 2011, Howden et al. 2010). However, the severity of the clinical signs varies considerably from asymptomatic to death of animals depending upon the species and the age of the infected animals with cattle infected as adult showing most severe clinical signs (Aubry and Geale 2011; Howden et al. 2010). The infected animals after recovery, become persistent carriers of the pathogen and play an important role in the epidemiology of the disease (M'ghirbi et al. 2016).

In India, it is one of the economically important disease affecting ruminants and the economic loss combined due to Babesiosis and Anaplasmosis has been estimated to be $ 57 million (Nair et al. 2013). However, the significance of the disease in endemic areas is underestimated owing to seasonal outbreaks and stable infection rates (M'ghirbi

✉ Dr. Samir Kumar Rana
skrana@nddb.coop

[1] National Dairy Development Board Research and Development Laboratory, IIL Campus, Gachibowli, Hyderabad, Telangana 500032, India

[2] National Dairy Development Board, Anand, Gujarat 388001, India

et al. 2016). The *Anaplasma* are transmitted by many species of ticks, predominantly by the genus *Rhipicephalus* (Tembue et al. 2011; Uilenberg 1995). The *Anaplasma* can also be transmitted mechanically by hematophagus arthropods (Kocan et al. 2003; Tembue et al. 2011). Further, iatrogenic transmission through blood contaminated fomites such as exposure to contaminated ear tagging, dehorning, castration instrument and needles has been reported to be important in the transmission of the organism in the farm (Morley and Hugh-Jones 1989; Oliveira et al. 2011; Tembue et al. 2011).

Following primary infection, the recovered animal usually becomes lifelong carrier. Diagnosis of carrier status is important for implementation of appropriate control measures. However, very few systematic study has been carried out in India on this neglected disease and most of them either used Giemsa staining method which cannot detect carrier status of the animals or molecular technique such as PCR method, but the sample size is low (Kumar et al. 2015; Sharma et al. 2015b; Maharana et al. 2016b; Ganguly et al. 2017, 2018, 2020). The aim of this cross-sectional study was to estimate the prevalence of the diseases in various organized herds as such information is required to assess the level of herd immunity to the disease, to know the enzootic stability of the disease which is necessary for implementation of disease control/ prevention measures in the farm (Gioia et al. 2018; Urdaz-Rodriguez et al. 2009; Paramanandham et al. 2019).

Materials and methods

Study design and sampling

For this study, the serum samples available in the NDDB R&D laboratory, Hyderabad (frozen at − 20 °C) were used. These serum samples were submitted by the organized farms for the periodic whole herd screening of animals for diagnosis of brucellosis and infectious bovine rhinotracheitis. In this cross-sectional study, 14 organized herds located at different parts of the country, India were selected. The sample size for the study was determined considering expected disease prevalence of 50%, acceptable error of 5%, a confidence level of 95%, desired sensitivity and specificity of test method to be 95% using epitools (http://epitools.ausvet.com.au) (Humphry et al. 2004; Sergeant 2017). As wide variation on prevalence of anaplasmasis has been reported in literature, 50% expected prevalence was used to get maximum sample size. Using the above specified inputs, the sample size required for the study was 475. In order to compensate the location variation and cluster effects, the sample size was increased by 50% and this leads to 713. The number of animals from each farm was determined by using the epitool epidemiological calculator for calculation of sample size to achieve specified herd level sensitivity (Sergeant 2017). In this calculation, the inputs were design prevalence 50%, test sensitivity 95%, population sensitivity and the population (herd) size of individual farm. After the required number of animals to be screened from each farm was determined, the individual animals to be screened for the study were selected by simple random sampling method using epitool epidemiological calculator (Sergeant 2017).

In this study, a total of 911 animals were screened for detection of serum antibodies to *Anaplasma marginale*. The sampled population contained more cattle (73.2%) than buffaloes (26.8%). In cattle population, the indigenous cattle (16.04%) were sampled less than the cross-breeds (41.08%) and imported breeds (42.87%). Further, the sampled population contained more male (61.5%) than female (38.5%).

Sample analysis

The serum samples were screened for presence of antibodies to *Anaplasma* species by using a commercially available competitive ELISA test (VMRD, USA). This test detects antibodies raised against an epitope of the major surface protein 5 (MSP5). The test was performed as per manufacturer's instruction. Briefly, 50 µL of the serum samples and the controls were added to the antigen coated ELISA plate and the plate was incubated for one hour at room temperature. After incubation, the plate was washed two times with wash buffer and 50 µL of conjugate (horseradish peroxidase (HRP)-labelled monoclonal antibody) was added to the test plate. Following 20 min of incubation the plate was washed four times with the wash buffer. Then 50 µL of substrate solution was added to test wells and incubated for 20 min at room temperature. After the incubation time 50 µL of stop solution was added and the optical density (OD) was measured in an ELISA reader (Tecan) at 650 nm. The percentage of inhibition (PI) was calculated for each sample as follows: PI = 100 [1 − (sample OD ÷ negative control OD)]. Test samples with PI value greater than or equal to 30% were considered positive and others having PI less than 30% were considered negative. The test procedure was considered proper if the mean OD of the negative control have OD value 1 to 2 and if the positive control have PI less than 30. The reported sensitivity and specificity of the kit is 96% and 95.2% respectively (Hairgrove et al. 2014; Seo et al. 2018; Torioni De Echaide et al. 1998).

Statistical analysis

The Chi-square test or Fisher's exact test were used to compare the prevalence results for species, breed, sex, agro-climatic region and farm. The difference is considered significant if the p value is less than 0.05. The calculations were performed using SPSS software (version 16). The apparent and true value was calculated by considering the imperfect test method with 96% sensitivity and 95.2% specificity by using online statistical software (epitools) (Sergeant 2017).

Results

Test results of 911 samples indicate the overall true seroprevalence of 48.72% and varies from 9.95–100% between farms (Table 1). The prevalence in cattle was 51.58% (95% CI 47.37–55.78%) whereas in buffaloes it was 40.89% (95% CI 34.21–47.86%) (Table 2). The analysis of the results on gender basis revealed female are more seropositive (52.37%, 95% CI 46.57–58.12%) than male (46.43%, 95% CI 41.88–51.03%) (Table 2). Sorting of results by cattle breed type revealed highest seropositivity in indigenous cattle (59.30%, 95% CI 48.91–69.01%) followed by cross-breeds (57.16%, 95% CI 50.67–63.44%) and imported breeds (42.28%, 95% CI 36.12–48.63%) (Table 2).

Discussion

In the present study, the overall true seropositivity detected in this study was 48.72% which is lower than those reported in Texas, USA (Hairgrove et al. 2015), Galapogas Island (90%) (Gioia et al. 2018). However, lower prevalence than this study has also been reported from many parts of the world *viz.*, Philippines (19.8%) (Ybanez et al. 2014), Tunisia (24.7%) (M'ghirbi et al. 2016). Although very scanty information is available in India, both higher (73.1%) (Singh et al. 2012) and lower prevalence (36.8%) (Sharma et al. 2015b) than this present study has been reported. In India, vaccination for control of bovine anaplasmosis is not practised and therefore, the seropositivity recorded in the study could be due to exposure to the *Anaplasma* species (Paramanandham et al. 2019). Further, to rule out the possibility of detection of maternal antibody in the serological assay, cattle and buffaloes above one year age were included in the study.

In this study, cELISA test targeting MSP5 antigen of *Anaplasma marginale* was used. This kit is considered to be most reliable screening test for diagnosis of

Table 1 Prevalence of antibodies to *Anaplasma* in different farms of India

Farm sl. no.	State	Climatic zone (Koppen classification system)	No. of samples tested	Apparent prevalence		True prevalence	
				% positivity	95% CI	% positivity	95% CI
Farm 1	Uttar Pradesh	Humid sub-tropical	90	34.44	25.45–44.72	32.72	22.72–44.13
Farm 2	Andhra Pradesh	Tropical wet and dry	29	62.07	44.00–77.31	63.41	43.34–80.35
Farm 3	Andhra Pradesh	Tropical wet and dry	44	61.36	46.62–74.28	62.63	46.25–76.98
Farm 4	Tamil Nadu	Tropical wet and dry	55	43.64	31.37–56.73	42.93	29.30–57.48
Farm 5	Gujarat	Semi-arid	68	36.76	26.30–48.64	35.29	23.67–48.49
Farm 6	Maharashtra	Tropical wet and dry	71	63.38	51.76–73.63	64.87	51.95–76.26
Farm 7	Chhattisgarh	Humid sub-tropical	48	47.92	34.47–61.67	47.69	32.75–62.97
Farm 8	Gujarat	Arid	59	86.44	75.46–92.97	90.49	78.29–97.74
Farm 9	Telangana	Tropical wet and dry	124	40.32	32.11–49.12	39.25	30.12–49.02
Farm 10	Haryana	Semi-arid	30	40.00	24.59–57.68	38.89	21.77–58.53
Farm 11	Gujarat	Semi-arid	86	13.95	08.17–22.82	9.95	03.52–19.80
Farm 12	Gujarat	Semi-arid	86	20.93	13.67–30.68	17.70	09.63–28.53
Farm 13	Telangana	Semi-arid	44	75.00	60.56–85.43	77.78	61.73–89.36
Farm 14	Uttarakhand	Humid sub-tropical	77	98.70	93.00–99.77	100.0	97.78–100.0
–	Total	–	911	48.85	45.61–52.09	48.72	45.13–52.32

Table 2 Prevalence of antibodies to *Anaplasma* in different variables

Type	Description	No. of samples tested	Apparent prevalence		True prevalence		*p* value
			% positivity	95% CI	% positivity	95% CI	
Species	Cattle	667	51.42	47.63–55.20	51.58	47.37–55.78	0.011
	Buffalo	244	41.80	35.79–48.07	40.89	34.21–47.86	
Cattle breed-type	Indigenous	107	58.87	49.41–67.74	59.30	48.91–69.01	< 0.001
	Cross breed	274	56.93	51.02–62.66	57.16	50.67–63.44	
	Exotic	286	43.35	37.74–49.15	42.28	36.12–48.63	
Sex	Male	560	46.79	42.69–50.93	46.43	41.88–51.03	0.118
	Female	351	52.14	46.92–57.31	52.37	46.57–58.12	
Climatic zone	Humid sub-tropical	215	60.47	53.80-66.76	61.63	54.22–68.62	< 0.001
	Tropical wet and dry	323	50.77	45.34–56.18	50.86	44.83–56.87	
	Semi-arid	314	31.85	26.94–37.19	29.83	24.38–35.77	
	Arid	59	86.44	75.46–92.97	90.49	78.29–97.74	
Total	–	911	48.85	45.61–52.09	48.72	45.13–52.32	

anaplasmosis (Knowles et al. 1996). USDA has approved this test for use in cattle (Dreher et al. 2005) and OIE also has suggested this test method for prevalence of infection/surveillance purpose (OIE 2015). It has been demonstrated that, the test can detect antibodies to *A. marginale* early during acute anaplasmaosis cases as well as during long-term persistence cases (Knowles et al. 1996). The specificity was found to be 100% (99% CI 98–100%) in sera collected in uninfected animals of non-endemic region (Knowle et al. 1996). The validation studies of the test method has revealed a sensitivity of 96% and specificity of 95% for diagnosis of anaplasmosis in cattle in endemic regions (Torini de Echaide et al. 1998). Fosgate et al. (2010) evaluated the diagnostic accuracy of MSP-5 cELISA, qPCR and card test in detection of *A. marginale* infection in dairy cattle of Puerto Rico and reported very high (99%) sensitivity of the cELISA test and suggested it as an appropriate screening test in detection of carrier animals.

The MSP5 is a 19 kDa surface protein and is highly considered among *Anaplasma* species (Visser et al. 1992; Torini de Echaide 1998). This cELISA test uses recombinant MSP5 (rMSP5) fused to maltose binding protein (MBP) as antigen and is detected by the monoclonal antibody AnaF16C1 as conjugate (Visser et al. 1992; Knowles et al. 1996). The cELISA test is based on the inhibition of Mab binding to rMSP5 by the test serum antibodies. However, as the MSP5 is a highly conserved protein among *Anaplasma* species and the epitope recognized by the mAb is common among *Anaplasma* species, so the cELISA test can detect antibodies to *A. bovis, A. centrale* and *A. phagocylaphilium* in addition to the targeted *A. marginale* strains (Knowles et al. 1996; Dreher et al. 2005). A negative test result suggest absence of *A. marginale* infection although the possibility of new infection or a transient period of very low *A. marginale* load cannot be ruled out especially in endemic setting (Dreher et al. 2005). A positive serological result implies the animal has developed antibodies to either *A. marginale* or the other cross-reactive pathogen *viz.*, *A. centrale* or *A. bovis* or *A. phagocytophlum*. Of these cross-reactivity agents, *A. centrale* is a less pathogenic organism whereas *A. ovis* is a pathogen of sheep but not infectious in cattle. *A. phagacylophilum* is an important species as it causes febrile disease in ruminant as well as human. However, to the best of our knowledge, *A. phagocytophilum* has not been reported from India. Therefore, in Indian scenario, the use of MSP5-cELISA is adequate for the screening purpose. If further differentiation is required, then molecular tests using specific primers from whole blood sample for detection of respective agents can be attempted.

A significant difference in seropositivity was observed between cattle breed types ($p < 0.001$). Somewhat surprisingly and in contrast to the previous reports, highest prevalence was detected in indigenous cattle (59.30%) followed by cross breed (57.16%) than imported cattle breeds (42.28%). However, the number of indigenous cattle screened in this study was less (n = 107) in comparison to cross breed (n = 274) and exotic breeds (n = 286). It is generally considered that local and cross-breed are more resistant to infection than pure imported breeds (Magona and Mayende 2002; Vetrivel et al. 2017). Further some authors have reported significant association of prevalence among indigenous, cross-breed ad exotic breeds (Ait Hamou et al. 2012). However, the finding of this study suggest, the relative susceptibility to infection is

less due to breed type than to the environment that the animal is born and reared. Animals born and reared in endemic areas acquire a natural immunity (presumption) at an early age (M'ghirbi et al. 2016) and further if the farm practices insecticide to control ticks and flies, then the animals can be maintained free of *Anaplasma* infection. Similar finding of higher seroprevalence in indigenous cattle breed has also been reported (Salih et al. 2009). They hypothesized the positivity is due to more vigorous and long lasting antibody response to *Anaplasma* in indigenous cattle breeds than cross-breeds and exotic breeds.

Analysis of the results revealed, although statistically not significant, female have higher seropositivity (52.37%) than male (46.43%). Previous studies has also reported higher prevalence of anaplasmosis in female than male with possible explanation that, females are preferred host for ticks over males and therefore, more susceptible to tick borne diseases (Seo et al. 2018). Similarly, in a study undertaken at Tamil Nadu, the prevalence of anaplasmosis in female (29.71%) was higher than their male (12.50%) counterpart (Vetrivel et al. 2017). In line with our findings, Tembue et al. (2011) has also reported no association of *Anaplasma* seropositivity with gender.

Sorting of the results by species of the animals screened suggest, cattle have higher seropositivity (51.58%) than buffaloes and this difference was statistically significant ($p = 0.011$). High prevalence of *Anaplasma* species infection in cattle than buffaloes has been reported from India (Sharma et al. 2015a; Filia et al. 2015; Paramanandham et al. 2019). Previous literature suggest, buffaloes are more resistant to *Anaplasma* infection than cattle, as they are mostly found on submerged wetlands and thus avoiding tick parasitism (Somparn et al. 2004; Terkawi et al. 2011). However, da Silva et al. (2014) in their study in water buffaloes in Northern Brazil could not observe any difference in susceptibility of different breeds of water buffaloes to infestation by ticks (*Rhipicephalus microplus*) or to infection with *Anaplasma* and they suggested the necessity of further studies related to geographical distribution tick and fly vector in survey areas. We are also of same opinion, rather than the species or cattle breed-type susceptibility to *Anaplasma* infection, the geographical location of the farm, the tick and fly management practices adopted by the farm are more important factor for infection and transmission of the infective agent.

Anaplasmosis is a tick borne disease and therefore, climate has an effect on the prevalence of the disease. In this study, 14 herds located at 9 different states of India were screened. The studied farms belonged to four different agro climatic zone as per the Koppan classification system: Humid sub-tropical (03 farms), Tropical wet and dry (05 farms), semi-arid (05 farms) and arid (01 farm). Sorting of data based on climatic zone revealed highest seropositivity in arid region (90.49%) followed by humid sub-tropical (61.63%), tropical wet and dry (50.86%) and semi-arid (29.83%) and this difference in seropositivity is statistically significant ($p < 0.001$). However, the number of farms studied in this study is very less with only one farm being screened from arid region. This finding is in contrast to M'ghirbi et al. (2016) who reported very low prevalence in arid (3.8%) and semi-arid (8.8%) zones and high prevalence in sub-humid (46.6%) and humid (25.6%) zones in cattle population of Tunisia. Similarly, in Morocco also higher prevalence was reported in sub-humid (52%) zones than humid (22.7%) and semi-arid zone (20%) (Ait Hamou et al. 2012). These differences in prevalence of *Anaplasma* infection in various agro-climatic zone could be due to diversity of tick fauna present in each locality and can also be due to tick and flies management strategies adopted at various farms (M'ghirbi et al. 2016).

In the present study, all the herds were found positive for *Anaplasma* infection with majority of the herds having more than 40% seropositivity. This suggests anaplasmasis is endemic in the country in line with other tropical and sub-tropical regions of the world. The true seropositivity range between the farms was very wide (9.95–100%) and is statistically significant ($p < 0.001$). Wide variation in the prevalence of anaplasmosis among various cattle management system has been reported (Kumar and Sangwan 2010). This could be due to managemental practices adopted by the farm such as use of acaricides to control ticks, drugs used by the veterinarians, zoo sanitary measures adapted by the farm and other scientific practices preventing intragenic transmission of the organism with in the farm.

Endemic stability concept has been proposed for vector borne diseases. In this epidemiological state, due to complex relationship between hosts, agent, vector and environment, the clinical cases in animals becomes rare occurrence despite of high infection level (Oliveira et al. 2011). In endemic regions, the animals are exposed to infection at an early age resulting in developing of immunity. This favours maintenance of the infection in the population with low tick infection level and very few clinical cases are observed (Guglielmone 1995; Oliveira et al. 2011). It has been reported in literature that herds with seroprevalence ranging from 1 to 40% are considered to be susceptible to new infection either from within the herd or from outside herd via vectors, or contaminated fomites (Tucker et al. 2016). Of the 14 farms studied here, 10 farms have more than 40% prevalence suggesting possibility of endemic stability. The other four farms have low prevalence which indicate the naïve adult animals are more susceptible to clinical cases (Guglielmone 1995; Oliveira et al. 2011). Therefore, suitable policies and procedures for

prevention and control of *Anaplasma* infection should be adopted in these farms to prevent disease outbreak.

Conclusions

In the present study, an overall seroprevalence of 48.72% was recorded. The presence of antibodies to *Anaplasma* in all the studied farms suggest endemicity of the disease in India, similar to other tropical and sub-tropical countries of the world. Endemic instability was recorded in some of the studied farms suggesting possibility of outbreak of new clinical cases resulting in economic loss. Therefore, suitable policies and procedures for prevention and control of *Anaplasma* infection should be adopted in these farms. Further, continuous monitoring of the disease status in the farms should be carried out to obtain information on the risk factors involved, the ticks and flies circulating in the farm for development of suitable tick control measures or vaccination of the animals and to evaluate the effectiveness of those adopted control measures.

Acknowledgements The authors are grateful to the management of the National Dairy Development Board (NDDB), Anand for providing necessary facilities and funding to carry out this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval In this study, serum samples submitted as part of routine disease screening program (Brucellosis and IBR) and stored in the repository of NDDB R&D laboratory, Hyderabad were used. These serum samples were collected by the organized herds as per the standard protocol. The present study does not come under the category of experimental research on animals, therefore, formal ethical approval is not required.

References

Ait Hamou S, Rahali T, Sahibi H, Belghyti D, Losson B, Goff W, Rhalem A (2012) Molecular and serological prevalence of *Anaplasma marginale* in cattle of North Central Morocco. Res Vet Sci 93:1318–1323

Aubry P, Geale DW (2011) A review of bovine anaplasmosis. Transbound Emerg Dis 58(1):1–30

da Silva JB, Vinhote WM, Oliveira CM, André MR, Machado RZ, da Fonseca AH, Barbosa JD (2014) Molecular and serological prevalence of *Anaplasma marginale* in water buffaloes in northern Brazil. Ticks Tick-Borne Dis 5(2):100–104

Dreher UM, de la Fuente J, Hofmann-Lehmann R, Meli ML, Pusterla N, Kocan KM, Woldehiwet Z, Braun U, Regula G, Staerk KDC, Lutz H (2005) Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. Clin Diagn Lab Immunol 12(10):1177–1183

Filia G, Mahajan V, Bal MS, Leishangthem GD, Singh A (2015) Seroprevalence of babesiosis and anaplasmosis in apparently healthy large ruminants of Punjab, India. Proc Natl Acad Sci India Sec B 85:885–888

Fosgate GT, Urdaz-Rodríguez JH, Dunbar MD, Rae DO, Donovan GA, Melendez P, Dobek GL, Alleman AR (2010) Diagnostic accuracy of methods for detecting *Anaplasma marginale* infection in lactating dairy cattle of Puerto Rico. J Vet Diagn Investig 22(2):192–199

Ganguly A, Bisla RS, Singh H, Vandna B, Kumar A, Kumari S, Maharana BR, Ganguly I (2017) Prevalence and haematobiochemical changes of tick-borne haemoparasitic diseases in crossbred cattle of Haryana, India. Indian J Anim Sci 87(5):552–557

Ganguly A, Maharana BR, Ganguly I, Kumar A, Potliya S, Arora D, Bisla RS (2018) Molecular diagnosis and hematobiochemical changes in Anaplasmamarginale infected dairy cattle. Indian J Anim Sci 88(9):989–993

Ganguly A, Maharana BR, Ganguly I (2020) Pentaplex PCR assay for rapid differential detection of Babesia bigemina, Theileria annulata, Anaplasma marginale and Trypanosoma evansi in cattle. Biologicals 63:81–88

Gioia GV, Vinueza RL, Marsot M, Devillers E, Cruz M, Petit E, Boulouis HJ, Moutailler S, Monroy F, Coello MA, Gondard M, Bournez L, Haddad N, Zanella G (2018) Bovine anaplasmosis and tick-borne pathogens in cattle of the Galapagos Islands. Transbound Emerg Dis 65(5):1262–1271

Guglielmone AA (1995) Epidemiology of babesiosis and anaplasmosis in south and central America. Vet Parasitol 57(1–3):109–119

Hairgrove TB, Schroeder ME, Budke CM, Rodgersb S, Chungd C, Uetie MW, Bounpheng MA (2015) Molecular and serological in-herd prevalence of *Anaplasma marginale* infection in Texas cattle. Prev Vet Med 119:1–9

Hairgrove TB, Craig TM, Budke CM, Rodgers SJ, Gill RJ (2014) Seroprevalence of *Anaplasma marginale* in Texas cattle. Prev Vet Med 116(1–2):188–192

Howden KJ, Geale DW, Paré J, Golsteyn-Thomas EJ, Gajadhar AA (2010) An update on bovine anaplasmosis (*Anaplasma marginale*) in Canada. Can Vet J 51(8):837–840

Humphry RW, Cameron A, Gunn GJ (2004) A practical approach to calculate sample size for herd prevalence surveys. Prev Vet Med 65:173–188

Knowles D, Torioni de Echaide S, Palmer G, McGuire T, Stiller D, McElwain T (1996) Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. J Clin Microbiol 34:2225–2230

Kocan KM, De La Fuente J, Guglielmone AA, Melendéz RD (2003) Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clin Microbiol Rev 16:698–712

Kumar PP, Sangwan AK (2010) Comparative prevalence of subclinical bovine anaplasmosis under different cattle management systems in Haryana. Haryana Vet 49:1–5

Kumar B, Maharana BR, Prasad A, Joseph JP, Patel B, Patel JS (2015) Seasonal incidence of parasitic diseases in bovines of south western Gujarat (Junagadh), India. J Parasit Dis 40(4):1342–1346

Magona JW, Mayende JSP (2002) Occurrence of concurrent trypanosomosis, theileriosis, anaplasmosis and helminthosis in Friesian, Zebu and Sahiwal cattle in Uganda. J Vet Res 69:133–140

Maharana BR, Tewari AK, Saravanan BC, Sudhakar NR (2016a) Important hemoprotozoan diseases of livestock: challenges in current diagnostics and therapeutics: an update. Vet World 9(5):487–495

Maharana BR, Kumar B, Prasad A, Patbandha TK, Sudhakar NR, Joseph JP, Patel BR (2016b) Prevalence and assessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India. Indian J Anim Res 50(5):733–739

M'ghirbi Y, Bèji M, Oporto B, Khrouf F, Hurtado A, Bouattour A (2016) *Anaplasma marginale* and *A. phagocytophilum* in cattle in Tunisia. Parasites Vectors 9(1):556

Morley RS, Hugh-Jones ME (1989) The effect of management and ecological factors on the epidemiology of anaplasmosis in the Red river plains and south-east areas of Louisiana. Vet Res Commun 13:359–369

Nair AS, Ravindran R, Lakshmanan B, Sreekumar C, Kumar SS, Raju R, Tresamol PV, Vimalkumar MB, Saseendranath MR (2013) Bovine carriers of *Anaplasma marginale* and *Anaplasma bovis* in South India. Trop Biomed. 30(1):105–112

OIE (Office international des epizooties) (2015) Bovine anaplasmosis. In: The manual of diagnostic tests and vaccines for terrestrial animals, O.I.E., pp 999–1013

Oliveira JB, Montoya J, Romero JJ, Urbina A, Soto-Barrientos N, Melo ES, Ramos CA, Araújo FR (2011) Epidemiology of bovine anaplasmosis in dairy herds from Costa Rica. Vet Parasitol 177(3–4):359–365

Paramanandham K, Mohankumar A, Puttahonnappa Suresh K, Susan Jacob S, Roy P (2019) Prevalence of *Anaplasma* species in India and the World in dairy animals: a systematic review and meta-analysis. Res Vet Sci 123:159–170

Salih DA, Abdel Rahman MB, Mohammed AS, Ahmed R, Kamal S, El Hussein AM (2009) Seroprevalence of tick-borne diseases among cattle in the Sudan. Parasit Res 104(4):845–850

Seo MG, Ouh IO, Lee SH, Son UH, Geraldino P, Rhee MH, Kwon OD, Kim TH, Kwa D (2018) Serological detection of antibodies against *Anaplasma* spp. in cattle reared in the Gyeongsangbuk-do, Korea. Korean J Parasitol 56(3):287–290

Sergeant ESG (2017) Epitools epidemiological calculators. Retrieved from http://epitools.ausvet.com.au/

Sharma A, Singla LD, Kaur P, Bal MS (2015a) PCR and ELISA Vis-à-vis Microscopy for detection of bovine anaplasmosis: a study on associated risk of an upcoming problem in North India. Sci World J 15:1–8

Sharma A, Singla LD, Tuli A, Kaur P, Bal MS (2015b) Detection and assessment of risk factors associated with natural concurrent infection of *Trypanosoma evansi* and *Anaplasma marginale* in dairy animals by duplex PCR in eastern Punjab. Trop Anim Health Prod 47:251–257

Singh NK, Singh H, Jyoti Haque M, Rath SS (2012) Prevalence of parasitic infections in cattle of Ludhiana district, Punjab. J Parasit Dis 36(2):256–259

Somparn P, Gibb MJ, Markvichitr K, Chaiyabutr N, Thummabood S, Vajrabukka C (2004) Analysis of climatic risk for cattle and buffalo production in northeast Thailand. Int J Biometeorol 49:59–64

Tembue AA, da Silva JB, da Silva FJ, Pires MS, Baldani CD, Soares CO, Massard CL, da Fonseca AH (2011) Seroprevalence of IgG antibodies against *Anaplasma marginale* in cattle from south Mozambique. Rev Bras Parasitol Vet 20(4):318–324

Terkawi MA, Huyen NX, Shinuo C, Inpankaew T, Maklon K, Aboulaila M, Ueno A, Goo YK, Yokoyama N, Jittapalapong S, Xuan X, Igarashi I (2011) Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water buffaloes in the northeast region of Thailand. Vet Parasitol 78:201–207

Torioni de Echaide S, Knowles DP, McGuire TC, Palmer GH, Suarez CE, McElwain TF (1998) Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzymelinked immunosorbent assay using recombinant major surface protein 5. J Clin Microbiol 36:777–782

Tucker TR, Aly SS, Maas J, Davy JS, Foley JE (2016) Investigation of *Anaplasma marginale* seroprevalence in a traditionally managed large California beef herd. Vet Med Int 16:6186078

Uilenberg G (1995) International collaborative research: Significance of tick-borne hemoparasitic diseases to world animal health. Vet Parasitol 57:19–41

Urdaz-Rodríguez JH, Fosgate GT, Alleman AR, Rae DO, Donovan GA, Melendez P (2009) Seroprevalence estimation and management factors associated with high herd seropositivity for *Anaplasma marginale* in commercial dairy farms of Puerto Rico. Trop Anim Health Prod 41:1439–1448

Vetrivel D, Pandian ASS, Shree JS (2017) A study on predisposing factors for the prevalence of anaplasmosis in dairy cattle. J Entomol Zool Stud 5(6):1228–1230

Visser ES, McGuire TC, Palmer GH, Davis WC, Shkap V, Pipano E, Knowles DP Jr (1992) The *Anaplasma marginale* msp5 gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. Infect Immun 60(12):5139–5144

Ybanez AP, Ybanez RHD, Cruz-Flores MJ, Xuenan X, Yokoyama N, Inokuma H (2014) High genetic diversity of *Anaplasma marginale* detected from Philippine cattle. J Vet Med Sci 76:1009–1014