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Full Length Article

First Report on Transplacental Transmission of *Anaplasma marginale* in Neonatal Dairy Calves from District Jhang, Punjab, Pakistan

Farhan Ahmad Atif[1,] *, Kashif Hussain[1,2], Muhammad Fiaz Qamar[3], Muhammad Sohail Sajid[2], Muhammad Arfan Zaman[3] and Muhammad Kamran Rafique[3]

[1]*Medicine Section, Department of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang; (Sub-campus) University of Veterinary and Animal Science, Lahore, Pakistan*
[2]*Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan*
[3]*Department of Pathobiology, College of Veterinary and Animal Sciences, Jhang; University of Veterinary and Animal Science, Lahore, Pakistan*
*For correspondence: farhan.atif@uvas.edu.pk; atifvet_2000@yahoo.com
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Abstract

The current study was planned to evaluate the transplacental transmission potentials of *Anaplasma marginale* among naturally infected adult dairy cattle in district Jhang, Punjab, Pakistan. A dairy farm was selected after a district level survey having highest number of blood smear, cELISA and PCR positives of *A. marginale* infected pregnant crossbred cows (Holstein Friesian × Cholistani). Blood samples were collected from dams (within 4 months of their pregnancies and at the time of parturitions) as well as from the new-born calves (before colostrum feeding within 3 h of the birth). Eighty pregnant cows were screened through the cELISA and PCR. Out of these, 54 were found positive for anaplasmosis. Further follow up revealed that 38 cows remained positive at the time of parturition. Finally, based on the criteria, 32 calves without colostrum feeding qualified for the vertical transmission trial. The results of present study revealed that 28 (9/32) and 13% (4/32) of the neonatal calves acquired intrauterine infection confirmed by cELISA and PCR, respectively. Overall occurrence of the transplacental transmission was 31%. Nevertheless, we concluded that transplacental transmission occurs and this route of transmission, can lead to a significant number of neonatal deaths. Hence, the transplacental route of disease transmission should also be considered for devising the prevention and control strategies regarding anaplasmosis in the dairy cattle.

Keywords: *Anaplasma*; Transplacental transmission; cELISA; PCR; Punjab; Pakistan

Introduction

Anaplasmosis is a tick-borne infectious disease, caused by an obligatory intracellular pathogen of genus *Anaplasma (A.)*; causing heavy economic losses, worldwide including Pakistan (Sajid *et al.* 2014; Atif 2016; Abbas *et al.* 2020; Spare *et al*. 2020). The bovine anaplasmosis (BA), mostly prevalent in the tropical, and the subtropical regions, is transmitted biologically by the ticks, mechanically by the mosquitoes, lice, biting flies, contaminated fomites and trans placentally through placenta from mother to the offspring (Aubry and Geale 2011; Costa *et al.* 2016; Karim *et al.* 2017; Rehman *et al.* 2019). *Rhipicephalus microplus* is the major vector of the BA, worldwide; nevertheless, competent vector of the BA in the region is not known. Transplacental transmission of anaplasmosis occurs mainly during the second and third trimesters of pregnancy (Zaugg and Kuttler 1984; Ribeiro *et al.* 1995; Grau *et al.* 2013) and may lead to death of the new-borne calves (Vos *et al.* 1976; Pypers *et al.* 2011; Santarosa *et al.* 2013). Transplacental transmission potential permits the bacterium to adapt different transmission strategies (Estrada-Peña *et al.* 2009). Usually, transplacental transmission has been commonly described during the case series, experimental and longitudinal studies (Pypers *et al.* 2011; Grau *et al.* 2013; Silva *et al.* 2015; Costa *et al.* 2016; Nazar *et al.* 2018; Henker *et al.* 2020). *Anaplasma* is being transferred from the dams to the calves through placenta (Grau *et al.* 2013; Silvestre *et al.* 2016; Costa *et al.* 2016). Different studies reported the mortality in calves of the infected dams due to vertical transmission of the pathogen (Pypers *et al.* 2011; Santarosa *et al.* 2013; Henker *et al.* 2020).

Molecular and serological techniques are more specific and sensitive towards the detection of anaplasmosis as compared to the conventional blood smear microscopy (Brito *et al.* 2007; Atif, 2016; Wen *et al*. 2016; Farooqi *et al.*

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2018; Rehman *et al.* 2019). The improved competitive ELISA (cELISA) is the most used serological test for the detection of *Anaplasma* antibodies in cattle with higher sensitivity (100%) and specificity (99.7%) (Chung *et al.* 2014). This uses a monoclonal antibody (MAb) specific for the surface protein 5 (MSP5). Confirmatory diagnosis is usually based on the serology followed by the molecular tests (Atif 2016). Only one study has reported the transplacental transmission in Khyber Pakhtunkhwa province, Pakistan (Nazar *et al.* 2018). However, status of the trans-placental transmission in the natural infection of anaplasmosis in the dairy cattle is lacking from Punjab, Pakistan. Therefore, the current study was planned to evaluate the trans-placental transmission potentials of *A. marginale* in the adult dairy cattle from Jhang district, Punjab, Pakistan.

Materials and Methods

Study location and sampling criteria

Adairy farm located at Moza KotSai Singh, Jhang was selected (31.2761° N, 72.3496° E), based on highest number of positive pregnant crossbred (Holstein Friesian x Cholistani) dairy cattle, identified from a district level survey. In Jhang district, molecular based herd prevalence of *A. marginale* in crossbred and exotic cattle was 35.48 and 56.76%; respectively (Annual Project Report, Pakistan Science Foundation, Pakistan, Project # PSF/NSLP-UVAS (967). First batch of the blood samples were collected from asymptomatic carrier cows (within 120 days of the gestation/before parturition). The second batch of the samples was collected just after parturition and subsequently, from their newborn calves (before colostrum feeding). However, there was a history of clinical disease seven months prior to sampling. Species of ticks were identified based on the morphological features using taxonomic key (Walker *et al.* 2014). The blood samples collected from the dairy farm were transported to laboratory in an ice box for further analysis.

Serology

The blood samples were collected in a Vacutainer (BD Vacutainer®SST™), containing polymer gel and spray-coated silica for separation of the sera. The samples were centrifuged for 5 mins at 5000 rpm. Sera were separated and stored at –20°C until used for cELISA.

Competitive ELISA

The cELISA was performed using Anaplasma Antibody Test Kit (cELISA v. 2; Catalog No. 283-2) as described by Veterinary Medical Research & Development (MRD) Inc., Pullman, WA, USA. The wells with no color change were considered as positive and those with blue color were considered as negative. The intensity of blue described the percentage of positivity. Furthermore, the results were recorded with the help of ELISA reader (Biobase-EL10A; China) at 630 nm wavelength. The samples with inhibition ≥ 30% were considered positive. Conversely, the samples with < 30% inhibition were considered negative.

Isolation of the genomic DNA from the blood samples

The DNA was extracted using Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermofisher Scientific; Catalogue No. K0782) following the manufacturer's guidelines. Briefly, 200 μL of the blood sample was filled in an Eppendorf tube and 'Proteinase K Solution' (20 μL) was added. Later, lysis solution (400 μL) was added and mixed by vortexing (MS-X DLAB; U.S.A.) followed by an incubation at 56°C in water bath (APin, Samheung Energy) for 10 mins and vortexed. Subsequently, 200 μL of ethanol (96–100%) was added followed by the reverse pipetting. The mixture was shifted to a spin column containing collection tube included in the kit, and centrifuged (8,000 rpm) for 1 min in microcentrifuge machine (D2012plus DLAB, USA). The column was washed twice with 500 μL Wash Buffer and centrifuged. At the end, 200 μL elution buffer was added to remove the genomic DNA. Finally, the spin column was disposed off after centrifugation (10,000 rpm for 1 min). The micro-centrifuge tube containing the purified DNA was stored at -20°C until used for further processing.

PCR

The PCR was based on amplification of MSP1b gene using master-mix (Dream taq green PCR master mix; catalogue No. K1081). The MAR1bB2 primers (forward: 5'-GCT CTA GCA GGT TAT GCG TC-3' and reverse primer 5'-CTG CTT GGG AGA ATG CAC CT-3') were utilized for the detection of 265 base pair DNA product, which specifically amplify *A. marginale* in the bovine blood samples (Bilgiç *et al.* 2013). A total of 35 cycles (initial heating and denaturation at 94°C for 3 mins, annealing at 55°C for 50 seconds and extension at 72°C for 1 min using thermal cycler (T100; Bio Rad, U.S.A.). Positive control was obtained from the Institute of Pure and Applied Biology, Bahauddin Zakariya University (BZU), Multan, Pakistan; isolated from whole frozen blood of *Bubalus bubalis* (Layyah district, Pakistan). Whereas sterile distilled water was used as a negative control. Furthermore, the PCR products along with positive and negative controls were analyzed on 1.3% agarose gel having ethidium bromide at the rate of 0.5 μg/μL of gel in 1X TAE buffer using 100 bp DNA ladder (Gene Ruler 100 bp DNA Ladder, Catalog No. SM0323; Thermo-Fisher Scientific, USA). Gel electrophoresis was performed at 90 V, and 400 amp (maximum) for 30 min or until the dye migrated to the two-third of the gel. Finally, the gel image was captured using

Transilluminator (Catalog no. MUVB-112; Major Scientific, USA).

Results

Transplacental transmission

From the sampling frame a dairy farm was selected for transplacental transmission study having highest number of positive pregnant crossbred cattle after district level survey. At an initial screening, 54 pregnant animals were found positive for *A. marginale* (within 120 days of gestation) and 38 cows remained positive until parturition with both detection methods (2nd blood sampling). We managed to get blood of 32 newborn calves (before colostrum's feeding) out of 38 positive dams. Blood samples of the six calves were not taken because they had ingested colostrum. In the present study, overall transplacental transmission rate of *A. marginale* in neonatal crossbred calves was 31%; whereas occurrence of 12 (4/32) and 28% (9/32) was noticed using PCR and cELISA, respectively. The DNA product with 265 bp was detected using PCR. Three calves found positive from both detection methods (Table 1; Fig. 1–3). The cutoff values for validation of negative control with optical density ranging from > 0.40 to < 2.10 and inhibition of >30% for positive controls was considered. Furthermore, *Rhipicephalus microplus* and *Hyalomma anatolicum* species of ticks were identified based on the morphological features from the selected dairy farm.

Discussion

Most of the earlier reports have demonstrated transplacental transmission for *A. marginale* and *A. phagocytophilum* during case series and experimental studies (Pypers *et al.* 2011; Grau *et al.* 2013; Silva *et al.* 2015; Costa *et al.* 2016; Nazar *et al.* 2018; Stuen *et al.* 2018; Henker *et al.* 2020). However, limited longitudinal studies have mentioned intra-uterine transmission during natural infection. So far, there is a single report of the vertical transmission from Khyber Pakhtunkhwa (KPK), Pakistan with occurrence of 13.7% transplacental transmission rate of *A. marginale* in cattle (Nazar *et al.* 2018). Additionally, during a survey from limited samples, prevalence of *A. marginale* was mentioned as 45.83 and 34.3% using qPCR (MSP1a gene) and indirect ELISA (iELISA), respectively from Peshawar (KPK). Nevertheless, they did not mention the sampling sources, disease status of dams (at parturition), breed of new-born calves and their dams as well as whether neonates have ingested colostrum before blood sampling. These are important aspects prior to validate transplacental transmission. In the present study, for the detection of anaplasmosis MSP1b and recombinant MSP5 (rMSP5) genes were utilized for PCR and cELISA; respectively. Furthermore, the different gene targets for various PCRs and serodiagnostic kits yield variable sensitivity and specificity (cELISA *vs*. iELISA) (Chung *et al.* 2014; Atif 2015).

Table 1: Results of competitive ELSIA with percent inhibition and their interpretation for the detection of anaplasmosis in selected cattle population of Jhang district, Punjab, Pakistan

Sr. No.	Sample ID on ELISA plate	OD Value	% Inhibition	Interpretation
1	A1	0.64	57.33333	Positive Control
2	A2	1.5	0	Negative Control
3	A3	1.601	-6.73333	Negative
4	A4	1.443	3.8	Negative
5	A5	1.283	14.46667	Negative
6	A6	0.718	52.13333	Positive*
7	A7	1.493	0.466667	Negative
8	A8	0.845	43.66667	Positive*
9	A9	0.708	52.8	Positive*
10	A10	1.72	-14.6667	Negative
11	A11	1.68	-12	Negative
12	A12	0.68	54.66667	Positive*
13	B1	1.32	12	Negative
14	B2	1.671	-11.4	Negative
15	B3	0.83	44.66667	Positive*
16	B4	1.29	14	Negative
17	B5	1.88	-25.3333	Negative
18	B6	1.364	9.066667	Negative
19	B7	1.674	-11.6	Negative
20	B8	0.902	39.86667	Positive*
21	B9	1.56	-4	Negative
22	B10	1.801	-20.0667	Negative
23	B11	0.742	50.53333	Positive*
24	B12	1.308	12.8	Negative
25	C1	1.532	-2.13333	Negative
26	C2	0.684	54.4	Positive*
27	C3	1.637	-9.13333	Negative
28	C4	1.781	-18.7333	Negative
29	C5	1.407	6.2	Negative
30	C6	0.821	45.26667	Positive*
31	C7	1.583	-5.53333	Negative
32	C8	1.734	-15.6	Negative
33	C9	1.625	-8.33333	Negative
34	C10	1.742	-16.1333	Negative

*These are positive samples (Percent inhibition greater than 30%) as mentioned by manufacturer a positive sample must have inhibition ≥ 30%

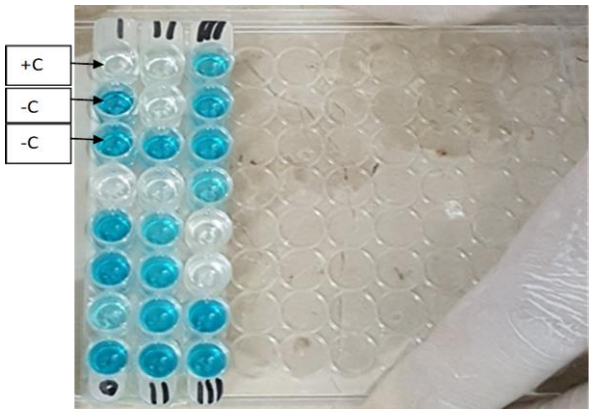

Fig. 1: The cELISA results showing color change after addition of stop solution and arrow heads indicate positive and negative samples. The –C and +C on top of the wells represent negative and positive controls; respectively

Ixodidae ticks, act as biological vector and play essential role in spread and propagation of disease during

different lifecycle stages. Biological transmission *A. marginale* is accomplished through ticks, mechanically through biting flies, fomites and tans-placental spread (during 3rd and 4th trimesters of pregnancy) (Dikmans 1950; Zaugg 1985; Rikihisa 1991). Biting insects chiefly from order Dipteran and Phthiraptera such as horse flies (Tabanus), Stable flies (Stomoxys), deer flies (chrysops), eye flies (Hippelates) and mosquitoes (Psorophora) contribute for mechanical transmission. Contrary to other tick-borne diseases anaplasmosis is also predominant in tick free areas. In tick free zones the flies meaningfully contribute for mechanical transmission of disease (Dikmans 1950; Ewing 1981; Hawkins *et al.* 1982; Silva *et al.* 2014). The exotic and crossbred cattle had genetic susceptibility to ticks and tick-bone diseases; remain persistently infected with higher potential for vertical transmission.

The cELISA detects *A. marginale* antibodies in undiluted serum samples by inhibiting the binding of horseradish peroxidase (HRP) labeled monoclonal antibody (conjugate) coated with each wells of microtiter plate. Recombinant major surface protein5 (rMSP5) along with Glutathione S-transferase fusion protein is attached with the plate wells as antigen. Glutathione S-transferase fusion protein help to minimize cross reaction with bacterial proteins. This test proved 99.7% specific and 100% specific (Chung *et al.* 2014). The discrepancy in our molecular and serological results may be due to the fact that dams were carrier during gestation. We detected higher persistently infected cows, transferred immunoglobulins to their calves as detected by cELISA. The calves that were positive through both of the detection methods (cELISA and PCR) were justified as they had the latent infection. Nevertheless, neonates who were positive with PCR and negative through cELISA were suggestive of the recent infection. However, young ones with positive cELISA and negative PCR possibly have had very low bacteremia and infection could have been controlled by the fetus (Zaugg and Kuttler 1984). Immunosuppressive conditions during the gestation period also contribute in the reoccurrence of infection in dams and increase the chances of transplacental transmission of infections. During peripartum period, transitional immunosuppression occurs which leads to the subclinical infection and may be the possible cause of an *in-utero* transmission of anaplasmosis (Silva and Fonseca 2014). Furthermore, Pypers *et al.* (2011) reported that there is a correlation between immunosuppression of dams and death in calves. Earlier reports published on the congenital anaplasmosis in calves had led to undiagnosed neonatal deaths (Grau *et al.* 2013).

Different diagnostic tests with variable detection limits can yield different vertical transmission rates. For example, Grau and associates has detected higher sero-positivity using indirect fluorescent antibody test (100%) as compared to the indirect ELISA (97%) during a survey. The PCR-based occurrence of the transplacental transmission was 10.5% in Braford calves from Pelotas, Brazil (Grau *et al.*

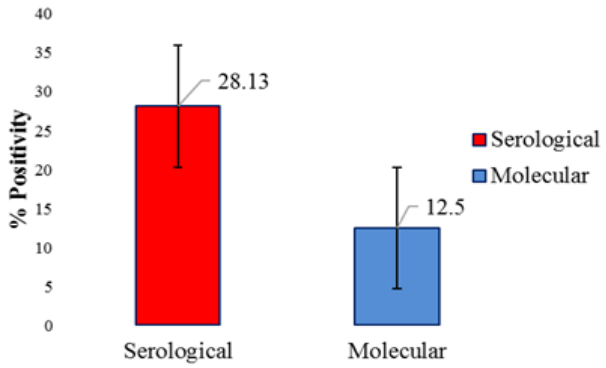

Fig. 2: Transplacental transmission of *A. marginale* detected by serological (cELISA) and molecular (PCR) techniques. The error bar indicates standard error

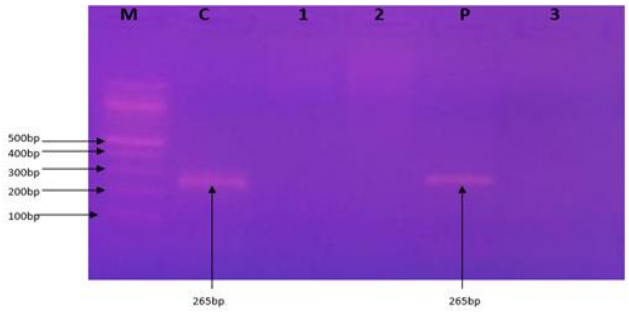

Fig. 3: Agarose gel electrophoresis of *A. marginale* targeting cytob1 gene with DNA product of 265 bp visualized on Transilluminator (M= Marker/Ladder; C=Control; P= Positive sample)

2013). Furthermore, they mentioned that not a single calf was found positive for anaplasmosis with ELISA and 10% of the calves were positive with IFAT. In contrast, we noticed the transmission rate of 28% with cELISA, perhaps due to larger number of the carrier animals in our study. Nevertheless, Silvestre *et al.* (2016) demonstrated occurrence of 10% vertical transmission in male Holstein calves from Minas Gerais, Brazil. They depicted lower transmission rate of *A. marginale* using nested PCR (MSP4) than our results, possibly due to difference in regional tick control and managemental practices. Likewise, transplacental transmission rate of 15.6% was reported by Potgieter and Rensburg (1987) in *Anaplasma*-infected calves kept under laboratory conditions in South Africa using a serological test, rapid card agglutination test. Conversely, Costa *et al.* (2016) mentioned higher 26.47% transplacental positivity in the crossbred neonatal calves using the nested PCR. Siva and his colleagues from Rio de Janeiro reported higher occurrence of the transplacental transmission 41% (Silva *et al.* 2015). Likewise, Salabarria and Pino (1988) from Cuba mentioned higher 86.4% (32/37) frequency of vertical transmission under clinical anaplasmosis in the last month of the gestation. The variation in results might be due to different of diagnostic

techniques, genetic diversity and different agro-climatic conditions of area (Costa *et al.* 2016). Taken together, lower transmission rate might be due to susceptibility of dams towards infection and environmental conditions in comparison to Jhang, Pakistan.

Our findings are supported by Pohl and their colleagues; they mentioned that the vertical transmission in cattle is mainly due to persistent infection in a population (Pohl *et al.* 2013). The rate of *in-utero* transmission depends upon the timing of fetal infection during gestation as the occurrence of transmission is higher at the end of gestation. Nonetheless, Henker and co-workers identified anaplasmosis/babesiosis infected cases of abortion; stillbirth and neonatal deaths in neonatal Angus/crossbred beef calves from Rio Grande do Sul (Southern Brazil). They stressed the importance of considering anaplasmosis in differential diagnosis (Henker *et al.* 2020). Concisely, the transmission potential may vary due to the detection methods (as well as their sensitivities), climatic conditions, region, host/breed, vectors, and pathogenic characteristics (Costa *et al.* 2016).

Conclusion

Anaplasmosis might be one of the major causes of mortality in young cattle calves in Pakistan. We reported first occurrence of the transplacental transmission of *A. marginale* in the pregnant dairy cows in Jhang district of Punjab, Pakistan using cELISA and PCR. This would be an important route of *Anaplasma* transmission in cattle and can lead to significant number of neonatal deaths. Based on our conclusion, following recommendations are suggested: (a) Anaplasmosis might be one of the major causes of mortality in young cattle calves, further studies are needed to explore the transplacental transmission potential of the disease in buffalo calves and other domestic animals. (b) Early treatment of the calves or preventive therapy can minimize the risk of mortality. (c) Enhancing dam's immunity in general or specifically against bovine anaplasmosis can help to reduce calf mortality.

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Author Contributions

FAA was involved in the conceptualization, planning, interpretation of results, and proof-reading; KH typed the manuscript, performed research work, and made illustrations; MSS planned the study design and proof-read; MFQ, MAZ and MKR helped in the conceptualization, and interpretation of the results.

References

Abbas MZ, M Saqib, F Deeba, MS Sajid (2020). Impact of sub-clinical anaplasmosis on haemato-biochemical parameters of naturally infected camels (*Camelus dromedaries*). *Pak J Agric Sci* 57:963–967

Atif FA (2016). Alpha proteobacteria of genus Anaplasma (Rickettsiales: Anaplasmataceae): Epidemiology and characteristics of *Anaplasma* species related to veterinary and public health importance. *Parasitol* 143:659–685

Atif FA (2015). *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. *Parasitol Res* 114:3941–3957

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

Bilgiç HB, T Karagenç, M Simuunz, B Shiels, A Tait, H Eren, W Weir (2013). Development of a multiplex PCR assay for simultaneous detection of *Theileria annulata*, *Babesia bovis* and *Anaplasma marginale* in cattle. *Exp Parasitol* 133:222–229

Brito LG, MCDS Oliveira, FGDS Netto, FA Cavalcante, AD Marim, GCRD Souza, JLD Silva, F Benitez, MMDF Moura (2007). Epidemiologia molecular de *Anaplasma marginale* em bovinos criados nos Estados de Rondônia e Acre. *Embr Rondôn Bolet Pesq Desenvolv* 49:8–29

Chung C, C Wilson, CB Bandaranayaka-Mudiyanselage, E Kang, DS Adams, LS Kappmeyer, DP Knowles, TF McElwain, JF Evermann, MW Ueti, GA Scoles, SS Lee, TC McGuire (2014). Improved diagnostic performance of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5-glutathione S-transferase fusion protein as antigen. *J Vet Diagn Invest* 26:61–71

Costa SCL, VCSD Magalhães, UVD Oliveira, FS Carvalho, CPD Almeida, RZ Machado, AD Munhoz (2016). Transplacental transmission of bovine tick-borne pathogens: Frequency, co-infections and fatal neonatal anaplasmosis in a region of enzootic stability in the northeast of Brazil. *Ticks Tick Borne Dis* 7:270–275

Dikmans G (1950). The transmission of anaplasmosis. *Amer J Vet Res* 11:5–16

Estrada-Peña A, V Naranjo, K Acevedo-Whitehouse, AJ Mangold, KM Kocan, JDL Fuente (2009). Phylogeographic analysis reveals association of tick-borne pathogen, *Anaplasma marginale*, MSP1a sequences with ecological traits affecting tick vector performance. *BMC Biol* 7; Article 57

Ewing SA (1981). Transmission of Anaplasmamarginale by arthropods. *In: The Natural History of Anaplasma Marginale. Vet Parasitol* 167:95–107

Farooqi SH, M Ijaz, MI Rashid, H Nabi, S Islam, AI Aqib, K Hussain, A Khan, SNB Rizvi, S Mahmood (2018). Molecular epidemiology of bovine anaplasmosis in Khyber Pakhtunkhwa, Pakistan. *Trop Anim Health Prod* 50:1591–1598

Grau HEG, NAD Cunha Filho, FG Pappen, NA da R. Farias (2013). Transplacental transmission of *Anaplasma marginale* in beef cattle chronically infected in southern Brazil. *Rev Bras Parasitol Vet* 22:189–193

Hawkins JA, JN Love, RJ Hidalgo (1982). Mechanical transmission of anaplasmosis by tabanids (Diptera: Tabanidae). *Amer J Vet Res* 43:732–734

Henker LC, MP Lorenzett, R Fagundes-Moreira, AG Cabrera Dalto, L Sonne, D Driemeier, JF Soares, SP Pavarini (2020). Bovine abortion, stillbirth and neonatal death associated with *Babesia bovis* and *Anaplasma* spp. infections in Southern Brazil. *Ticks Tick-borne Dis* 11; Article 101443

Karim S, K Budachetri, N Mukherjee, J Williams, A Kausar, MJ Hassan, S Adamson, SE Dowd, D Apanskevich, A Arijo, ZU Sindhu, MA Kakar, RMD Khan, S Ullah, MS Sajid, A Ali, Z Iqbal (2017). A study of ticks and tick-borne livestock pathogens in Pakistan. *PLoS Negl Trop Dis* 11; Article e0005681

Nazar M, MA Khan, AA Shah, SU Rahman, I Khan, A Ullah, IU Khan, M Shuaib (2018). Occurrence and transplacental transmission of *Anaplasma marginale* in dairy cattle. *Slov Vet Res* 55:183–191

Pohl AE, A Cabezas-Cruz, MFB Ribeiro, JAGD Silveira, C Silaghi, K Pfister, LMF Passos (2013). Detection of genetic diversity of *Anaplasma marginale* isolates in Minas Gerais, Brazil. *Rev Bras Parasitol Vet* 22:129–135

Potgieter FT, LV Rensburg (1987). The persistence of colostral *Anaplasma* antibodies and incidence of in utero transmission of *Anaplasma* infections in calves under laboratory conditions. *Onderstepoort J Vet Res* 54:557–560

Pypers AR, DE Holm, JH Williams (2011). Fatal congenital anaplasmosis associated with bovine viral diarrhoea virus (BVDV) infection in a crossbred calf. *J S Afr Vet Assoc* 82:179–182

Rehman A, FJ Conraths, C Sauter-Louis, J Krücken, AM Nijhof (2019). Epidemiology of tick-borne pathogens in the semi-arid and the arid agro-ecological zones of Punjab province, Pakistan. *Transbound Emerg Dis* 66:526–536

Ribeiro MF, JD Lima, AM Guimaräes, MA Scatamburlo, NE Martins (1995). Transmissäo congênita da anaplasmose bovina. *Arq Bras Med Vet Zootec* 47:297–304

Rikihisa Y (1991). The tribe Ehrlichieae and ehrlichial diseases. *Clin Microbiol Rev* 4:286–308

Sajid MS, RM Siddique, SA Khan, Z Iqbal, MN Khan (2014). Prevalence and risk factors of anaplasmosis in cattle and buffaloe populations of district Khanewal, Punjab, Pakistan. *Glob Vet* 12:146–153

Salabarria FF, R Pino (1988). Vertical transmission of *Anaplasma marginale* in cows affected in late pregnancy. *Rev Cub Cienc Vet* 19:179–182

Santarosa BP, GN Dantas, DOL Ferreira, NS Rocha, RC Gonçalves, RM Amorim, SB Chiacchio (2013). Infecção neurológica por Babesia bovis em bovino neonato: Relato de caso. *Vet Zootec* 20:9–14

Silva JB, LR Goncalves, ADM Varani, MR André, RZ Machado (2015). Genetic diversity and molecular phylogeny of *Anaplasma marginale* studied longitudinally under natural transmission conditions in Rio de Janeiro, Brazil. *Ticks Tick Borne Dis* 6:499–507

Silva JBD, AHD Fonseca (2014). Risk factors for anaplasmosis in dairy cows during the peripartum. *Trop Anim Health Prod* 46:461–465

Silva JBD, WMS Vinhote, CMC Oliveira, MR Andre, RZ Machado, AHD Fonseca, JD Barbosa (2014). Molecular and serological prevalence of Anaplasma *marginale* in water buffaloes in northern Brazil. *Ticks Tick-borne Dis* 5:100–104

Silvestre BT, JAG Silveira, RM Meneses, EJ Facury-Filho, AU Carvalho, MFB Ribeiro (2016). Identification of a vertically transmitted strain from *Anaplasma marginale* (UFMG3): Molecular and phylogenetic characterization, and evaluation of virulence. *Ticks Tick Borne Dis* 7:80–84

Spare MR, GA Hanzlicek, KL Wootten, GA Anderson, DU Thomson, MW Sanderson, RR Ganta, KE Reif, RK Raghavan (2020). Bovine anaplasmosis herd prevalence and management practices as risk factors associated with herd disease status. *Vet Parasitol* 3; Article 100021

Stuen S, W Okstad, AM Sagen (2018). Intrauterine transmission of *Anaplasma phagocytophilum* in persistently infected lambs. *Vet Sci* 5; Article 25

Vos AJVD, GD Imes, JS Cullen (1976). Cerebral babesiosis in a new-born calf. *Onderstepoort J Vet Res* 43:75–78

Wen XB, HT Jiang, YL Zhang, XY Lang, J Liu, HB Ni (2016). Rapid and sensitive diagnosis of cattle anaplasmosis by loop-mediated isothermal amplification (LAMP). *Pak Vet J* 36:174–178

Walker AR, A Bouattour, JL Camicas, A Estrada-Peña, IG Horak, AA Latif, RG Pegram, PM Preston (2014). *Ticks of Domestic Animals in Africa: A Guide to Identification of Species*, pp:3–210. Bioscience Reports, Edinburgh, Scotland, UK

Zaugg JL (1985). Bovine anaplasmosis: Transplacental transmission as it relates to stage of gestation. *Amer J Vet Res* 46:570–572

Zaugg JL, KL Kuttler (1984). Bovine anaplasmosis: *In utero* transmission and the immunologic significance of ingested colostral antibodies. *Amer J Vet Res* 45:440–443