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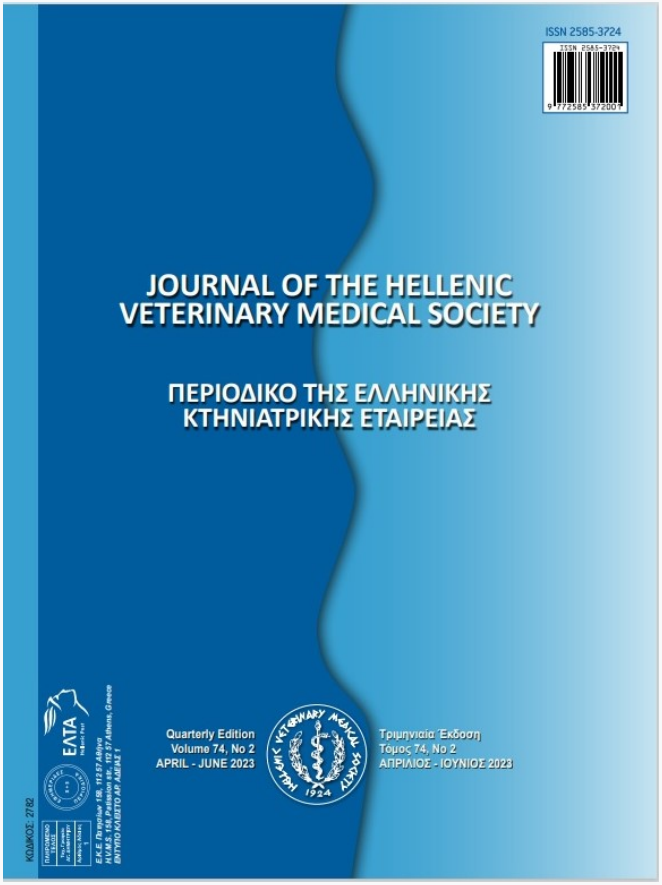

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Research article
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A study of biochemical changes in river buffaloes (*Bubalus bubalis*) naturally infected with *Anaplasma marginale*

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ABSTRACT: *Anaplasma marginale* is an intracellular rickettsia, endemic in tropical and subtropical regions of the world. This study was performed to determine *A. marginale* infection, molecularly, and to investigate its effects on some serum biochemical parameters in river buffaloes referred to Ahvaz abattoir, Southwest Iran. A total of 103 apparently healthy river buffaloes were randomly sampled. Whole blood samples were subjected to PCR analysis and blood smears were examined microscopically for the presence of *Anaplasma* inclusions. Serum biochemical parameters including total protein, albumin, glucose, urea, iron, total bilirubin, total cholesterol, calcium, phosphorus and magnesium concentrations, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities and also serum electrolytes comprising Na and K were assessed. The results revealed that 16 and 32 samples were infected with *A. marginale* in microscopic and PCR assessment, respectively. Serum biochemical analysis showed no significant difference between *A. marginale* infected and non-infected buffalo groups. It can be concluded that infection with *A. marginale* does not lead to much pathogenicity in river buffaloes, so that in infected animals, no abnormalities in biochemical parameters could be detected. However, according to the high infection prevalence, the possible role of this species in the epidemiology and transmission of anaplasmosis to other species remains significant. Further studies are needed to investigate the role of this species as a reservoir.

***Keywords*:** *Anaplasma marginale*; Buffalo; Serum biochemistry; Ahvaz

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INTRODUCTION

Anaplasma marginale is an obligate intracellular parasite that infects erythrocytes of superior vertebrates, especially ruminants. It is the causative agent of bovine anaplasmosis, which has been reported in domestic and wild animals including buffalo, American bison, African antelope, and deer (Kocan et al., 2003). This rickettsia germ can be transmitted both mechanically (flies bite or fomites contaminated with blood) and biologically (ticks) (Kocan et al., 2004; Rymaszewska and Grenda, 2008). The hallmark of clinical anaplasmosis is anemia due to phagocytosis of infected erythrocytes. The severity of clinical symptoms is related to the degree of anemia and includes discoloration of the skin and mucous membranes and increased heart rate and respiration (Constable et al., 2017, Kocan et al., 2003). In addition, infection with *A. marginale* was demonstrated to cause major changes in serum biochemical analytes in cattle (Coskun et al., 2012; Sharma et al., 2013).

Diagnosis of bovine anaplasmosis may be made based on geographic area, season and clinical signs, or necropsy findings in infected animals. Laboratory tests are needed to confirm the diagnosis, such as microscopic examination of stained blood smears, serological or molecular diagnostic methods (Kocan et al., 2003).

Anaplasmosis in domestic ruminants is highly prevalent in Iran due to the biodiversity of tick species and the variety of ecological and climatic conditions. The prevalence of *Anaplasma* infection in domestic ruminants is estimated to be 34%, with Khouzestan province (with 54% positive rate of infection) being the most prevalent area in Iran (Soosaraei et al., 2020; Jalali et al., 2013, 2016; Noaman et al., 2009; Razmi et al., 2006).

Few studies on *A. marginale* contamination in river buffaloes are available. In a PCR study in South Africa, the rate of buffalo infection with *A. marginale* was reported to be 17.37% (Sisson et al., 2017). Additionally, in another study by Saetiew et al., (2015) the rate of *A. marginale* infection in buffaloes was 8% in Thailand. The results of a study in Brazil also showed that buffaloes can carry this parasite which raises concerns regarding the role of this species as reservoirs of *A. marginale* for cattle living in the same area (Silva et al., 2014a). Obregón et al., 2018 also reported genetic similarities between *A. marginale* strains from cohabiting cattle and water buffalo, proposing the possible cross-species transmission.

River buffalo (*Bubalus bubalis*) is an economically and culturally important livestock in Iran. River buffalo plays a critical role in social and cultural contributions and possess the highest potential for producing milk and meat due to its promising gene pool. There are three main buffalo breeds in Iran: Azeri, Khuzestani and Mezandrani, with 119,000, 81,000 and 4000 individuals, respectively. The Khuzestani is reared in the west and southwest (mainly in Khuzestan province) of the country and raised outdoors throughout (Borghese, 2005). Iranian buffalo is mostly kept for its milk production (Borghese 2005). In comparison with cattle, buffalo milk is richer in protein, fat, lactose and energy. Water buffalo provide about 293,000 tons (2.8% of Iran's total milk production) and 24,700 tons (2.5%) of meat (Mokhber et al., 2018). Average milk production of buffaloes in a 202-day lactation is 2017 Kg in Khuzestan (Sanjabi et al, 2011). Their unique adaptability to unstable conditions in terms of temperature and moisture, their resistance to diseases and parasites as well as their long productive life, and ability to consume low-quality forage made buffaloes as a remarkable species (Qanemi 1998; Ghavi Hossein-Zadeh 2016).

The effects of *Anaplasma* infection on hematological parameters in river buffaloes in Ahvaz have already been published (Nikvand et al., 2020). However, the various aspects of pathogenicity and the effects of this microorganism on other vital organs have not been identified in this animal. Hence, the present study was performed with the aim of molecularly investigating *A. marginale* infection by PCR and its effects on some serum biochemical parameters in river buffaloes referred to Ahvaz abattoir.

MATERIALS AND METHODS

Sampling

Sampling was performed from September to October 2017 from Ahvaz abattoir. A total of 103 apparently healthy river buffaloes, 36 female and 67 male which aged between one to 10 years, were randomly sampled. Blood samples were taken from jugular vein and collected into EDTA containing tubes for microscopic examination of blood smears and molecular analysis and also plain tubes for serum separation. Whole blood and serum samples were preserved in -20º C until further assessment.

Microscopic examination of blood smears

Blood smears were examined microscopically

after staining with Giemsa for the presence of *Anaplasma* inclusion bodies. More than 30 microscopic fields of blood films were examined under a ×100 objective lens. Approximately 20,000 erythrocytes were carefully searched per slide (Jalali et al., 2016).

Molecular assessment

DNA extraction

DNA extraction from whole blood samples was performed using Rahazist Padtan, Iran, based on the manufacturer's instructions. DNA samples were preserved at -20º C until further molecular assessment.

PCR analysis

A nested- PCR method was used to detect *A. marginale* in blood samples. Two pairs of oligonucleotide primers, based on the groEL gene sequence of *A.* marginale were employed (Ybanez et al., 2013). The sequences of primers are presented in table 1.

The PCR was performed in two rounds. First round PCR was conducted in a total reaction volume of 15 µl containing 7.5 µl mastermix (Ampliqon Taq DNA Polymerase 2x Master Mix RED containing Tris-HCl pH 8.5, (NH4)2S04 , 3 mM MgCl2 , 0.2% Tween 20, 0.4 mM of each dNTP, Ampliqon Taq DNA polymerase and Inert red dye and stabilizer) , 0.2 µM of F1 and R1 primers and 2 µl(5 ng) of extracted DNA suspension as the template. Second round PCR was performed in a total reaction volume of 25 µl containing 12.5 µl mastermix, 0.3 µM of F2 and R21 primers and 1 µl of first round PCR product as the template.

The amplification was performed in an automated gradient thermocycler (Eppendorf, Germany). The thermal profile of PCR is described in table 2.

Biochemical analysis

Serum biochemical parameters including total protein, albumin, glucose, urea, iron, total bilirubin, total cholesterol, calcium, phosphorus and magnesium concentrations, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were assessed using a biochemistry autoanalyser (BT-1500, Biotechnica, Italy) and Parsazmun kits (Iran). The serum concentration of electrolytes including Na and K were also measured by flame photometry (Sherwood Scientific, England). The accuracy of the biochemical results for river buffalo samples with the applied kits and the analyzers was approved by checking the results against the reference intervals (Kaneko et al., 2008).

Statistical analysis

Independent samples T-test was used to compare and determine statistical differences in laboratory-obtained values between two groups. All values were expressed as mean and standard error (SE), and $p<0.05$ was considered as statistically significant.

RESULTS

Microscopic results

The results of microscopic evaluation of 103 blood samples showed that 16 samples (15.53%) were infected with *Anaplasma*-like bodies.

Table 1. Primer sequences specific for *groEL* gene for detection of *A. marginale* in the studied buffaloes.

Primer	Nucleotide Sequence
AM265F1	5'-GACTACCACATGCTCCATACTGACTG-3'
AMA424F2	5'-GTCTGAAGATGAGATTGCACAGGTTG-3'
AM1574R1	5'-GACGTCCACAACTACTGCATTCAAG-3'
AM1289R2	5'-CCTTTGATGCCGTCCAGAGATGCA-3'

Table 2. PCR program used for the amplification of a *groEL* gene fragment of *A. marginale*.

Step	Temperature	Time	Cycle
Initial denaturation	95	9 min	1
Denaturation	95	30 sec	
Anealing	75	30 sec	5
Extension	72	90 sec	
Denaturation	95	30 sec	
Anealing	65	30 sec	30
Extension	72	30 sec	
Final extension	72	5 min	1

PCR results

In PCR test on 103 buffalo blood samples using specific primers of *A. marginale* groEL gene, 32 samples (31.1%) were positive, of which 9 samples in microscopic test were found to have anaplasmic-like bodies.

Statistical analysis

Infection rates were assessed based on age and sex. There was no significant relationship between age and infection (P> 0.05) and also no significant relationship between sex and infection was obtained based on chi-square test (P> 0.05) (Table 3).

In addition, despite some variations, serum biochemical analysis showed no significant difference between A. marginale infected and non-infected buffalo groups (p>0.05) (Table 4).

There was also no significant difference in the mean of various minerals or electrolytes assessed in two groups of buffaloes with and without *A. marginale* infection (p>0.05) (Table 5).

DISCUSSION

A. marginale as an intracellular rickettsia is endemic almost all over the world, especially in tropical and subtropical regions (Kocan et al., 2010). Although clinical anaplasmosis due to *A. marginale* often occurs in cattle, other ruminants, including river buffalo, can also be infected (Kuttler, 1984). According to the results of this study, the rate of infection in river buffaloes was 15.53% and 31.1% (16 and 32 out of 103 samples) in microscopic examination and PCR assessment, respectively. There were 9 microscopically positive samples which were negative in PCR analysis. These samples might have contained other similarly shaped inclusions or artifacts that were mistakenly presumed as *Anaplasma* like inclusions. It must be considered that while light microscopy of stained thin blood smears may facilitate detection of *A. marginale* organisms in the erythrocytes, this technique may be unreliable in carrier or persistently infected animals (Kocan et al., 2010). Moreover, it is difficult to differentiate the organism from other similar structures like Howell-Jolly bodies, or stain-

Table 3. Number (percentage of frequency) of river buffaloes by age and sex infected (PCR+) and non-infected (PCR-) with *A. marginale*.

	Age				Total	
	<2.5 year (immature)		>2.5 year (mature)			
	PCR +	PCR-	PCR-	PCR-	PCR +	PCR-
Female	8 (57.1%)	6	8 (36.4%)	14	16 (44.4%)	20
Male	3 (20%)	12	13 (25%)	39	16 (23.9%)	51
Total	11 (37.9%)	18	21 (39.6%)	53	32 (31.1%)	71

Table 4. Serum constituents of river buffaloes as mean ± SE in two groups based on A. marginale infection detected by PCR test.

Analyte	Unit	*Anaplasma* positive (n=32)	*Anaplasma* negative (n=71)	P value
ALT	U/l	26.02 ± 5.69	29.58 ± 4.38	0.63
ALP	U/l	284.67 ± 23.31	264.03 ± 13.62	0.41
Total bilirubin	mg/dl	0.36 ± 0.02	0.38 ± 0.01	0.13
Total cholesterol	mg/dl	48.67 ± 4.45	55.37 ± 2.91	0.20
Glucose	mg/dl	51.75 ± 3.93	55.11 ± 2.89	0.51
Total protein	g/dl	6.76 ± 0.17	6.78 ± 0.08	0.92
Albumin	g/dl	3.35 ± 0.06	3.45 ± 0.04	0.21
Urea	mg/dl	34.29 ± 1.93	32.20 ± 1.25	0.35

Table 5. Serum minerals and electrolytes of river buffaloes as mean ± SE in two groups based on A. marginale infection detected by PCR test.

	Unit	*Anaplasma* positive (n=32)	*Anaplasma* negative (n=71)	P value
Calcium	mg/dl	8.90 ± 0.10	9.03 ± 0.08	0.32
Phosphorus	mg/dl	6.44 ± 0.21	6.46 ± 0.18	0.95
Magnesium	mg/dl	3.04 ± 0.20	2.86 ± 0.15	0.49
Iron	µg/dl	129.12 ± 21.86	114.16 ± 7.66	0.42
Sodium	mEq/l	140.73 ± 3.74	138.32 ± 2.82	0.61
Potassium	mEq/l	4.93 ± 0.30	4.77 ± 0.19	0.65

ing artifacts, especially in animals with low level of rickettsiaemia (Shompole et al., 1989; Ndung'u et al., 1995). Hence, nucleic acid-based assays are currently the most reliable technique for demonstration of this tick-borne rickettsia (Kocan et al., 2010). Accordingly, the statistical comparisons in this study were performed based on PCR results.

it seems that while *A. marginale* infection in river buffaloes has a relatively high prevalence in Ahvaz region located in southwestern Iran, this infection is not associated with significant changes in serum biochemical parameters. In a previous study the authors also did not record any significant change in hematological parameters including anemia which is one of the important complications of anaplasmosis in other ruminant species (Nikvand et al., 2020).

Therefore, it can be inferred that despite the significant virulence of *A. marginale* in domestic ruminants, especially cattle, which in cases of acute infection, due to extravascular hemolysis, is associated with anemia, jaundice, fever, abortion, etc. (Kocan et al., 2010; Jones and Brock, 1966), in river buffaloes, it does not lead to much pathogenicity, so that in infected animals, no abnormalities in biochemical parameters could be detected.

Several studies in cattle have shown that natural or experimental infection with *A. marginale* is associated with significant changes in serum biochemical parameters comprising elevation in creatinine and bilirubin concentrations, some liver enzyme activities and some acute phase proteins and decline in serum total protein and albumin concentration (Allen et al., 1981; Coskun et al., 2012; Sharma et al., 2013; Jassem and agar, 2015; Ganguly et al., 2018).

In contrast, limited studies have been performed on the pathogenesis of *A. marginale* in river buffalo and to the best of our knowledge no data was documented on serum biochemical status in the infected animals of this species. Most studies have only examined the prevalence of this infection by molecular or serological methods (Silva et al., 2014a; 2014b; Mamun et al., 2010; Sarangi et l., 2020; Amira et al., 2020; Lima et al., 2019). Although clinical signs have been reported in some experimental studies (Sharma ,1987; Reddy et al., 1988) and natural infections (Srivastava and Ahluwalia, 1974; Vatsya et al., 2013) in buffaloes, however, in other investigations that have been done in this area, similar results to the present study have been reported in which *A. marginale* only caused subclinical infection with low parasitemia in river buffaloes (Singh and Gill, 1977; Guatam et al., 1970; Obregón et al., 2018). In addition, in an experimental study, buffalo infestation with *A. marginale was associated with no clinical and blood abnormalities and also lower* frequency of infection in blood PCR tests compared to cattle (Lima et al., 2019). Hence the researchers suggested that buffaloes may be an alternative to raising cattle in areas with high frequency of clinical anaplasmosis (Lima et al., 2019). Other studies similarly reported lower values of natural infection in buffaloes (Silva et al. 2014a, 2014b) in comparison to the detected in cattle (Bacanelli et al. 2014).

The cause of this natural resistance of river buffalo species to *Anaplasma* pathogenesis has not been identified. There are some possible reasons for this, including: Different strains of *Anaplasma* infecting cattle and buffaloes with varying degrees of pathogenicity (Obregón et al., 2018); Elimination or reduction of the number of blood rickettsiae as well as other hemoparasites by the buffalo innate immunity (Benitez et al., 2018). However, more studies are needed to accurately identify the causes and mechanisms involved in this buffalo resistance to this rickettsia.

Despite the findings of this study and other previous studies on the non-pathogenicity of *A. marginale* in buffalo, the role of this species in the epidemiology and transmission of anaplasmosis to other species remains significant (Obregón et al., 2018).

CONCLUSION

It can be concluded that infection with *A. marginale* does not lead to much pathogenicity in river buffaloes, so that in infected animals, no abnormalities in biochemical parameters could be detected. However, according to the high infection prevalence, the possible role of this species in the epidemiology and transmission of anaplasmosis to other species remains significant. Further studies are needed to investigate the role of this species as a reservoir.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Allen PC, Kuttler KL, Amerault TE (1981) Clinical chemistry of anaplasmosis: blood chemical changes in infected mature cows. Am J Vet Res 42(2): 322-325.

Amira AH, Răileanu C, Tauchmann O, Fischer S, Nijhof AM, Silaghi C (2020) Epidemiology and genotyping of *Anaplasma marginale* and co-infection with piroplasms and other Anaplasmataceae in cattle and buffaloes from Egypt. Parasites Vectors 13(1): 1-11.

Bacanelli GM, Ramos CAN, Araújo FR (2014) Molecular diagnosis of *Anaplasma marginale* in cattle: quantitative evaluation of a real-time PCR (Polymerase Chain Reaction) based on msp5 gene. Pesq Vet Bras 34(1):29-33. http://dx.doi.org/10.1590/S0100-736X2014000100005

Benitez D, Mesplet M, Echaide I, Torioni de Echaide S, Schnittger L, Florin-Christensen M (2018) Mitigated clinical disease in water buffaloes experimentally infected with Babesia bovis. Ticks Tick Borne Dis 9(5):1358-1363. <http://dx.doi.org/10.1016/j.ttbdis.2018.04.012> <PMid:29724619>

Borghese A (2005) Buffalo Production and Research. FAO Ed. REU Tech Series 67:1-316.

Constable PD, Hinchcliff KW, Done SH, Grunberg W (2017) Anaplasmosis. In: Veterinary Medicine, 11th ed., Elsevier, China P. 769-775.

Coskun A, Ekici O D, Guzelbektes H, Aydogdu U, Sen I (2012) Acute phase proteins, clinical, hematological and biochemical parameters in dairy cows naturally infected with *Anaplasma marginale*. Kafkas Univ Vet Fak Derg 18(3): 497-502.

Ganguly A, Maharana BR, Ganguly INDRAJIT, Kumar ANKIT, Potlya S, Arora D, Bisla RS (2018) Molecular diagnosis and haemato-biochemical changes in *Anaplasma marginale* infected dairy cattle. Indian J Anim Sci 88(9): 989-993.

Ghavi Hossein-Zadeh N (2016) Analysis of population structure and genetic variability in Iranian buffaloes (*Bubalus bubalis*) using pedigree information. Anim Prod Sci 56: 1130–1135.

Guatam OP, Sharma RD, Singh B (1970) Anaplasmosis-II. Clinical cases of anaplasmosis in cattle, buffaloes and sheep. Indian Vet J 47:1012-1019.

Jalali SM, Khaki Z, Kazemi B, Bandehpour M, Rahbari S, Razi Jalali M, Yasini S (2013) Molecular detection and identification of Anaplasma species in sheep from Ahvaz, Iran. Iran J Vet Res 14 (1): 50–56.

Jalali SM, Bahrami S, Rasooli A, Hasanvand S (2016) Evaluation of oxidant/antioxidant status, trace mineral levels, and erythrocyte osmotic fragility in goats naturally infected with *Anaplasma ovis*. Trop Anim Health Prod 48 (6): 1175–1181.

Jassem GA, Agaar OA (2015) Molecular and biochemical study of *Anaplasma marginale* in cattle in Wassit Province of Iraq. African J Bacteriol Res 7(4): 36-41.

Jones EW, Brock WE (1966) Bovine anaplasmosis: its diagnosis, treatment, and control. JAVMA 149:1624–1633.

Kaneko JJ, Harvey JW, Bruss M (2008) Veterinary clinical biochemistry of domestic animals. 6th ed. London, Academic press PP: 882-888.

Kocan KM, de la Fuente J, Blouin EF, Coetzee JF, Ewing SA (2010) The natural history of *Anaplasma marginale*. Vet Parasitol 167(2-4): 95-107.

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

Kocan KM, de la Fuente J, Blouin EF, Garcia-Garcia JC (2004) *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitol 129: S285–S300.

Kuttler KL (1984) Anaplasma infections in wild and domestic ruminants: a review. J Wildlife Dis 20: 12–20.

Lima DH, Vinhote W, Ubiali DG, Soares PC, Cordeiro MD, Silva JB, Fonseca AH, Barbosa JD (2019) Experimental infection by *Anaplasma marginale* in buffaloes and cattle: clinical, hematological, molecular and pathological aspects. Pesq Vet Bras 39(9): 700-709.

Mamun MAA, Begum N, Bari MA, Mondal MMH (2010) Haemoprotozoa of Buffaloea (*Bubalus bubalis*) in Kurigram. Bangladesh J Prog Sci Tech 8: 209-212.

Mokhber M, Moradi-Shahrbabak M, Sadeghi M, Moradi-Shahrbabak H, Stella A, Nicolzzi E, Rahmaninia J, Williams JL (2018) A genome-wide scan for signatures of selection in Azeri and Khuzestani buffalo breeds. BMC genomics 19(1):1-9.

Nikvand AA, Hasanpour Besati E, Gharibi D, Jalali SM (2020) Molecular and Hematologic Survey on *Anaplasma marginale* Infection in Slaughtered Water Buffaloes (Bubalous bubalis) in Ahvaz City, Iran. J Vet Res 75(2): 192-199.

Ndung'u LW, Aguirre C, Rurangirwa FR, McElwain TF, McGuire TC, Knowles DP, Palmer GH (1995) Detection of *Anaplasma ovis* infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. J Clin Microbiol 33: 675-679.

Noaman V, Shayan P, Aminina N (2009) Molecular diagnostic of *Anaplasma marginale* in carrier cattle. Iran J Parasitol 4 (1): 26–33.

Obregón D, Corona BG, de la Fuente J, Cabezas-Cruz A, Gonçalves LR, Matos CA, Armas Y, Hinojosa Y, Alfonso P, Oliveira MC, Machado RZ (2018) Molecular evidence of the reservoir competence of water buffalo (Bubalus bubalis) for *Anaplasma marginale* in Cuba. Vet Parasitol: Reg Stud 13: 180-187.

Qanemi A (1998) Buffalo population and production in Iran. Buffalo Newsletter 10: 12–14.

Razmi GR, Dastjerdi K, Hossieni H, Naghibi A, Barati F, Aslani M (2006) An epidemiological study on Anaplasma infection in cattle, sheep, and goats in Mashhad Suburb, Khorasan Province, Iran. Ann NY Acad Sci 1078 (1): 479–481.

Reddy GR, More T, Sharma SP, Singh LN (1988) The oxidant defense system in water-buffaloes (Bubalus bubalis) experimentally infected with *Anaplasma marginale*. Vet Parasitol 27(3/4):245-249. <http://dx.doi.org/10.1016/0304-4017(88)90039-8> <PMid:3369075>

Rymaszewska A, Grenda S (2008) Bacteria of the genus Anaplasma–characteristics of Anaplasma and their vectors: a review. Vet Med 53(11): 573-584.

Saetiew N, Simking P, Inpankaew T, Wongpanit K, Kamyingkird K, Wongnakphet S, Stich RW, Jittapalapong S (2015) Prevalence and genetic diversity of *Anaplasma marginale* infections in water buffaloes in Northeast Thailand. J Trop Med Parasitol 38: 9-16.

Sanjabi M, Alemi F, Naderfard H (2011) Estimated breeding value in Iranian buffaloes. J Agric Sci Technol A 1:570–574.

Sarangi LN, Rana SK, Prasad A, Ponnanna NM, Sharma GK (2020) Prevalence of antibodies to Anaplasma in cattle and buffaloes of different organized herds in India. J Parasit Dis 12:1-7.

Sharma SP (1987) Characterization of *Anaplasma marginale* infection in buffaloes. Indian J Anim Sci 57:76-78.

Sharma A, Singla LD, Kaur P, Bal MS, Batth BK, Juyal PD (2013) Prevalence and haemato-biochemical profile of *Anaplasma marginale* infection in dairy animals of Punjab (India). Asian Pacific J Trop Med 6(2): 139-144.

Shompole S, Waghela SD, Rurangirwa FR, McGuire TC (1989) Cloned DNA probes identify *Anaplasma ovis* in goats and reveal a high prevalence of infection. J Clin Microbiol 27: 2730-2735

Silva JB, Fonseca AH, Barbosa JD, Cabezas-Cruz A, de la Fuente J (2014a). Low genetic diversity associated with low prevalence of *Anaplasma marginale* in water buffaloes in Marajó Island, Brazil. Ticks Tick Borne Dis 5: 801-804.

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

Singh A, Gill BS (1977) Note on the prevalence of subclinical anaplasmosis (*Anaplasma marginale*) in three herds of cattle and buffaloes in Punjab State. Indian J Anim Sci 47: 224-226.

Sisson D, Hufschmid J, Jolles A, Beechler B, Jabbar A (2017) Molecular characterisation of *Anaplasma* species from African buffalo (syncerus caffer) in kruger national park, South Africa. Ticks Tick Borne Dis 8: 400-406.

Soosaraei M, Haghi MM, Etemadifar F, Fakhar M, Teshnizi SH, Asfaram, S, Esboei BR (2020) Status of Anaplasma spp. infection in domestic ruminants from Iran: A systematic review with meta-analysis. Parasite Epidemiol Cont e00173.

Srivastava R, Ahluwalia SS (1974) A clinical case of anaplasmosis in buffalo. Indian Vet J 51:371-374.

Vatsya S, Kumar RR, Singh VS, Arunraj MR (2013) *Anaplasma marginale* infection in a buffalo: a case report. Vet Res Int 1(2):51-53.

Ybanez AP, Sivakumar T, Ybanez RHD, Ratilla JC, Perez ZO, Gabotero SR, Inokuma H (2013) First molecular characterization of *Anaplasma marginale* in cattle and *Rhipicephalus (Boophilus) microplus* ticks in Cebu, Philippines. J Vet Med Sci 75: 27-36.