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Original article

Occurrence of anaplasmosis among sheep (*Ovis aries*) and goats (*Capra hircus*) in Madina and Tabuk, Saudi Arabia

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ABSTRACT

Anaplasma spp. is a group of intra-erythrocytic bacteria that various species of ticks can transmit. Information regarding the prevalence of infections in sheep and goats caused by this group of organisms is scarce in Saudi Arabia. The present study was carried out during 2020–2021 to examine the prevalence of anaplasmosis among sheep and goats in two cities in western Saudi Arabia. The study included samples from 177 sheep (77 from Madina, 100 from Tabuk) and 226 goats (123 from Madina, 103 from Tabuk). The samples were investigated using direct microscopy method as well as a competitive Enzyme-Linked Immuno-Assay (cELISA) for the detection of anti-*Anaplasma* spp. antibodies. A total of 93 (23.1%) of the samples were positive on direct microscopy, whereas 84 (20.7%) were positive on cELISA. Of those samples positive on direct microscopy, 44 (19.5%) were from goats while 49 (27.7%) were from sheep. Of the positive samples on cELISA; 38 (17.0%) were from goats, and 46 (26.0%) were from sheep. A significant difference in the prevalence of anaplasmosis was reported ($p < 0.05$) using microscopic and cELISA in goats and sheep in both regions studied, having a higher prevalence in Tabuk. There was no significant difference in the prevalence of anaplasmosis in males and females from Madina and Tabuk using both methods ($p > 0.05$). However, there was a significant difference in the prevalence of anaplasmosis in older goats (>2 years) and in the summer compared to the winter in samples collected from Tabuk ($p < 0.05$). Seroprevalence of anaplasmosis was detected for the first time In the Tabuk region from sheep and goats. The difference in the prevalence in the two locations studied was probably due to the variation in climatic conditions and the availability of the vector responsible for the transmission of anaplasmosis.

1. Introduction

Members of the genus *Anaplasma* are obligate, intraerythrocytic, gram-negative, tick-borne rickettsial organisms that infect a wide range of domestic and wild mammals, including humans. There are nine species, as well as seven candidate species, included in the genus *Anaplasma* (Dumler et al., 2001; Vanstreels et al., 2018). Of these, *A. ovis*, *A. capra,* and *A. phagoytophilum* infect sheep and goats, with the latter infecting multiple hosts, including man. *A. marginale* primarily infects cattle. However, it was also found to infect sheep and goats (Yousefi et al., 2017; Barbosa et al., 2021). Ticks of various genera can transmit the infection from one individual animal to another. Infection may result in anemia when transmitted to hosts outside of their natural cycle (Parola et al., 2005; Nicholson et al., 2010).

The zoonotic potential of infection with *A. ovis* has been proven, and some variants of *A. ovis* were detected in human patients from Iran and Cyprus in recent studies (Chochlakis et al., 2010; Hosseini-Vasoukolaei et al., 2014). *A. ovis* has a cosmopolitan distribution and is responsible for considerable losses in sheep and goats as a result (Renneker et al., 2013). In Saudi Arabia, limited studies on the disease and its epidemiological burden have been conducted. Hemoparasite investigation resulted in the detection of several blood protozoan parasites in camels, sheep, goats, and cattle in different regions of Saudi Arabia (El-Azazy et al., 2001; Diab et al.,

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2006). Al-Khalifa et al. (2009) conducted a microscopic examination of blood from several animals in the Riyadh region, Southern region, and the Eastern and Northern frontiers of Saudi Arabia. They found evidence of *A. ovis* in 2% of the sheep samples collected from the Eastern and Northern regions of the Kingdom of Saudi Arabia. Furthermore, they found *A. marginale* in 3.4% of cattle from the Eastern region. The overall prevalence of anaplasmosis in some areas like Pakistan was 29.63% and 1.66 % in sheep and goats, respectively (Muhammad et al., 1999). Prevalence in Mosul, Iraq, was found to be (62.6%) in sheep (Sulaiman et al., 2010).

The most common method for diagnosing hemoparasites such as *Theileria* spp. or intraerythrocytic bacteria such as *Anaplasma* spp. depends on the demonstration of the parasite or the bacteria in Giemsa-stained thick or thin blood smears using light microscopy (Ali et al., 1996; Nagore et al., 2004). In addition, indirect methods such as different serologic tests or DNA-based molecular methods of the agent are used to detect the specific antibodies or pathogens (Goff et al., 1990; Sumbria and Singla, 2015).

Enzyme Linked Immunosorbent Assay (ELISA) is routinely used for the detection of specific parasitic antibodies, antigens, and immune complexes. It is commonly used as the basis for epidemiological surveys (Passos et al., 1998; Sekiya et al., 2013). Nowadays, various studies have been performed in small ruminants and other domestic livestock for sensitive detection of hemoparasites and intraerythrocytic bacteria**.** Serological evidence has been provided for anaplasmosis in dogs, horses, and camels from Algeria, Morocco, and Tunisia (M'ghirbi et al., 2009; Ben Said et al., 2014; Azzag et al., 2015; Elhamiani Khatat et al., 2017). *A. marginale* has been reported in cattle from Sudan, Morocco, and Egypt (Salih et al., 2008; Hamou et al., 2012; Fereig et al., 2017).

The present study aimed to investigate the occurrence of anaplasmosis among sheep and goats in Medina and Tabuk, KSA using direct microscopic bacterial examination and competitive ELISA. In addition, the study also aimed to investigate the role of some risk factors that might be associated with direct infection or previous exposure to the species members of *Anaplasma*.

2. Materials and methods

2.1. Study sites and samples collection

Blood samples were collected from sheep and goats in Madina (24.8404° N, 39.3206° E) and Tabuk (28.2453° N, 37.6387° E) regions in the north and northwestern areas of the Kingdom of Saudi Arabia.

A total of 403 blood samples were collected from apparently healthy sheep (n = 177; 77 from Madina and 100 from Tabuk) and goats (n = 226; 123 from Madina and 103 from Tabuk). Samples were collected from animals kept for breeding and production. Some were obtained from Veterinary clinics affiliated with the Ministry of Environment, Water, and Agriculture at Madina and Tabuk. The samples were collected during the summer season (n = 219) and the winter season (n = 184). Sheep and goats' age, gender, location, system of rearing, and season during which samples were collected are regarded as risk factors for infection with anaplasmosis.

Blood samples were collected from the jugular vein using a 20 g × ½ inch needle into 5 ml syringes. Each sample was then transferred into two clean vacutainers, one of which was coated with ethylene diamine tetra-acetic acid (EDTA) to be used in preparing direct blood thin smears for microscopic examination. The second vacutainers (non-anticoagulant tubes) were used to obtain serum.

Serum samples were obtained after obtaining the blood samples which were collected in plain vacutainers being clotted overnight at room temperature and then centrifuged for 10 min at 2500 g using a tabletop centrifuge (Gemmy Industrial Corp. Taiwan associated with Cannic, Inc. USA). Serum samples were then transferred into clean Eppendorf tubes and stored at – 20 °C until used.

2.2. Microscopic examination

A drop of blood (up to 5 μl) was placed on a clean glass slide and spread along the slide, then air-dried, stained with 5% Giemsa stain, and examined microscopically using X100 objective lens for the detection of intraerythrocytic bodies typical of *Anaplasma* spp.

2.3. Serological methods

Anti-*Anaplasma* spp. antibodies in serum samples from sheep and goats were detected using a competitive enzyme-linked immunosorbent assay (cELISA) for *Anaplasma* spp. antigen using (VMRD, ANAPLASMA ANTIBODY TEST KIT, cELISA v2 Pullman, WA 99163 USA) according to the manufacturer's protocol. A volume of 50 μl of negative and positive controls and undiluted samples were transferred to the *Anaplasma* spp. antigen-coated plates. In the positive serum samples, the antibodies block the binding of the secondary antibody (the horseradish peroxidase labeled monoclonal antibody conjugate). Hence, when adding the substrate, there will be no color development on the positive wells and a dark color in the negative wells. The plate was read in SpectraMax M series multimode microplate reader (Molecular Devices, LLC. 3860 N First Street, San Jose, CA 95134) with a wavelength of 630 nm. The % of inhibition (I) was calculated according to the following formula:

$$\%I = \frac{(1 - \text{ODofsample})}{(\text{ODofnegativecontrol})} X100$$

Samples with an inhibition of ≥30% were considered positive, while samples with inhibition of <30% were considered negative.

2.4. Sensitivity and specificity of tests calculations

The microscopic examination is considered the gold standard method. The sensitivity and specificity were calculated as the proportion of all the positive samples on ELISA out of all the samples positive in microscopic examination.

2.5. Statistical analysis

Results were analyzed using the Chi-square test in the statistical software SPSS 20.0 (IBM, White Plain NYS, USA). Significant differences were indicated when the p value was less than 0.05. The likelihood of detection or exposure as an indicator for the risk to the parasite was quantified using the odds ratio.

3. Results

Intraerythrocytic organisms suggestive of Anaplasma spp. (Fig. 1) were microscopically demonstrated in 23.1% of the total examined animals (44 goats and 49 sheep) (Table 1). Serological investigations revealed that antibodies to anaplasmosis were detected in 20.7% (84/403) of the studied populations, with 46 and 38 positives from sheep and goats respectively. There was a significant difference in the prevalence of anaplasmosis using microscopic and cELISA tests in goats and sheep in both regions studied, having higher prevalence in Tabuk ($p < 0.05$).

The prevalence of anaplasmosis in goats and sheep is shown in Tables 2 and 3. There was no significant difference in the preva-

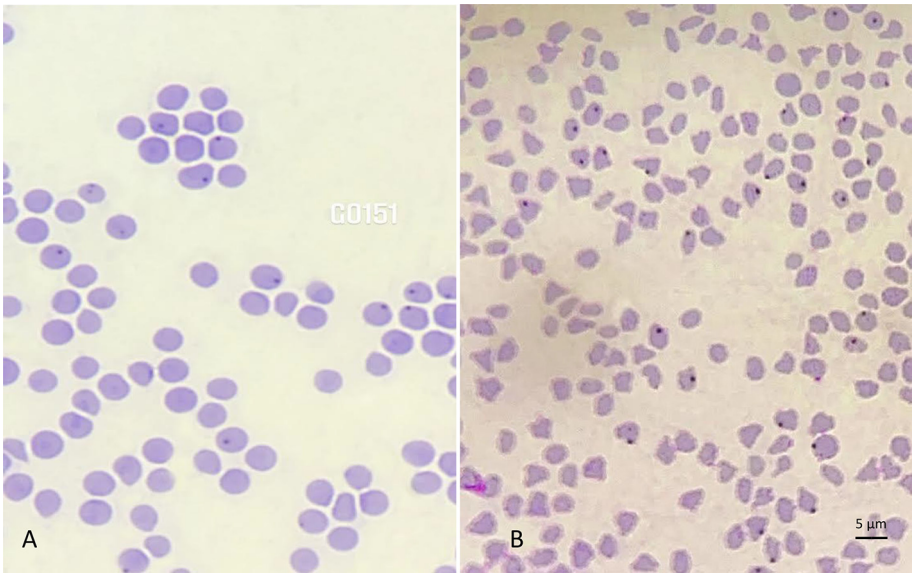

Fig. 1. Intraerythrocytic *Anaplasma* spp. on blood smears stained with Giemsa from sheep (A) and goat (B). Scale bar 5 µm for both photographs.

Table 1
Results of the microscopic and antibody (Ab) ELISA of *Anaplasma* spp. Infection in sheep and goats from Medina and Tabuk regions in Saudi Arabia.

Animals Examined		Results of Microscopy			Results of Ab ELISA	
		Positive (%)	*p* value	Odds Ratio (95% CI)	Positive (%)	*p* value
Goats	Madina (124)	14 (11.3)	**<0.05**	0.3 (0.2, 0.6)	12 (9.7)	**<0.05**
	Tabuk (102)	30 (29.1)			26 (25.5)	
Sheep	Madina (77)	8 (10.4)	**<0.05**	0.2 (0.1, 0.3)	6 (7.8)	**<0.05**
	Tabuk (100)	41 (41)			40 (40)	

Table 2
Results of microscopic and serological screening for *Anaplasma* spp. Infections in goats in Medina and Tabuk during 2019–2020, correlation with sex, locality and season.

Variable	No Examined		Results of Microscopy				Results of Ab ELISA			
	Madina	Tabuk	Positive (%)		*p* value		Positive (%)		*p* value	
			Medina	Tabuk	Medina	Tabuk	Medina	Tabuk	Medina	Tabuk
Sex										
Male	64	39	8 (12.5)	9(23.1)	>0.05	>0.05	8 (12.5)	8 (20.5)	>0.05	>0.05
Female	60	63	6 (10)	21(33.3)			4 (6.7)	18 (28.6)		
Age (Months)										
<2 years	34	60	2 (5.9)	18 (30)	>0.05	>0.05	3(8.8)	11 (18.3)	>0.05	**<0.05**
≥2 years	90	42	12 (13.3)	12 (28,6)			9(10)	15 (35.7)		
Season										
Summer	49	58	3 (6.1)	25 (43.1)	>0.05	**<0.05**	4 (8.2)	24 (41.4)	>0.05	**<0.05**
Winter	75	44	11(14.7)	5 (11.4)			8 (10.7)	2 (9.1)		
Rearing System										
Open	50	50	6(12)	5(10)	>0.05	**<0.05**	3(6)	2(4)	>0.05	**<0.05**
Intensive	74	52	8(10.8)	25(48)			9(12)	24(46)		

lence of anaplasmosis using both microscopic and ELISA methods in male and female goats in the Madina and Tabuk regions ($p > 0.05$). Likewise, there was no significant difference ($p > 0.05$) in the prevalence in sheep with different ages in both regions studied. However, there was a significant difference in the prevalence of anaplasmosis in older goats (>2 years old) using the cELISA method ($p < 0.05$) in the Tabuk region. The prevalence of anaplasmosis in goats in Tabuk was significant ($p < 0.05$) in the summer compared to the winter (Table 2) using the cELISA method (Table 2). There was no significant difference in the prevalence of anaplasmosis in different seasons in goats from Madina using both methods. Goats and sheep kept under intensive conditions had a higher prevalence compared to animals kept under open systems using both methods in Madina and Tabuk. However, the difference was significant ($p < 0.05$) in goats and sheep from Tabuk using both microscopic as well as ELISA methods (Tables 2 and 3).

The majority of animals showed positive antibodies (34.5%) against anaplasmosis with inhibition between 30 and 40%. While 13.1% showed inhibition between 90 and 100%.

4. Discussion

In the present study, we investigated the occurrence and the associate risk factors of a tick-borne disease pathogen anaplasmosis in sheep and goats using direct microscopy and the cELISA method in two regions of the Kingdom of Saudi Arabia. To our

Table 3
Results of microscopic and serological screening for *Anaplasma* spp. Infections in sheep in Medina and Tabuk during 2019–2020, correlation with sex, locality and season.

Variable	No Examined		Results of Microscopy				Results of Ab ELISA			
	Madina	Tabuk	Positive (%)		*p* value		Positive (%)		*p* value	
			Medina	Tabuk	Medina	Tabuk	Medina	Tabuk	Medina	Tabuk
Sex										
Male	36	48	6 (16.7)	15(31.3)	>0.05	**>0** 0.05	1(2.8)	15(31.3)	>0.05	>0.05
Female	41	52	2(4.9)	26(50)			5(12.2)	25(48)		
Age (Months)										
<2 years	16	43	4(25)	15(34.9)	> 0.05	>0.05	2(12.5)	15(34.9)	>0.05	>0.05
≥2 years	61	57	4(6.5)	26(45.6)			4(6.6)	25(43.9)		
Season										
Summer	57	55	2(3.5)	19(34.5)	**< 0.05**	>0.05	0(0)	18(32.7)	–	>0.05
Winter	20	45	6(30)	22(48.9)			6(30)	22(48.9)		
Rearing System										
Open	42	51	2(4.8)	31(60.8)	>0.05	**< 0.05**	0(0)	27(52.9)	–	**<0.05**
Intensive	35	49	6(17.1)	10(20.4)			6(17.1)	13(26.5)		

knowledge, this is the first report of anaplasmosis from the Tabuk region in Saudi Arabia. Using direct microscopic examination of Giemsa-stained blood smears, we detected a prevalence of 23.1%, whereas using the cELISA method, antibodies against anaplasmosis were detected in 20.7% of goats and sheep screened from both regions studied. Ghafar and Amer (2019) detected a prevalence of *A. ovis* as low as 9% of the goats investigated from Taif using molecular techniques. On the other hand, Shabana et al. (2018) detected a much higher prevalence of antibodies against anaplasmosis in 43.6% of the sheep and goats investigated in the western part of Saudi Arabia. In Pakistan, a prevalence of 22.2% of the sheep and goats studied have shown antibodies against anaplasmosis (Khan et al., 2019). Obaidat and Salman (2019) reported as high as 90% seroprevalence in sheep and goats in Jordan. Lower rates, but higher than what has been reported in the present study, were detected from sheep in Portugal (82%), Sudan (42%), Iraq (67%), and Turkey (31%) (Renneker et al., 2013) using molecular techniques. Detection of anaplasmosis in the blood of sheep and goats depends mainly on the method used for the detection, i.e., if it was direct or indirect. Direct methods such as demonstrating the organisms in blood smears is useful; however, it requires expertise and high levels of bacteremia. Low bacteremia can simply result in false-negative results. Indirect methods such as cELISA and molecular techniques detect circulating antibodies and the organism's DNA, respectively. Only two previous reports have dealt with direct microscopy in estimating the prevalence of anaplasmosis infection in small ruminants in the Kingdom of Saudi Arabia (Al-Khalifa et al., 2009; Shabana et al., 2018). Al-Khalifa et al. (2009) reported a prevalence of 2% in sheep from eastern and northern provinces, whereas Shabana et al. (2018) reported a much higher prevalence of 40.7% and 45.5% in goats and sheep, respectively from the western part of Saudi Arabia.

It is interesting, in the present study, to note that 13.1% of the samples investigated showed high levels of antibodies with optical densities greater than 90%, whereas most of other samples revealed optical densities between 30 and 40.

Previous studies related to the prevalence of anaplasmosis in small ruminants have shown contradicting results between males and females (Rajput et al., 2005; Shabana et al., 2018; Khan et al., 2019). Our study observed similar results with no significant difference between the prevalence in males and females in both regions studied. However, the prevalence was higher in females in Tabuk and in males in Madina. In Pakistan, Nasreen et al. (2016) attributed the higher prevalence of anaplasmosis in sheep to some stress factors such as malnutrition and pregnancy in females which may be the case in our study.

We observed that there was a high prevalence of anaplasmosis in older sheep and goats (>2 years) compared to younger animals (<2 years) in both regions. However, the difference was not statistically significant. Similar findings were reported from sheep by Khan et al. (2019) in Pakistan as well as in bovine anaplasmosis in the USA (Aubry and Geale, 2011; Okafor et al., 2019). One explanation for this finding was due to the fact that younger animals were less exposed to tick infestation as they were handled differently compared to adults. Furthermore, as animals and humans get older, they develop fewer T-lymphocytes, and the MHC class II response is lower over time (Graham et al., 2006).

The prevalence of anaplasmosis in different animal species is affected by the season. We found that goats in Tabuk, unlike sheep, showed significantly high prevalence in the summer compared to the values obtained from the winter. Whereas in Madina, sheep showed higher prevalence in the winter. Mohammed et al. (2021) reported a high prevalence of a tick-borne piroplasm in hedgehogs in Saudi Arabia during the summer compared to the winter. El-Bahy et al. (2008) reported a higher prevalence of *Theileria ovis* in central Saudi Arabia. There may be different susceptibilities to sheep and goats for tick infestation, as in the case of the hedgehog and sheep in studies conducted by El-Bahy et al. (2008) and Mohammed et al. (2021).

The system of rearing is important in determining the prevalence of anaplasmosis infection, where in the present study, animals reared under intensive rearing system showed higher prevalence compared to free-range animals. An intensive rearing system or what is called zero-grazing would enable keeping the animals in a pen or a paddock where food is provided without allowing the animals to mix with others. It is likely that there will be an accumulation of tick vectors among hosts in an intensive system if the management practices are poor, such as not following preventive measures regarding external parasites. Khan et al. (2019) reported that animals that are at zero grazing are not affected by anaplasmosis and they attributed that to the control of ticks.

More animals were positive by direct microscopy (23.1%) compared to the cELISA method (20.7%). Hence, the sensitivity of the cELISA was found to be 84.5%, while the specificity was found to be 93.1%. The cELISA method used in the present study employs using the Main Surface Protein 5 (MSP5), which is highly sensitive, specific, and conserved for *Anaplasma* spp. (Visser et al., 1992; Knowles et al., 1996).

5. Conclusion

The occurrence of anaplasmosis was investigated in sheep and goats both in the Madina and Tabuk regions of the Kingdom of

Saudi Arabia using direct microscopy and cELISA. Evidence of anaplasmosis was revealed for the first time in the Tabuk region from both sheep and goats using a serological method with se seroprevalence of 40 % and 25.5 % in sheep and goats, respectively. Occurrence of anaplasmosis in the Madina region was significantly higher than in the Tabuk region ($p < 0.05$) (7.8 % and 9.7 % from sheep and goats orderly). Risk factors, such as sender, age, system of rearing and season were found to be contributory risk factors for the occurrence of the diseases in both regions studied. Further work is recommended in order to determine which species of *Anaplasma* is causing anaplasmosis in both regions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ali, S., Sugimoto, C., Onuma, M., 1996. Equine Piroplasmosis. J. Equine Sci. 7 (4), 67–77.
Al-Khalifa, M.S., Hussein, H.S., Diab, F.M., Khalil, G.M., 2009. Blood parasites of livestock in certain regions in Saudi Arabia. Saudi J. Biol. Sci. 16 (2), 63–67.
Aubry, P., Geale, D.W., 2011. A review of bovine anaplasmosis. Trans. Emerg. Dis. 58 (1), 1–30.
Azzag, N., Petit, E., Gandoin, C., Bouillin, C., Ghalmi, F., Haddad, N., Boulouis, H.-J., 2015. Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers. Comp. Immunol. Microbiol. Infect. Dis. 38, 1–7.
Barbosa, I.C., André, M.R., Amaral, R.B.d., Valente, J.D.M., Vasconcelos, P.C., Oliveira, C.J.B., Jusi, M.M.G., Machado, R.Z., Vieira, T.S.W.J., Ueti, M.W., Vieira, R.F.C., 2021. *Anaplasma marginale* in goats from a multispecies grazing system in northeastern Brazil. Ticks Tick-borne Dis. 12 (1), 101592.
Ben Said, M., Belkahia, H., Sayahi, L., Aloui, M., Jemli, M.H., Hadj Mohamed, B., et al., 2014. First serological study of the prevalence of *Anaplasma phagocytophilum* in dromedary (*Camelus dromedarius*) in Tunisia. Bull. Soc. Pathol. Exot. 107, 1–6.
Chochlakis, D., Ioannou, I., Tselentis, Y., Psaroulaki, A., 2010. Human anaplasmosis and *Anaplasma ovis* variant. Emerg. Infec. Dis. 16 (6), 1031–1032.
Diab, F.M., Al-Khalifa, M.S., Al-Asgah, N.A., Hussein, H.S., Khalil, G.A., 2006. Ticks (Acari: Argasidae, Ixodidae) infesting livestock in Saudi Arabia. Fauna Saudi Arabia 22, 233–244.
Dumler, J.S., Barbet, A.F., Bekker, C., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and "HGE agent" as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. 51, 2145–2165.
El-Azazy, O.M.E., El-Metenawy, T.M., Wassef, H.Y., 2001. *Hyalomma impeltatum* (Acari: Ixodidae) as a potential vector of malignant theileriosis in sheep in Saudi Arabia. Vet. Parasitol. 99 (4), 305–309.
El-Bahy, M.M., Omer, O.H., Al-Sadrani, A.A., 2008. Temperature difference and parasite infection at Qassim region, Saudi Arabia. Res. J. Parasitol. 3 (4), 114–122.
Elhamiani Khatat, S., Khallaayoune, K., Errafyk, N., Van Gool, F., Duchateau, L., Daminet, S., Kachani, M., El Amri, H., Azrib, R., Sahibi, H., 2017. Detection of *Anaplasma* spp. and *Ehrlichia* spp. anibodies, and *Dirofilaria immitis* antigens in dogs from seven locations of Morocco. Vet. Parasitol. 239, 86–89.
Fereig, R.M., Mohamed, S.G.A., Mahmoud, H.Y.A.H., AbouLaila, M.R., Guswanto, A., Nguyen, T.-T., Ahmed Mohamed, A.E., Inoue, N., Igarashi, I., Nishikawa, Y., 2017. Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies in cattle in southern Egypt. Ticks Tick Borne Dis. 8 (1), 125–131.
Ghafar, M.W., Amer, S.A., 2019. Molecular survey of zoonotic *Anaplasma phagocytophilum* and genetic evidence of a putative novel *Anaplasma* species in goats from Taif, Saudi Arabia. Int. J. One Health 5, 54–59.
Goff, W.L., Stiller, D., Roeder, R.A., Johnson, L.W., Falk, D., Gorham, J.R., McGuire, T.C., 1990. Comparison of a DNA probe, complement- fixation and indirect immunofluorescence tests for diagnosing *Anaplasma marginale* in suspected carrier cattle. Vet. Microbiol. 24 (3-4), 381–390.
Graham, J.E., Christian, L.M., Kiecolt-Glaser, J.K., 2006. Stress, age, and immune function: toward a lifespan approach. J. Behav. Med. 29, 389–400.
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 (3), 1318–1323.
Hosseini-Vasoukolaei, N., Oshaghi, M.A., Shayan, P., Vatandoost, H., Babamahmoudi, F., Yaghoobi-Ershadi, M.R., Telmadarraiy, Z., Mohtarami, F., 2014. *Anaplasma* infection in ticks, livestock and human in Ghaemshahr, Mazandaran Province, Iran. *J. Arthropod-borne Dis.* 8, 204–211.
Khan, A., Nasreen, Mitchell, R.D., Niaz, S., Ayaz, S., Khattak, I., Naeem, H., de León, A. A.P., Zaman, M.A., 2019. Seroprevalence of *Anaplasma* spp. among sheep and goats in Charsadda District, Pakistan. *Small Rumin. Res.* 176, 5–10.
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 (9), 2225–2230.
M'Ghirbi, Y., Ghorbel, A., Amouri, M., Nebaoui, A., Haddad, S., Bouattour, A., 2009. Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. Parasitol. Res. 104 (4), 767–774.
Mohammed, O.B., Amor, N.M.S., Omer, S.A., Alagaili, A.N., 2021. Molecular detection and characterization of *Theileria* sp. from hedgehogs (Paraechinus aethiopicus) in Saudi Arabia. Lett. Appl. Microbiol. 72 (4), 476–483.
Muhammad, G., Saqib, M., Athar, M., Khan, M.Z., Asi, M.N., 1999. Clinico-epidemiological and therapeutic aspects of bovine theilleriasis. Pakistan Vet. J. 19, 64–69.
Nagore, D., García-Sanmartín, J., Garcıa-Pérez, A.L., Juste, R.A., Hurtado, A., 2004. Identification, genetic diversity and prevalence of Theileria and Babesia species in a sheep population from Northern Spain. Int. J. Parasitol. 34 (9), 1059–1067.
Nasreen, K.S., Khan, A., Niaz, S., Akhtar, N., 2016. Serodiagnosis and haematological effect of anaplasmosis in goats and sheep of district Mardan, Khyber Pakhtunkhwa, Pakistan. *World. J. Zool.* 11, 67–80.
Nicholson, W.L., Allen, K.E., McQuiston, J.H., Breitschwerdt, E.B., Little, S.E., 2010. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol. 26 (4), 205–212.
Obaidat, M.M., Salman, A.E.B., 2019. *Anaplasma* spp. in dairy ruminants in Jordan: high individual and herd-level seroprevalence and association with abortions. J. Vet. Diagn. Invest. 31, 481–484.
Okafor, C.C., Collins, S.L., Daniel, J.A., Coetzee, J.F., Whitlock, B.K., 2019. Factors associated with seroprevalence of bovine anaplasmosis in Mississippi, USA. Vet. Parasitol. 17, 100301.
Parola, P., Davoust, B., Raoult, D., 2005. Tick-and flea-borne rickettsial emerging zoonoses. Vet. Res. 36 (3), 469–492.
Passos, L.M.F., Bell-Sakyi, L., Brown, C.G.D., 1998. Immunochemical characterization of in vitro culture-derived antigens of *Babesia bovis* and *Babesia bigemina*. Vet. Parasitol. 76 (4), 239–249.
Rajput, Z.I., Hu, S.-H., Arijo, A.G., Habib, M., Khalid, M., 2005. Comparative study of *Anaplasma* parasites in tick carrying buffaloes and cattle. J. Zhejiang Univ. Scie. 6B (11), 1057–1062.
Renneker, S., Abdo, J., Salih, D.E.A., Karagenç, T., Bilgiç, H., Torina, A., Oliva, A.G., Campos, J., Kullmann, B., Ahmed, J., Seitzer, U., 2013. Can *Anaplasma ovis* in small ruminants be neglected any longer. Trans. Emerg. Dis. 60, 105–112.
Salih, D.A., Liu, Z., Bakheit, M.A., Ali, A.M., El Hussein, A.M., Unger, H., Viljoen, G., Seitzer, U., Ahmed, J.S., 2008. Development and evaluation of a loop-mediated isothermal amplification method for diagnosis of tropical theileriosis. Trans. Emerg. Dis. 55 (5-6), 238–243.
Sekiya, M., Zintl, A., Doherty, M.L., 2013. Bulk milk ELISA and the diagnosis of parasite infections in dairy herds: a review. Irish Vet. J. 66, 1–12.
Shabana, I.I., Alhadlag, N.M., Zaraket, H., 2018. Diagnostic tools of caprine and ovine anaplasmosis: a direct comparative study. BMC Vet. Res. 14, 1–8.
T. Al-Obaidi, Q., H. Arslan, S., G. Sulaiman, E., Daham, E., 2010. Clinical, haematological and biochemical studies of babesiosis in native goats in Mosul Iraq. J. Vet. Sci. 24 (1), 31–35.
Sumbria, D., Singla, L.D., 2015. Recent diagnostic and control approaches in equine piroplasmosis. Vet. J. 2, 1–6.
Vanstreels, R.E.T., Yabsley, M.J., Parsons, N.J., Swanepoel, L., Pistorius, P.A., 2018. A novel candidate species of *Anaplasma* that infects avian erythrocytes. Parasites Vectors 11, 1–7.
Visser, E.S., McGuire, T.C., Palmer, G.H., Davis, W.C., Shkap, V., Pipano, E., Knowles, D. P., 1992. The *Anaplasma marginale* msp5 gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. Infect. Immun. 60 (12), 5139–5144.
Yousefi, A., Rahbari, S., Shayan, P., Sadeghi-dehkordi, Z., Bahonar, A., 2017. Molecular detection of *Anaplasma marginale* and *Anaplasma ovis* in sheep and goat in west highland pasture of Iran. Asian Pac. J. Trop. Biomed. 7 (5), 455–459.