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RESEARCH ARTICLE

SEROPREVALENCE OF *ANAPLASMA* sp. IN SHEEP (*OVIS ARIES)* BY ELISA IN PESHAWAR, PAKISTAN

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ABSTRACT

In the present study, sero-prevalence of anaplasma sp. in sheep, *Ovisaries* (L) was done from January-May, 2012 in Peshawar, Pakistan. The information concerning anaplasmosis in sheep is scare. For this purpose, 376 serum samples were obtained randomly from4 different breeds of sheep, from different areas of Peshawar, and an indirect ELISA using recombinant MSP-5 as antigen of *Anaplasmamarginale* (T), was performed. Totally, 92/376 (24.47%) of the overall sheep sera were positive for antibodies against *A. marginale*. In 4 breeds of sheep, (i.e. Balkhai, Watanai, Punjabai and Turkai) Turkai were found highly infected i.e. 27/376 (7.20%). This is the first record of *A. marginal* infection in Sheep in Peshawar, Pakistan, which is very high. This research should be useful in epidemiological applications.

INTRODUCTION

Sheep, *Ovisaries* (L) is one of the initial animals, domesticated for agricultural purposes; it is raised for meat, (hogget or mutton, lamb) milk and fleece production. These quadru-pedal ruminant mammals are members of the order *Artiodactyla*, the even-toed ungulates typically kept as livestock. It has great economic potential because of their early maturity and high fertility as well as their adaptability to moist environment (Ademosun, 1988). However, the benefits derived are too low from the expected due chiefly to low productivity. Numerous factors are involved in this low productivity, in which the major one is disease (Akerejola *et al.,* 1979). Diseases caused by heamoparasites are most apparent. These heamoparasites are parasites found in the blood of mammals in which *A. marginale* is also include. Ticks are biological vectors of *Anaplasma* sp.; tick, mammalian or bird hosts with persistent *Anaplasma* sp. infection can serve as reservoir of infection naturally. *Anaplasma* sp. is intracellular, gram-negative bacteria and representatives of the order *Rickettsiales* classified into *Rickettsiaceae* and *Anaplasmataceae* families (Dumler *et al.,* 2001). The tick vector distribution is the factor influencing the transmission of tick-borne diseases (Bazarusanga *et al.,* 2007a). However, for *A. marginale*, mechanical transmission through contaminated hypodermic needles and biting flies plays an important role (Potgieterand Stoltsz, 2004). Erythrocytes are phagocyted by reticulo-endothelial cells during infection. Animals may die older than 2 years due to the infection (Kocan *et al.,* 2003). Nevertheless, concerning ovine anaplasmosis, little information is available, in despite of the expressive number of sheep, goat and expansion of small ruminant herds in this country. Diagnosis of anaplasmosis in small ruminants mainly based in the identification of the rickettsia in stained blood smears. However, below 0.1% rickettsemias in chronic carriers are not detected by this method (Palmer, 1992). Serological assays, based on Major Surface Protein 5 (MSP-5) of *A. marginale* have been successfully used, for the detection of antibodies against *Anaplasma* sp. (Strik *et al.,* 2007). In this study, we observed for the first time sero-prevalence of *Anaplasma* sp., in different breeds of sheep using an indirect ELISA based on MSP5 recombinant of *A. marginale,* in Peshawar, Pakistan. This research should be particularly useful for epidemiological applications such as prevalence studies, awareness, education, research and control programs in this region.

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MATERIALS AND METHODS

Samples collection

Conveniently, 376 blood sampling was collected from the overall sheep population of different areas of Peshawar from

Table 1. Sex wise positive samples of sheepanaplasmosis by indirect Enzyme Linked Immunosorbent Assay (iELISA) from January to May, 2012 from Peshawar, Pakistan

S. No.	Sheep Breeds	Age						
		n^1	Adult n^2	Positive*(%)		Young n^3	Positive**(%)	
1	Balkhai	124	78	14	(17.94)	46	9	(19.56)
2	Watanai	97	70	18	(25.71)	27	8	(29.62)
3	Punjabai	76	42	8	(19.04)	34	8	(23.52)
4	Turkai	81	36	10	(27.77)	45	17	(37.77)

n^1: total numbers of samples examined; **n^2** : total numbers of male samples examined
n^3 : total numbers of female samples examined**; (*) (**)** represents positive samples for *A.marginale* of male and female respectively

Table 2. Age wise positive samples of sheepanaplasmosis by indirect Enzyme Linked Immunosorbent Assay (iELISA) from January to May, 2012 from Peshawar, Pakistan

S. No.	Sheep Breeds	Sex						
		n^1	Male n^2	Positive*(%)		Female n^3	Positive**(%)	
1	Balkhai	124	93	17	(18.28)	31	6	(19.35)
2	Watanai	97	71	21	(29.57)	26	5	(19.23)
3	Punjabai	76	58	12	(20.68)	18	4	(22.22)
4	Turkai	81	65	22	(33.85)	14	7	(50.00)

n^1 : total numbers of samples examined; **n^2**: total numbers of adult samples examined
n^3 : total numbers of young samples examined**; (*) (**)** represent positive samples for *A.marginale* of adult and young respectively

January to May 2012. About 5 ml blood samples were collected from the jugular vein of each sheep with a sterile hypodermic syringe into an evacuated tube containing gel breed, age, and sex were noted. The blood sample was then centrifuge for 5 minutes at 12000 rpm to separate serum and stored at −35°C until further use. The SVANOVIR®*A. marginale*-Ab ELISA kit (Svanova Biotech AB, Uppsala, Sweden) was used for the diagnosis of specific antibodies against *A. marginale* in bovine serum samples. The kit procedure was based on the Indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA). The whole procedure was done according to the protocol given with the kit.

Protocol for Indirect Enzyme Linked Immunosorbent Assay (iELISA)

All reagents were equilibrated to room temperature 18 to 25 °C before use. Pre-dilution of control and samples 1/40 in PBS-tween buffer (e.g., 10 µl sample in 390 µl of PBS-tween buffer). Hundred micro liter of pre-diluted serum sample was added to selected wells. The plate was then seal and incubate at 37 °C for 30 minutes. The plate was rinse 4 times with PBS-tween buffer. Hundred micro liter of conjugate dilution was added to each well and then sealed the plate and incubate on 37 °C for 30 minutes. Again, and clot activator. Some information like the plate was rinse 4 times with PBS-tween buffer. Hundred micro liter substrate solution was added to each well and then incubated for 30 minute at room temperature (18 to 25 °C). Hundred micro liter of stop solution was added to each well and mixed thoroughly. The Optical Density (OD) of the controls and sample was measured at 405 nm in a micro-plate photometer (BIOTEK Instruments Inc., Winooski, Vermont, U.S.A.). Mean OD values were calculated for each of the control and samples.

Data analysis

The following formula was used for the Percent Positivity (PP): PP= [(sample OD ×100)/Mean positive control OD]

Interpretation of the results

The calculated Percent Positivity (PP) if less than 25%, the sample was consider as negative and if PP was equal or more than 25%, then the sample was consider as positive.

RESULTS

There were overall 92 (24.47%) positive and 284 (75.53%) negative blood samples for A. marginale of sheep breeds were: Balkhai—18.55% (23/124), Watanai—26.80% (26/97), Punjabai—21.05% (16/76) and Turkai35.80% (29/81) (Figure 2). Sex wise, in total 124 Balkhai samples, 17/93(18.28%) male positive and 6/31 (19.35%) were female positive. In total 97 Watanai samples, 21/71 (29.58%) male positive and 5/26 (19.23%) were female positive. In Punjabai 76 samples, 12/58 (20.69%) male positive and 4/18 (22.22%) were female positive. In Turkai 81, 22/65 (33.85%) male positive and 7/14 (50.00%) were female positive (Table 1). Age vise in total 124 Balkhai, they were 9/46 (19.56%) young positive and 14/78 (17.95%) were adult positive. In total 97 Watanai, there were 8/27 (29.63%) young positive and 18/70 (25.71%) were adult

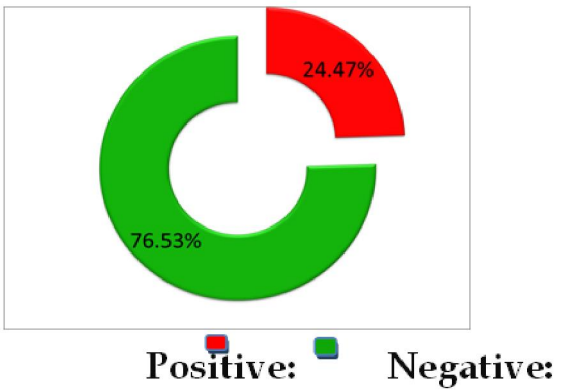

Fig. 1. Number of positive (24.47%) and negative (76.53%) samples of sheep (*Ovisaries*) of *A.marginale*by indirect Enzyme Linked Immunosorbent Assay (iELISA) from January to May, 2012 from Peshawar, Pakistan

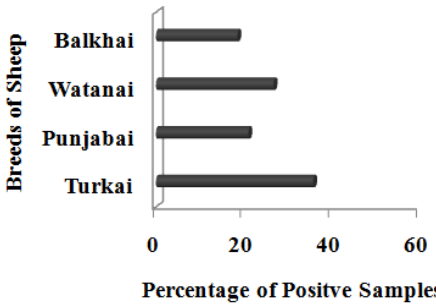

Fig. 2. Breeds wise percentage of positive samples of sheep (*O. aries*) of *A. marginale*by indirect Enzyme Linked Immunosorbent Assay (iELISA) from January to May, 2012 from Peshawar, Pakistan

positive. In total 76 Punjabai, there were 8/34 (23.53%) young positive and 8/42 (19.05%) were adult positive. In total 81 Turkai, they were 17/45 (37.77%) young positive and 10/36 (27.77%) were adult positive (Table 2).

DISCUSSION

The research on sheep anaplasmosis (*A. marginale*) is rare and little literature is available. The frequency of sero- (Figure 1). The frequencies in the four positivity of sheep anaplasmosisin this research were (24.47%) which is very low as compared to the prevalence of sero-positive sheep found by Hornok *et al.* (2007) (99.4%) in Hungry and high as compared to the prevalence of sero-positive sheep found by Cabral *et al.* (2009) (8.92%). Sero-prevalence were found by Ramos *et al.* (2008) (16.17%) in Ibimirim county, semi-arid region of Pernambuco State, Brazilusing monoclonal antibody ANAF16C1 and De La Fuente *et al.* (2005) (75.0%), in sicily, Italy, using competitive ELISA, based on recombinant MSP-5 of *A. marginale*. The low sero-prevalence rate in this research work can be the cause of low tick vector population in Peshawararea. However, some ticks were also observed in sheep during blood samples collection. This result represents the first description of antibodies for *Anaplasma* sp. in sheep from Peshawar, Pakistan. Further studies are require to know the epidemiology of *Anaplasma* sp. infection in sheep, in Pakistan, particularly to define which species is involved, possible impacts and vectors in animal production and in public health.

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REFERENCES

Ademosun AA. 1988. Appropriate management systems for West African dwarf sheep and goats in humid tropics. In: O.B. Smith and H.G. Basman (eds.), Goat Production in the Tropics, Proc. Workshop at the University of Ife, Ile-Ife, Nigeria 20 – 24.

Akerejola OO, Schillhorn van VTW, Njoku CO. 1979. Ovine and Caprine diseases in Nigeria: a review of economic losses. Bulletin of Animal Health and Production in Africa 27, 65 – 70.

Bazarusanga T, Geysen D, Vercruysse, Madder M. 2007a. An update on the ecological distribution of Ixodid ticks infesting cattle in Rwanda: country-wide cross-sectional survey in the wet and the dry season. Experimental and Applied Acarology, 43, 279–291.

Cabral DA, Araújo, Flábio Ribeiro de, Ramos, Carlos Alberto do Nascimento, Alves LC, Porto Wjn, Faustino MA da Gloria. 2009. Serological survey of *Anaplasma* sp. in sheep from State of Alagoas, Brazil, Revista Brasileira Saúde Producao Animal, 10(3), 708-713.

Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, Rikihisa Y and Rurangirwa FR. 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 *Ehrlichiaequi* and 'HGE agent' as synonyms of *Ehrlichiaphagocytophila*. International Journal of Systematic Evolutionary Microbiology, 51, 2145–2165. http://dx.doi.org/10.1099/00207713-51-6-2145.

Hornok S, Elek V, De La Fuente J, Naranjo V, Farkas R, Majoros G, Foldvári G. 2007. First serological and molecular evidence on the endemicity of *A. ovis* and *A. marginale* in Hungary. Veterinary Microbiology, 122(4), 316-322.

Kocan KM, De La Fuente J, Guglielmone AA, Mele´ndez RD. 2003. Antigens and alternatives for control of *A. marginale* infection in cattle. Clinical Microbiology Reviews, 16, 698–712. http://dx.doi.org/10.1128/CMR.16.4.698-712.2003.

Palmer GH. 1992. Development of diagnostic reagents for anaplasmosis and babesioses. In: Dolan, T.T. Recent developments in the control of anaplasmosisbabesioses and cowdriosis. English Press, International Laboratory for Animal Diseases, Nairobi: pp 56-66.

Potgieter FT, Stoltsz WH. 2004. Bovine anaplasmosis. In: Coetzer JAW, Tustin RC, (Eds.), Infectious Diseases of Livestock, vol. I. Oxford University Press, Southern Africa, Cape Town, pp. 594–616.

Ramos RAN, Ramos CAN, Araújo FR, Melo ESP, Tembue AAS, Faustino MAG, Alves LC, Rosinha GMS, Elisei C and Soares CO. 2008. Detecção de anticorpospara Anaplasma sp. empequenosruminantes no semi-áridodo Estado de Pernambuco, Brasil. Revista Brasileira de Parasitologia Veterinária, 17(2), 115-117.

Strik NI, Alleman AR, Barbet AF, Sorenson HL, WAMSLEY HL, Gaschen FP, Luckschander N, Wong S, Chu F, Foley JE, Bjoersdorff A and Stuen S. 2007. Characterization of *A. phagocytophilum* major surface proteins 5 and the extent of its cross-reactivity with *A. marginale*. Clinical and Vaccine Immunology, 14(3), 262-268.

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