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Geographical seroprevalence of *Anaplasma marginale* infection (anaplasmosis) by ELISA in *Ovis aries,* in district Peshawar, Pakistan

Authors: Muhammad Kashif and Munawar Saleem Ahmad

Muhammad Kashif
Department of Zoology,
Hazara University,
Mansehra-23100, Pakistan.
E-mail: Mkashiflap1@gmail.com

Munawar Saleem Ahmad
Department of Zoology,
Hazara University,
Mansehra-23100, Pakistan.
E-mail: saleemsbs@gmail.com

Correspondence:
Muhammad Kashif
Department of Zoology,
Hazara University,
Mansehra-23100, Pakistan.
E-mail: Mkashiflap1@gmail.com

Abstract

Geographical seroprevalence of *Anaplasma marginale*(T) in sheep, *Ovis aries* (L) was done from January-May, 2012 in district Peshawar which is a crowded area of Pakistan. In this area sheep's infection with *A. marginale* is not reported before. For this purpose, 376 serum samples were obtained conveniently from 4 different breeds of sheep, from different geographical areas of Peshawar. An indirect ELISA using recombinant MSP-5 as antigen of *A. marginale*, was performed. Totally, 92/376 (24.47%) of the overall sheep sera were positive. In 6 areas of Peshawar, Peshthakhara and Mashokhel area were found highly infected i.e. 32.00% and 32.00% respectively, while Ghazi baba area was less infected comparatively. This is the first record of *A. marginale* showing high rate infection in sheep in Peshawar, Pakistan, This research should be useful in epidemiological applications.

Keywords: Epidemiology; *A. marginale*; MSP-5; Peshawar

1. Introduction

Sheep, *Ovis aries* (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 [1]. 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 [2].

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 [5]. The tick vector distribution is the factor influencing the transmission of tick-borne diseases [3]. However, for *A. marginale*, mechanical transmission through contaminated hypodermic needles and biting flies plays an important role [9].

Erythrocytes are phagocyted by reticulo-endothelial cells during infection. Animals may die older than 2 years due to the infection [7]. 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 [9]. 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. [11]. 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.

2. Materials and Methods

2.1 Samples Collection

Conveniently, 376 blood sampling was collected from the overall sheep population of different areas of Peshawar from 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 and clot activator. Some information like 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.

2.2 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, 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.

2.3 Data analysis

The following formula was used for the percent positivity (PP):

PP= [(Sample OD ×100)/Mean positive control OD]

2.4 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.

3. Results

There were overall 92 (24.47%) positive samples for *A.marginale* of *O.aries.* In Ghazi Baba 19 (19.00%) positive cases were detected in which 6 (13.33%) were Balkhai, 4 (16.00%) Watanai, 1 (16.67%) Punjabi and 8 (30.77%) Turkai. In Warsak road 17 (22.66%) positive cases were detected in which 3 (12.00%) were Balkhai, 7 (31.82%) Watanai, 3 (18.75%) Punjabi and 4 (33.33%) Turkai. In Badabher 19 (25.33%) positive cases were detected in which 5 (20.83%) were Balkhai, 6 (50.00%) Watanai, 4 (16.67%) Punjabi and 4 (26.67%) Turkai. In Peshtakhara 16 (32.00%) positive cases were detected in which 4 (40.00%) were Balkhai, 1 (8.33%) Watanai, 3 (23.10%) Punjabi and 8 (53.33%) Turkai. In Mashokhel 16 (32.00%) positive cases were detected in which 4 (33.33%) were Balkhai, 4 (25.00%) Watanai, 3 (33.33%) Punjabi and 5 (38.46%) Turkai. In Barha 8 (32.00%) positive cases were detected in which 2 (28.57%) were Balkhai, 3 (27.027%) Watanai, 3 (37.5%) Punjabi and 0 (0.00%) Turkai (Table 1). The infection was high in Peshtakhara, Mashokhel and Barha, while lower in Ghazi baba as compare to other areas.

Table 1: Area wise collected and positive blood samples for *A. marginale* by indirect Enzyme Linked Immunosorbent Assay (iELISA) in sheep during January -May, 2012 in Peshawar, Pakistan.

S No.	Area	Total sample	Positive (%)	Balkhai		Watanai		Punjabi		Turkai	
				n^1	P(%)	n^2	P (%)	n^3	P (%)	n^4	P (%)
1	**Ghazi Baba, Ring road**	100	19 (19.00)	45	6 (13.33)	25	4 (16.00)	6	1 (16.67)	26	8 (30.77)
2	**Warsak Road**	75	17 (22.66)	25	3 (12.00)	22	7 (31.82)	16	3 (18.75)	12	4 (33.33)
3	**Badabher**	75	19 (25.33)	24	5 (20.83)	12	6 (50.00)	24	4 (16.67)	15	4 (26.67)
4	**Peshtakhara**	50	16 (32.00)	10	4 (40.00)	12	1 (8.33)	13	3 (23.10)	15	8 (53.33)
5	**Mashokhel**	50	16 (32.00)	12	4 (33.33)	16	4 (25.00)	9	3 (33.33)	13	5 (38.46)
6	**Barha**	26	8 (32.00)	7	2 (28.57)	11	3 (27.27)	8	3 (37.5)	0	0 (0.00)

n^1, n^2, $n^3$and n^4: Shows the total number of collected samples of Balkhai,Watanai, Punjabi and Turkaibreed respectively.
P: Indicate the positive samples for *A. marginale.*

4. Discussion

The research on sheep anaplasmosis (A. marginale) is rare and little literature is available. The frequency of sero-positivity of sheep anaplasmosis in this research were (24.47%) which is very low as compared to the prevalence of sero-positive sheep found by Hornoket al.[6] (99.4%) in Hungry and high as compared to the prevalence of sero-positive sheep found by Cabral et al. [4] (8.92%). Sero-prevalence were found by Ramos et al.[10] (16.17%) in Ibimirim county, semi-arid region of Pernambuco State, Brazil using monoclonal antibody ANAF16C1 and De La Fuente et al. [5] (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 Peshawar area. 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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6. References

1. Ademosun AA. 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. 1988; 20 – 24.
2. Akerejola O.O, Schillhorn van V.T.W., Njoku, C.O. Ovine and Caprine diseases in Nigeria: a review of economic losses. Bulletin of Animal Health and Production in Africa 27, 1979; 65 – 70.

3. Bazarusanga T., Geysen D, Vercruysse, Madder M. 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, 2007a; 43: 279–291.
4. Cabral, D.A., Araújo, FlábioRibeiro de, RAMOS, Carlos Alberto do Nascimento, Alves L.C., Porto W.J.N., Faustino M.A. da Gloria. Serological survey of Anaplasma sp. in sheep from State of Alagoas, Brazil, RevistaBrasileiraSaúdeProducao Animal, 2009; 10(3), 708-713.
5. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, Rikihisa Y. Rurangirwa FR. 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. 2001; 51: 2145–2165.
6. Hornok S, Elek V, De La Fuente, J., Naranjo, V, Farkas R, Majoros G, Foldvári, G. First serological and molecular evidence on the endemicity of A. ovis and A. marginale in Hungary. Veterinary Microbiology. 2007; 122(4): 316-322.
7. Kocan K.M, de la Fuente J., Guglielmone, A.A., Mele´ndez, R.D. Antigens and alternatives for control of A. marginale infection in cattle. Clinical Microbiology Reviews. 2003; 16: 698–712.
8. Palmer GH. 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: 1992; pp56-66.
9. Potgieter FT, Stoltsz WH. Bovine anaplasmosis. In: Coetzer JAW, Tustin RC, (Eds.), Infectious Diseases of Livestock, vol. I. Oxford University Press, Southern Africa, Cape Town. 2004; pp. 594–616.
10. Ramos RAN, Ramos CAN, Araújo FR, Melo ESP, Tembue AAS, Faustino MAG, Alves LC, Rosinha, GMS, Elisei C, Soares CO. Detecção de anticorposparaAnaplasma sp. empequenosruminantes no semi-árido do Estado de Pernambuco, Brasil. RevistaBrasileira de ParasitologiaVeterinária, 2008; 17(2): 115-117.
11. Strik NI, Alleman AR, Barbet AF, Sorenson HL, Wamsley HL, Gaschen FP, Luckschander N, Wong S, Foley JE, Bjoersdorff A, Stuen S. Characterization of A. phagocytophilum major surface proteins 5 and the extent of its cross-reactivity with A. marginale. Clinical and Vaccine Immunology. 2007; 14(3): 262-268.

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