

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

¹Muhammad Kashif, ¹Munawar Saleem Ahmad, ²Ghufran Ullah and ³Iftikhar Fareed

¹Department of Zoology, Hazara University, Mansehra-23100, Pakistan

²Director General, Veterinary Research Institute, Peshawar-25000, Pakistan

³Department of Natural Resource Engineering and Management, University of Kurdistan, Kurdistan, Iraq
mkashiflap1@gmail.com

Abstract: Geographical sero-prevalence 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.

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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 (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 (Potgieter and Stoltz, 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% rickettssemias 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.

2. Materials and Methods

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.

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.

Data analysis

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

$$\text{PP} = \frac{[\text{Sample OD} \times 100]}{\text{Mean positive control OD}}$$

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.

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%) Punjabai 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%) Punjabai 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%) Punjabai 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%) Punjabai 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%) Punjabai 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%) Punjabai 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.

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 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, Brazil using 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 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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Corresponding Author:

Dr. Munawar Saleem Ahmad
Department of Zoology
Garden Campus, Hazara University
Mansehra-23100, Pakistan
Saleemsbs@gmail.com

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 | | Punjabai | | 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) |

n¹, n², n³ and n⁴: Shows the total number of collected samples of Balkhai, Watanai, Punjabai and Turkai breed respectively.

P: Indicate the positive samples for *A. marginale*.

References:

- Ademosun, AA. Appropriate management systems for West African dwarf sheep and goats in humid tropics. In: OB. Smith and HG. Basman (eds.), Goat Production in the Tropics, Proc. Workshop at the University of Ife, Ile-Ife, Nigeria, 1988; 20 – 24.
- Akerejola OO., Schillhorn van VTW., Njoku, CO.. Ovine and Caprine diseases in Nigeria: a review of economic losses. Bulletin of Animal Health and Production in Africa, 1979; 27, 65 – 70.
- Bazarusanga, T., Geysen, D., Vercruyssen, 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.
- Cabral, DA., Araújo, Flávio Ribeiro de, RAMOS, Carlos Alberto do Nascimento, Alves LC., Porto WJN, Faustino MA. da Gloria. Serological survey of Anaplasma sp. in sheep from State of Alagoas, Brazil, Revista Brasileira Saúde Producao Animal, 2009; 10(3), 708-713.
- Dumler, JS., Barbet, AF., Bekker, CPJ., Dasch, GA., Palmer, GH., Ray, SC., Rikihisa, Y. and Rurangirwa, F.R. 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 synonyms of Ehrlichia phagocytophila. International Journal of Systematic Evolutionary Microbiology, 2001; 51, 2145–2165.
- 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.
- Kocan, KM., de la Fuente J., Guglielmone, AA., Mele'ndez, RD. Antigens and alternatives for control of *A. marginale* infection in cattle. Clinical Microbiology Reviews, 2003; 16, 698–712.
- Palmer, GH. Development of diagnostic reagents for anaplasmosis and babesiosis. In: Dolan, TT. Recent developments in the control of anaplasmosis babesiosis and cowdriosis. English Press, International Laboratory for Animal Diseases, Nairobi, 1992; pp 56-66.
- Potgieter, FT., Stoltz, 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. and Soares, CO. Detecção de anticorpos para *Anaplasma* sp. em pequenos ruminantes no semi-árido do Estado de Pernambuco, Brasil. *Revista Brasileira de Parasitologia Veterinária*, 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. and 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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