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Research Article

Incidence of Bovine Anaplasma Marginale in Sindh, Pakistan

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Abstract: The goal of the study was to look at the Incidence of Anaplasma marginale in cattle in the Thatta, Tharparkar and Hyderabad districts of Sindh, Pakistan. Using a multistage cluster random sampling technique, a total of n=1500 samples were obtained from chosen small holders and private animal farms. Using a competitive enzyme-linked immunosorbent test, the overall Incidence of Anapalsma marginale infection was 38.13% (cELISA). The district with the greatest Incidence was Tharparkar (42.4%), followed by Thatta (37.2%) and Hyderabad (34.8%). Different age groups and breeds were shown to have a significant association. Competitive ELISA based on major surface protein-5 has a significant advantage over other serological markers for anaplasmosis because of its superior sensitivity (96%) and specificity (95%) for anaplasmosis. In all of the study districts, Incidence was considerably greater in small holders than in private livestock farms. In the summer, small-holder crossbred cattle over the age of four years from the Tharparkar district are more susceptible to Anaplasma marginale infection. The Tharparkar district's moderate climate encourages vector tick proliferation and multiplication. Furthermore, the higher Incidence of R. (Boophilus) microplus and stall feeding methods at Tharparkar may have contributed to the higher Incidence of Anaplasma marginale infection. The large variance in Incidence between study districts is explained by the fact that major parts of study districts have unique agro-ecological zones.

Keywords: Incidence, Anaplasma Marginale, Cattle, Sindh

1. Introduction

Anaplasmosis is a serious tick-borne disease that affects cattle worldwide and has a high economic cost [2]. Bovine anaplasmosis causes fever, weight loss, decreased milk supply, pale mucous membranes, severe anaemia, jaundice, brownish urine, hyperexcitability abortion, and mortality without hemoglobinemia or hemoglobinuria [1, 3]. Agglutination test, indirect fluorescent antibody test, complement fixation, and indirect ELISA are examples of serological assays routinely used for Anaplasma marginale serodiagnosis [5]. These tests had sensitivity, repeatability, and interpretation issues, as well as non-specific reactions [13, 14]. Because of the higher sensitivity (96%) and specificity (95%) of competitive ELISA, it offers a clear advantage [25]. A serological survey of Anaplasma marginale infection in cattle using a competitive enzyme linked immunosorbent assay in Sindh is scarce, if at all [7, 8]. The majority of previous Pakistani reports were based on the analysis of stained blood smears [6]. Using microscopic examination of blood smears, the Incidence of Anaplasma marginale in Pakistan was found to be 7.36-75.71% [13]. Detection of chronically infected carriers with low parasitaemia levels is difficult with this technique [15]. Identification of carriers is critical from both an epidemiological and a disease prevention and control perspective. As a result, the goal of this study was to find out how common Anaplasma marginale infection is in the different districts of Sindh, specifically in Tharparkar, Thatta and Hyderabad.

2. Material and Methods

2.1. Study Areas

Epidemiological investigations were undertaken in the Sindh districts of Tharparkar, Thatta, and Hyderabad, Pakistan. The average temperature of Thatta district is 32-49°C in the summer and 5-25°C in the winter, with 496 millimeters of rain per year. The hottest and driest district is Tharparkar. Summer temperatures range from 34 to 51°C elsius, while winter temperatures range from 10 to 29 degrees Celsius, with an average annual precipitation of 421 millimeters. The district of Hyderabad is classified as an arid high rainfall zone [9, 15]. Summer temperatures range from 23-46.5°C, while winter temperatures range from 8-19.5°C, with an annual average rainfall of 764 millimeters [5, 12].

2.2. Sampling Method

During April 2020 to March 2021, a serological survey on the Incidence of Anaplasma marginale was done in the Sindh, on indigenous and crossbred cattle in the Tharparkar, Thatta and Hyderabad districts. Using the multistage cluster random sampling technique [24], a total of 1500 blood samples were obtained from randomly selected small holders (n=120) and private livestock farms (n=26) and serum was separated. Each district's primary, secondary, and tertiary sampling units. Animals of various ages were sampled, including those aged 1 year, 1-2 years, > 2-4 years, and > 4 years. Small holders and private livestock farms were chosen based on the following criteria: a) small holder with 1-10 cattle; b) livestock farm with 50 cattle; c) distance between small holder farms less than 5 kilometers; d) distance between livestock farms greater than 10 kilometers.

2.3. Serological Examination

MSP-5 competitive enzyme linked immunosorbent assay (cELISA) was used to detect antibodies against A. marginale in serum using a commercially available Anaplasma Antibody Test Kit, cELISA (VMRD Inc., Pullman, WA, USA) confirmed earlier [25]. The test was carried out as directed by the manufacturer (VMRD Inc., Pullman, WA, USA). At a wavelength of 620 nm, an ELISA reader (Awareness Technologies, Inc.'s Statfax® 2100 Microplate Reader) was used to determine the optical density. To distinguish between positive and negative samples, a 30% inhibition limit was applied. Serum samples with less than 30% inhibition were deemed positive, whereas samples with more than 30% inhibition were deemed negative. The % inhibition was determined using the following formula:

$$\text{I. P} = 100\ \frac{\text{Sample Optical density}}{\text{Mean Negative Control optical density}} \times 100$$

Incidence was estimated using formula: P=d/n × 100; where I. P Inhibition Percentage, P=Incidence, d=No. of animals found positive, n=Total no. of animals sampled [23].

2.4. Statistics Analysis

The data was statistically examined using the Statistical Package for Social Services (SPSS) version 13.0 and the Chi square test. Statistical significance was defined as a $P<0.05$.

3. Results

Anaplasma marginale was detected in 572 (38.13%) of the 1500 samples tested. Tharparkar district has the highest Incidence (42.4%), followed by Thatta (37.2%) and Hyderabad (34.8%) (Table 1).

Table 1. *Incidence of bovine Anaplasma Marginale.*

District	No of Animals Examined	Positive Sample	Positive (%)
Tharparkar	500	212	42.4
Thatta	500	186	37.2
Hyderabad	500	174	34.8
Total	1500	572	38.13

The seroIncidence of Anaplasma marginale differed significantly between areas. Regardless of the study district, a significant ($P<0.001$) relationship between different age groups was discovered. When compared to other age groups, such as 2-4 years, the Incidence of Anaplasma marginale was higher in those aged >4 years (Table 2).

Table 2. *Incidence of age wise bovine Anaplasma Marginale.*

Age	No of Animals Examined	Positive Sample	Positive (%)
< 1 year	177	37	20.90
1-2 Years	283	87	30.74
2-4 years	487	186	38.19
> 4 Years	553	262	47.38
Total	1500	572	38.13

Though females had higher Incidence than males, the difference was statistically significant ($P<0.05$) (Table 3).

Table 3. *Incidence of gender wise bovine Anaplasma Marginale.*

Sex	No of Animals Examined	Positive Sample	Positive (%)
Male	424	128	30.19
Female	1076	444	41.26
Total	1500	572	38.13

Anaplasma marginale Incidence was shown to be significantly different amongst breeds, regardless of the research district. Crossbred cattle had a greater Incidence than indigenous cattle (Table 4).

Table 4. *Incidence of breed wise bovine Anaplasma Marginale.*

Breed	No of Animals Examined	Positive Sample	Positive (%)
Indigenous	750	232	30.93
Crossbred	750	340	45.33
Total	1500	572	38.13

Similarly, Incidence of Anaplasma marginale infection among smallholders was shown to be significantly associated in all districts, although Incidence at livestock farms was not significantly associated (Table 5).

Table 5. *Incidence of population wise bovine Anaplasma Marginale.*

Variables	No of Animals Examined	Positive Sample	Positive (%)
Small Hold	650	214	32.92
Cattle Farm	850	358	42.12
Total	1500	572	38.13

Summer had the highest Incidence of Anaplasma marginale (49.51%) in all research districts (Table 6). Seasonal Incidence was shown to be substantial in Tharparkar, Thatta and Hyderabad districts.

Table 6. *Incidence of season wise bovine Anaplasma Marginale.*

Season	No of Animals Examined	Positive Sample	Positive (%)
Autumn	320	134	41.88
Winter	390	111	28.46
Spring	380	124	32.63
Summer	410	203	49.51
Total	1500	572	38.13

4. Discussion

It was difficult to identify any previous research on the Incidence of Anaplasma marginale infection in Sindh, Pakistan. In different geographical regions, the distribution of A. marginale infection differed. All of the study districts had statistically significant Incidence. The large variance in Incidence between study districts is explained by the fact that major parts of study districts have unique agro-ecological zones [15]. The Tharparkar district's moderate climate encourages vector tick proliferation and multiplication. Furthermore, the higher Incidence of R. (Boophilus) microplus and stall feeding methods at Tharparkar may have contributed to the higher Incidence of Anaplasma marginale infection. Using competitive inhibition ELISA, [11] found an Incidence of 26% in semi-arid, sweet, and sour rangeland in South Africa. The Incidence is comparable to that of the arid Hyderabad and Thatta districts. The current study's Incidence was 38.13%, indicating that the region is endemically unstable. Endemic stability is most likely to occur in areas where 70% of the animal population has serum antibodies. A competitive ELISA was also used to identify serum antibodies for the confirmation of anaplasmosis in bovine, as suggested by the World Animal Health Organization [13, 14]. Competitive ELISA based on major surface protein-5 has a significant advantage over other serological markers for anaplasmosis because of its superior sensitivity (96%) and specificity (95%) for anaplasmosis [25]. The cELISA antigen is a recombinant major surface protein (MSP5) of 19 kDa that is substantially consistent among Anaplasma species [10]. Anaplasma marginale Incidence as a Function of Age [21, 22]. Various strains have been proven to have a significant impact on Anaplasma marginale Incidence. Similarly, [8] found that cross-bred cattle had a greater Incidence of tick-borne disease (19.4%) than native Red Sindhi (17%) and Dhanni (14%) breeds. Fewer Incidence in native cattle indicates tick resistance, resulting in lower Anaplasma marginale infection [12]. Tick resistance was most likely a factor in Anaplasma marginale infection being lower. Due to a higher tick infestation, European breeds are more susceptible to tick-borne diseases [4]. Small holders have a higher Incidence of Anaplasma marginale (34.89%), which supports the findings of [23, 25], who also found a rising trend in positivity among small holders compared to medium and large animal farms. [20] Discovered no link between herd size and Incidence, however [19] showed that Incidence in dairy cows rose as herd size grew. According to [16, 18], increased Incidence in small holdings may be linked to a specific age group. The link among livestock population and Anaplasma marginale positivity was found to be significant (P0.05) in this study. Bad management, a lack of tick control measures, and inadequate economic sustainable development of poor resource small holder farmers for the application of appropriate management and animal care measures were all factors contributing to the higher incidence [12, 17].

5. Conclusion

According with research, small-holder crossbred cattle above the age of four years in the Tharparkar district are more vulnerable to Anaplasma marginale disease in the summer. The Tharparkar district's moderate climate encourages vector tick proliferation and multiplication. Furthermore, the higher Incidence of R. (Boophilus) microplus and stall feeding methods at Tharparkar may have contributed to the higher Incidence of Anaplasma marginale infection. Competitive ELISA based on major surface protein-5 has a significant advantage over other serological markers for anaplasmosis because of its superior sensitivity (96%) and specificity (95%) for anaplasmosis. The cELISA antigen is a recombinant major surface protein (MSP5) of 19 kDa that is substantially consistent among Anaplasma species. Anaplasma marginale Incidence as a Function of Age. Various strains have been proven to have a significant impact on Anaplasma marginale Incidence.

Conflicts of Interest

The authors declare that they have no competing interests.

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