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Comparative study of *Anaplasma* parasites in tick carrying buffaloes and cattle

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Abstract: A comparative study on the prevalence of *Anaplasma* parasite was conducted on ticks carrying buffaloes and cattle. Five hundred blood samples of both animals (250 of each) were collected during February, March and April. Thin blood smears on glass slides were made, fixed in 100% methyl alcohol and examined. Microscopic examination revealed that 205 (41%) animals had *Anaplasma* parasites, out of which 89, 44 and 72 animals had *Anaplasma marginale*, *Anaplasma centrale* and mixed infection respectively. Infected buffaloes and cattle were 75 and 130 respectively. The infection in female was 53 and 92 in buffaloes and cattle respectively. Twenty-two and 92 blood samples of male were found positive in buffaloes and cattle respectively. Comparative study revealed that the cattle were 26.82% more susceptible than buffaloes. The parasite prevailing percentage in female of both animals was slightly higher than that of the male. This investigation was aimed at studying the comparative prevalence of *Anaplasma* parasite in tick carrying buffaloes and cattle.

Key words: *Anaplasma marginale*, *Anaplasma centrale*, Buffaloes, Cattle, Ticks
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INTRODUCTION

The world population of buffaloes (*Bubalus bubalis*) has been estimated at over 172 million head, more than 97% (167.6 million) of which are in Asia and the Pacific region, mainly in India (97.7 million) and Pakistan (25.5 million) (FAO, 2004). About 98% of buffaloes in the region are raised by small farmers owning less than two hectares of land and less than five buffaloes (Mudgal, 1992). In general, the water buffalo is regarded as more productive, healthier and more useful than the cow, especially for the poorest "backyard" farmers in Asia (Bhat, 1999). According to the 1997 FAO report, the buffalo are recognized as "Black Gold of Asia" (Khushk and Memon, 2004). In comparison to research on cattle, research on water buffaloes has been much neglected (Johan, 2001-2002).

Buffaloes are the principal source of milk and meat production in Pakistan while cattle comprise a second source. More than 71% of milk in Pakistan is obtained from dairy buffaloes availability per capita being 155.6 L/year (Economic Survey of Pakistan, 2004-2005). Despite good genetic potential most dairy animals in Pakistan have considerably low quantity of milk due to lack of disease control. Buffaloes are susceptible to most of the diseases and parasites that afflict cattle, but the effect is often less serious than on cattle in the same ecosystem. In Pakistan, parasitic diseases, including tick born diseases, are considered a major obstacle in the health and product performance of cattle and buffaloes.

Ticks comprise a burning veterinary problem because they transmit diseases, induce paralysis or toxicosis and cause physical damage to livestock.

Anaplasma is one of the most important parasites transmitted by at least 20 ticks species, including *Argas persicus*, *Ornithodoros lahorensis*, *Boophilus annulatus*, *B. decoloratus*, *B. microplus*, *Dermacentor albipictus*, *D. andersoni*, *D. occidentalis*, *D. variabilis*, *Hyalomma excavatum*, *Ixodes ricinus*, *Rhipicephalus bursa*, *R. sanguineus* and *R. simus* (Marchette and Stiller, 1982) but mostly *Boophilus microplus* causing *Anaplasmosis* (TFRC, 1996). Various other biting arthropods have been implicated as mechanical vectors. Experimental transmission has been demonstrated with a number of species of *Tabanus* (horseflies), and with mosquitoes of the genus *Psorophora* (Ristic, 1968). *Anaplasma* species were originally regarded as protozoan parasites, but later research showed they had no significant attributes to justify this description. Since 1957, they have been classified in the family *Anaplasmataceae* of the order Rickettsiales. Reorganisation of the family, which in the past has included the genera *Anaplasma*, *Aegyptianella*, *Haemobartonella* and *Eperythrozoon* (Ristic and Kreier, 1984), has been proposed based on a combination of 16S ribosomal RNA, groESL, and surface protein gene sequence analysis (Walker and Dumler, 1996; Dumler *et al*., 2001). In the reorganized family, *Anaplasmataceae* would now include all alpha subdivision Proteobacteria presently in the genera *Ehrlichia*, *Anaplasma*, *Cowdria*, *Wolbachia*, and *Neorickettsia*, with provisional retention of *Aegyptianella*.

There are many *Anaplasma* species parasites but *Anaplasma marginale* (*A. marginale*) and *Anaplasma centrale* (*A. centrale*) are the most important species (TFRC, 1996). *Anaplasma* parasite is responsible for a severe hemolytic disease, anaplasmosis. Bovine anaplasmosis is usually caused by *Anaplasma marginale* (Bram, 1975; Kocan *et al*., 2000; Dumler *et al*., 2001). *A. centrale* can induce a moderate degree of anaemia, with clinical outbreaks in the field being extremely rare (Kreier and Ristic, 1963). Clinical disease is most notable in cattle, but other ruminants including water buffalo, bison, African antelope, and mule deer can become persistently infected with *A. marginale* (Kuttler, 1984) which under microscope look like black, irregular shape dots (Stewart *et al*., 1981). The only known site of development of *A. marginale* is in bovine erythrocytes (Richey, 1981). After invasion into red blood cells (RBC), they divide into up to eight initial bodies and enlarge within its thin outer membrane forming a large dot. When infected red blood cells rupture, the parasite's membrane also ruptures, releasing the initial bodies into the blood stream to invade other RBCs. As the infection progresses, more and more RBCs contain parasites and are destroyed (Stewart *et al*., 1981). The disease is characterized by fever, severe anemia, jaundice, brownish urine, loss of appetite, dullness or depression, rapid deterioration of physical condition, muscular tremors, constipation, yellowing of mucous membrane and labored breathing (Bram, 1983).

Different vaccines are available in many countries for control. No effective vaccine against this parasite is available in Pakistan. In some countries, *Anaplasma centrale* is used in vaccine production. Theiler (1912) described the use of *A*. centrale, as a live vaccine to minimize the severity of *A. marginale* infection. He conducted many experiments and concluded that *A. centrale* vaccination did not prevent *A. marginale* infection upon challenge but rather resulted in lower levels of *A. marginale* rickettsemia and a reduction in severity of clinical signs compared to unvaccinated controls. Based on these findings, *A. centrale* live vaccines were used to protect cattle imported from England to South Africa in 1909 and have since remained in use in tropical and subtropical regions (Potgeiter, 1979; Losos, 1986; Pipano *et al*., 1986; Turton *et al*., 1998).

In the present investigation, the prevalence of *Anaplasma* parasite was studied in buffaloes and cattle in Hyderabad district of Pakistan. This study was aimed at investigating and comparing the prevalence of *Anaplasma* parasite in tick carrying buffaloes and cattle.

MATERIALS AND METHODS

Five hundred tick carrying animals (250 cattle and 250 buffaloes) of different villages were investigated during February, March and April. One hundred and seventy-five females and 75 males of each species of cattle and buffaloes were studied. Blood samples were collected from the jugular vein of the

animals. These samples were transported immediately to the Parasitology Laboratory in test tubes containing anticoagulant, ethylene diamine tetra-acetic acid (EDTA), with a ratio of 0.5 mg per ml of blood in icebox as suggested by Veterinary Research Institute Lahore. In some cases, blood smears were prepared on the spot.

Thin blood smears were deposited on glass slides adopting the procedure as suggested in the manual of Tick Fever Research Center, DPI Queensland, Australia (TFRC, 1996).

The blood samples on the slides were fixed in 100% methyl alcohol for 2 min.

The slides were stained 10% solution of Giemsa's stain for five minutes then rinsed with distilled water.

The slides were examined in the laboratory, Department of Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam at X40 than X100 oil immersion objectives under binocular electric microscope. The parasite identification was done with the help of keys mentioned in the book titled "Helminths, Arthropods and Protozoa of Domestic Animals" (Soulsby, 1982).

RESULTS

Microscopic examination revealed that 205 (41%) examined animals had *Anaplasma* parasites (Table 1), and that 89 (17.8%), 44 (8.8%) and 72 (14.4%) animals had *Anaplasma marginale*, *Anaplasma centrale* and mixed infection respectively (Table 2). The numbers of infected buffaloes and cattle were found to be 75 (30%) and 130 (52%) respectively (Table 2). Infection acquired by males was found to be 22 (29.33%) and 38 (50.66%) in buffaloes and cattle respectively (Table 3). Fifty-three (30.28%) and 92 (52.57%) blood samples of females were found positive in buffaloes and cattle respectively (Table 4).

Comparative study revealed that the cattle were 26.82% more susceptible than buffaloes. In total infected animals (205) the prevalence rate in female (70.73%) of both animals was slightly higher than that in male (29.26%) (Table 5). The results also revealed that both sexes of cattle are more susceptible than buffaloes.

Anaplasma species-wise comparative study revealed that 34 (13.6%) and 55 (22%) samples of *Anaplasma maginale*; 21 (8.4%) and 23 (9.2%) samples of *Anaplasma centrale*; 20 (8%) and 52 (20.8%) samples of mixed species were positive in buffaloes and cattle respectively (Table 2).

DISCUSSION

Very little work has been done on *Anaplasma* parasite in buffalo, the most important animal of Asia, especially in Pakistan and India. Buffaloes just like cattle, are susceptible to *Anaphema* infection, but the incidence and severity is less. This parasite is transmitted by at least 20 species of ticks (TFRC, 1996). The present study was conducted during February, March and April, the favorable period for ticks' infestation. In Pakistan, the native breeds of cattle and buffaloes are resistant to ticks. So Pakistan villagers usually do not worry much about ticks' infestation. The present findings revealed that tick carrying cattle had higher percentage (63.41%) of prevalence of the parasite than buffaloes (36.59%) (Table 1). Because cattle are the main hosts of *Anaplasma* parasite (Kuttler, 1984) and although buffaloes are susceptible to most of the diseases and parasites that afflict cattle, the effect is often less serious than that on cattle in same ecosystems. Water buffaloes are generally considered as healthier animal in comparison to cattle (Johan, 2001-2002). The present finding on the occurrence frequency of ticks' infestation in cattle and buffaloes is 41% (Table 2) which does not in accord with the findings of Haider and Bilqees (1988), as their result was 61% in Karachi and adjoining areas. This difference may be due to the moderate climate and high relative humidity in Karachi that favor ticks' infestation. In the present study, the prevailing percentage of parasite in buffaloes was 30% (Fig.1) which accords with the study of Buriro *et al*.(1994) conducted in the same area showing that 30% buffaloes had infection. Ocaido *et al*.(2005) found 11.8% prevalence of *A. marginale* in cattle of Sorti District, Uganda, while in the present study it was 22% (Fig.1). This difference may be attributed to the differences in climatic conditions and intensity of ticks' infestation in the area. Sex-wise comparative study of both

animals revealed that female cattle had higher (52.57%) prevailing percentage of *Anaplasma* than buffaloes (30.28%) but the difference was not significant (Fig.2). The male of cattle also had higher prevalence (50.66%) of *Anaplasma* than buffaloes male (29.33%) (Fig.3).

Table 1 Comparison of positive samples of cattle and buffaloes

Total number of animals found positive ($A+B=n$)	Buffaloes found positive		Cattle found positive	
	Number (A)	($A\times100$)/n (%)	Number (B)	($B\times100$)/n (%)
205	75	36.59	130	63.41

Table 2 Comparison of *Anaplasma* parasite prevalence in buffalo and cattle

Animal	Number of samples collected	Number of positive samples			Total ($a+b+c$)	
		A. Marg* (a)	*A. Cent*** (b)	Mixed*** (c)	No.	%
Buffalo	250	34 (13.6%)	21 (8.4%)	20 (8%)	75	30
Cattle	250	55 (22%)	23 (9.2%)	52 (20.8%)	130	52
Total	500	89 (17.8%)	44 (8.8%)	72 (14.4%)	205	41

*: *Anaplasma marginale*; **: *Anaplasma centrale*; ***: Both species

Table 3 Comparison of *Anaplasma* parasite prevalence in male buffalo and cattle

Animal	Number of samples collected	Number of positive samples			Total ($a+b+c$)	
		A. Marg* (a)	*A. Cent*** (b)	Mixed*** (c)	No.	%
Buffalo	75	9 (12%)	7 (9.33%)	6 (8%)	22	29.33
Cattle	75	14 (18.66%)	9 (12%)	15 (20%)	38	50.66
Total	150	23 (15.33%)	16 (10.66%)	21 (14%)	60	40

*: *Anaplasma marginale*; **: *Anaplasma centrale*; ***: Both species

Table 4 Comparison of *Anaplasma* parasite prevalence in female buffalo and cattle

Animal	Number of samples collected	Number of positive samples			Total ($a+b+c$)	
		A. Marg* (a)	*A. Cent*** (b)	Mixed*** (c)	No.	%
Buffalo	175	25 (14.28%)	14 (8%)	14 (8%)	53	30.28
Cattle	175	41 (23.43%)	14 (8%)	37 (21.14%)	92	52.57
Total	350	66 (22%)	28 (9.33%)	51 (17%)	145	48.33

*: *Anaplasma marginale*; **: *Anaplasma centrale*; ***: Both species

Table 5 Sex wise distribution of infected samples

Total number of positive samples	Male				Female			
	Buffaloes (A)	Cattle (B)	Total ($A+B$)		Buffaloes (A)	Cattle (B)	Total ($A+B$)	
			No.	%			No.	%
205	22	38	60	29.26	53	92	145	70.73

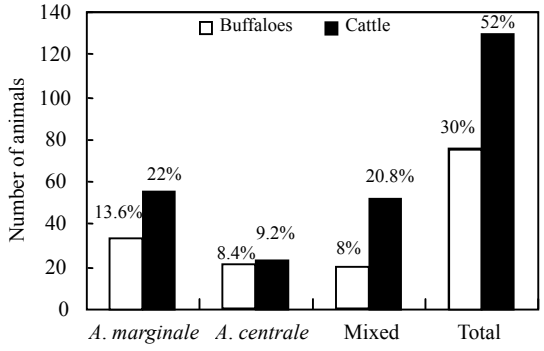

Fig.1 Comparison of *Anaplasma* parasite prevalence in buffaloes and cattle

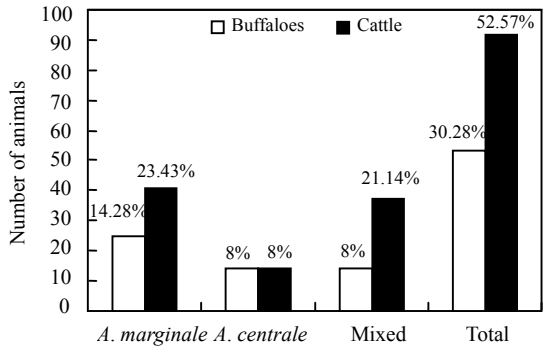

Fig.2 Comparison of *Anaplasma* parasite in female buffaloes and cattle

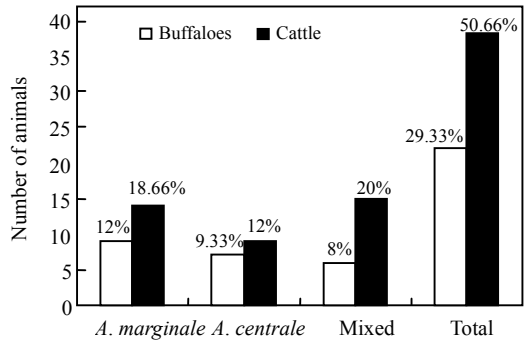

Fig.3 Comparison of *Anaplasma* parasite in male buffaloes and cattle

CONCLUSION

It is concluded that cattle and buffaloes are susceptible to *Anaplasma* parasite infestation but that cattle are more susceptible than buffaloes.

Male and female of cattle are equally more susceptible than either sex of buffaloes.

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