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Clinical and haemato-biochemical changes in cattle with *Anaplasma marginale* infection

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Abstract
The present study was conducted in cattle brought to Large Animal Medicine Unit of Teaching Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research and Ambulatory clinic, Puducherry. Out of which 15 cattle were found positive for *A. marginale* infection by blood smear and PCR. Pale mucosa, tachycardia and pyrexia were the predominant clinical findings of anaplasmosis in cattle. Mean Haemoglobin of 6.84 ± 0.29g/dL, PCV of 20.71 ± 0.92 and mean RBC count of 3.50 ± 0.10 × 10^6 cells/mm^3 of cattle with anaplasmosis were significantly (P<0.01) less when compared to their respective control mean. Major haematology changes included hypochromic, macrocytic and regenerative anemia. However, significant changes in blood biochemistry were not recorded.

Keywords: Cattle, *Anaplasma marginale*, macrocytic anemia, regenerative anemia

Introduction
Anaplasma marginale is a Gram-negative obligate intracellular bacterium and the etiologic agent of bovine anaplasmosis, a debilitating infection that is transmitted biologically by ticks, mechanically via fly bites or blood-contaminated fomites, and vertically from mother to calf (Aubry and Geale, 2011) [3]. Acute anaplasmosis, caused by *A. marginale* invades and multiplies in erythrocytes. The disease is usually sub-acute especially in young animals, but as the disease progresses, infected and even uninfected erythrocytes are destroyed in the liver and spleen, resulting in anaemia and even death in severe cases. The mucous membrane is icteric and show marked pallor, particularly after the acute stage is passed, but there is no hemoglobinuria. Cattle that recover from acute infection become carriers and the parasite can persist most probably in the blood for the lifetime (Vahid and Parviz, 2010) [9].
Peracute cases, with a sudden onset of high fever, anemia, icterus, severe dyspnea and death occur within 24 hours. Affected animals were often hyperexcitable and tend to attack attendants just before death. Pregnant cows frequently abort (Radostits *et al*., 2010) [6]. Hence, it becomes important for both herd health management and movement of animals in and out of the endemic areas (Tarek *et al*., 2009) [8]. The present study describes the clinical signs and haemato-biochemical alterations in cattle naturally infected with *Anaplasma marginale* in the Puducherry state.

Materials and Methods
The present study was conducted in cattle brought to Large Animal Medicine Unit of Teaching Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research and Ambulatory clinic, Puducherry. A total of 73 cattle with clinical signs suggestive of anaplasmosis were screened by blood smear examination and polymerase chain reaction.
Two milliliter of blood was collected from jugular vein in a dry vial containing 3.6 mg of EDTA for complete haemogram and was estimated as per standard methods (Schalm *et al*., 2010) [7]. Four millilitre of blood was collected, allowed to clot and the serum was separated by centrifugation. The serum value of Total Protein (Biuret method), Albumin (BCG method), Globulin, Alanine Transaminase (Kinetic Assay), Bilirubin - Total and Direct (DMSO method) was estimated by spectrophotometer (Spectronic 200) for cattle affected by *A. marginale.*
Six apparently healthy cattle that turned negative for parasitic ova / oocyst and found negative for anaplasmosis by blood smear examination and polymerase chain reaction were considered as control group.

Statistical Analysis

The data obtained in this study were subjected to statistical analysis using Graph pad prism and SPSS software.

Results

Out of 73 cattle with clinical signs suggestive of anaplasmosis, 15 cattle were found positive for *A. marginale* infection by blood smear and PCR.

The various clinical signs noticed in cattle with anaplasmosis were illustrated in (Table 1 and Fig. A-G). The clinical signs observed in these animals were reduced milk yield (71.4%), tick infestation (66.67%), pyrexia (53.33%), swollen lymph nodes (73.33%), pale conjunctival mucous membrane (67.67), icterus conjunctival mucous membrane (6.67%), bruxism (26.67%), nasal discharge (20%), dyspnea (13.3%) and tachycardia (53.33%).

The erythron and leukogram of apparently healthy cattle and cattle with anaplasmosis were given in Table 2.

Erythron of apparently healthy animals showed mean Hb of 10.50 ± 0.44g/dL, mean PCV of 31.50 ± 1.54%, mean RBC count of 5.70 ± 0.20 × 10^6 cells/mm^3, mean MCV of 55.26 ± 1.74fl, mean MCH of 18.43 ± 0.45pg, mean MCHC of 33.42 ± 0.56% and mean leucocyte of 10.14 ± 0.88 × 10^3 cells/mm^3. The absolute neutrophil, lymphocyte, eosinophil and monocyte counts in apparently healthy animals were 3.06 ± 0.58 × 10^3 cells/mm^3, 6.95 ± 1.09 × 10^3 cells/mm^3, 0.02 ± 0.02 × 10^3 cells/mm^3 and 0.12 ± 0.04 × 10^3 cells/mm^3.

Mean Haemoglobin of 6.84 ± 0.29g/dL, PCV of 20.71 ± 0.92 and mean RBC count of 3.50 ± 0.10 × 10^6 cells/mm^3 of cattle with anaplasmosis were significantly (P<0.01) less when compared to their respective control mean. The mean MCV of 59.17 ± 1.18fl, MCH of 17.48 ± 0.39pg, MCHC of 33.10 ± 0.40%, WBC of 11.96 ± 0.69 × 10^3cells/mm^3, neutrophils of 5.08 ± 0.63 × 10^3 cells/mm^3, lymphocytes of 6.39 ± 0.46 × 10^3cells/mm^3, eosinophils of 0.31 ± 0.10 × 10^3 cells/mm^3 and monocytes of 0.14 ± 0.06 × 10^3 cells/mm^3 count of anaplasmosis animals did not show any significant difference compared to the control mean.

The mean serum biochemical values of apparently healthy cattle and cattle with anplasmosis were given in Table 3.

Apparently healthy animals had a mean total protein of 6.65 ± 0.33g/dL, albumin of 3.42 ± 0.02g/dL, globulin of 3.23 ± 0.32g/dL, albumin: globulin of 1.11 ± 0.10, bilirubin (total) of 0.32 ± 0.02 mg/dl, bilirubin (direct) of 0.20 ± 0.02 mg/dL, bilirubin (indirect) of 0.13 ± 0.02mg/dL and ALT of 29.23 ± 0.77 IU/L respectively.

The mean total protein of 6.73 ± 0.20g/dL, albumin of 3.23 ± 0.09g/dL, globulin of 3.50 ± 0.19g/dL, albumin: globulin ratio of 0.97 ± 0.07, bilirubin (total) of 0.33 ± 0.02mg/dL, bilirubin (direct) of 0.21 ± 0.01mg/dL, bilirubin (indirect) of 0.12 ± 0.01mg/dL and ALT of 31.59 ± 1.28IU/L in these animals did not show any significant difference from that of their respective control mean.

Table 1: Clinical manifestation of anaplasmosis in cattle

Clinical Manifestation	Numbers (n=15)	Percentage (%)
Reduced milk yield	11	73.33
Swollen lymph nodes	11	73.33
Pale mucous membrane	10	66.67
Tick infestation	10	66.67
Pyrexia	8	53.33
Tachycardia	8	53.33
Bruxism	4	26.67
Anorexia	4	26.67
Nasal discharge	3	20
Dyspnea	2	13.3
Icteric mucous membrane	1	6.67

Table 2: Comparison of haematology in control Vs disease group

Parameters	Apparently healthy cattle (n=6)	Cattle with anaplasmosis (n=15)	P value
Haemoglobin (g/dL)	10.50 ± 0.44	6.84 ± 0.29	0.0001**
Packed Cell Volume (%)	31.50 ± 1.54	20.71 ± 0.92	0.0001**
RBC (10^6cells/mm^3)	5.70 ± 0.20	3.50 ± 0.10	0.0001**
MCV (fl)	55.26 ± 1.74	59.17 ± 1.18	0.2781^{NS}
MCH (pg)	18.43 ± 0.45	17.48 ± 0.39	0.1704^{NS}
MCHC (%)	33.42 ± 0.56	33.10 ± 0.40	0.6638^{NS}
WBC (10^3cells/mm^3)	10.14 ± 0.88	11.96 ± 0.69	0.1489^{NS}
Neutrophils (10^3 cells/mm^3)	3.06 ± 0.58	5.08 ± 0.63	0.0674^{NS}
Lymphocytes (10^3cells/mm^3)	6.95 ± 1.09	6.39 ± 0.46	0.5820^{NS}
Eosinophils (10^3 cells/mm^3)	0.02 ± 0.02	0.31 ± 0.10	0.0756^{NS}
Monocytes (10^3 cells/mm^3)	0.12 ± 0.04	0.14 ± 0.06	0.8076^{NS}

**Significant (P≤0.01); *Significant (P≤0.05); NS Not significant

Table 3: Comparison of serum biochemistry in control Vs disease group

Parameters	Apparently healthy cattle (n=6)	Cattle with anaplasmosis (n=15)	P value
Total protein (g/dL)	6.65 ± 0.33	6.73 ± 0.20	0.8410^{NS}
Albumin (g/dL)	3.42 ± 0.02	3.23 ± 0.09	0.1823^{NS}
Globulin (g/dL)	3.23 ± 0.32	3.50 ± 0.19	0.4749^{NS}
Albumin : Globulin	1.11 ± 0.10	0.97 ± 0.07	0.1306^{NS}
Bilirubin (Total) (mg/dL)	0.32 ± 0.02	0.33 ± 0.02	0.6895^{NS}
Bilirubin (Direct) (mg/dL)	0.20 ± 0.02	0.21 ± 0.01	0.8183^{NS}
Bilirubin (Indirect) (mg/dL)	0.13 ± 0.02	0.12 ± 0.01	0.9236^{NS}

ALT (IU/L)	29.23 ± 0.77	31.59 ± 1.28	0.2657^{NS}

**Significant (P≤0.01); *Significant (*P*≤0.05); NS Not significant

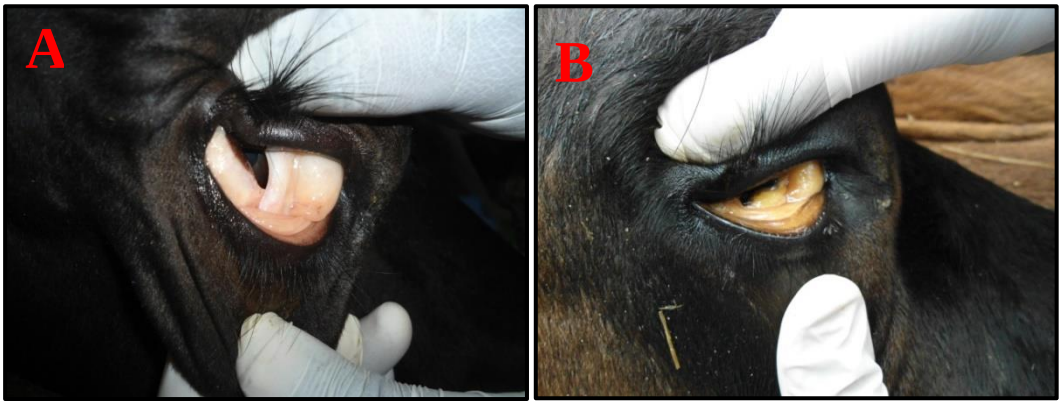

Fig. A, B: Cattle with *A. marginale* infection showing pale and icteric conjunctival mucous membrane

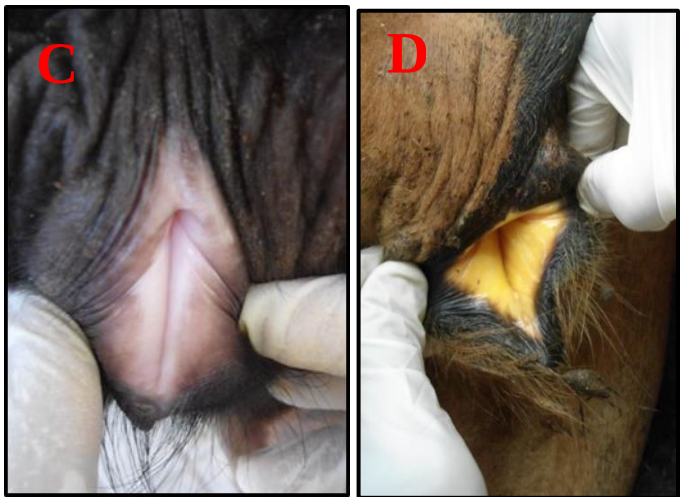

Fig C & D: Cattle with *A. marginale* infection showing pale and icteric vaginal mucous membrane

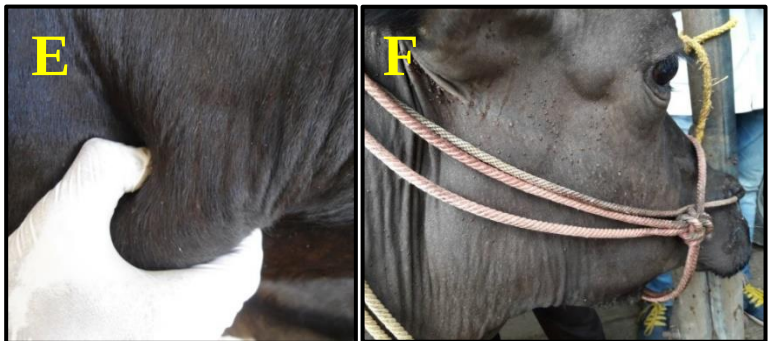

Fig E & F: Swollen pre – scapular lymph node & severe tick infestation in cattle with anaplasmosis

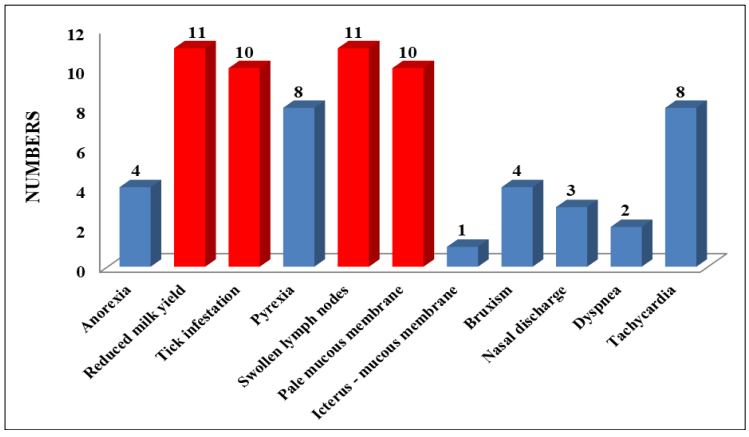

Fig G: Clinical manifestations of anaplasmosis in cattle

Discussion

Anaemia, jaundice and sudden death are the characteristic signs of anaplasmosis. Fever is attributed to removal of parasitized erythrocytes by phagocytosis in the reticular endothelial system, with release of acute phase inflammatory reactants and the consequent development of pyrexia. Infected cells in anaplasmosis were removed by the monocyte-macrophage system, resulting in mild to marked anemia and jaundice. Continued destruction of *A. marginale* infected or uninfected RBCs are caused by phagocytosis by leucocytes resulting in the development of anaemia and icterus. Intravascular hemolysis is minimal, and therefore animals have neither hemoglobinemia nor hemoglobinuria.

However, marked bilirubinemia results in bilirubinuria, which gives an intense yellow-brown color to urine. The clinical signs recorded viz. anemia, icterus, high fever, weakness, weight loss and sometimes the death of the affected animals may be due to the endogenous pyrogens liberated by *A. maginale* causing the destruction of erythrocytes and triggering various haemopoietic and thermoregutory centers of the body as reported by Radostits *et al*. (2010) [6] and Ashuma *et al*. (2013).

Mean Hb, PCV and RBC count of cattle with anaplasmosis were significantly (P<0.01) less when compared to their respective control mean. This could be due to a decrease in erythrocyte life span and extravascular erythrophagocytosis as also reported by Doyle *et al*. (2016) [4].

Khan *et al.* (2016) [5] and Ashuma *et al.* (2013) recorded hematological parameters of infected cattle population. The results of the present study, showed a decrease in all blood indices while MCV was found to be higher in infected cattle as compared to healthy control. The decrease of MCHC and increase of MCV was noticed as compared to controls. Hence anemia in anaplasmosis is classified as hypochromic and macrocytic. The increase in MCV is usually the indication of regenerative anaemia. After rapid destruction of RBCs by phagocytosis the immature RBCs are released from bone marrow due to increase in demand. The immature RBCs are larger in size than mature red blood cells resulting in increased MCV. In the present study, although MCV was elevated when compared to healthy control, no significant changes could be observed. However, it is understood that regenerative anaemia could be a positive picture in bovine anaplasmosis.

Mean total protein, albumin, globulin, albumin: globulin ratio, bilirubin total, direct bilirubin, indirect bilirubin and ALT did not show any significant difference from that of their respective control values. There was no significant increase or decrease in total serum proteins. However, in the present study the globulin fraction was slightly elevated with a decline in albumin values which had a insignificant impact in the albumin globulin ratio. Cattle infected with *Anaplasma* results in an elevated gamma globulin levels and decrease in albumin levels as reported by Allen *et al*. (1981) which is in accordance with the above findings.

Conclusion

Pale mucosa, tachycardia and pyrexia were the predominant clinical findings of anaplasmosis in cattle. Major haematology changes included hypochromic, macrocytic and regenerative anemia. However, significant changes in blood biochemistry were not recorded.

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References

1. Allen PC, Kuttler KL and Amerault BS. Clinical chemistry of anaplasmosis: Blood chemical changes in infected mature cows. Am. J Vet. Res. 1981, 42:322-325
2. Ashuma, Amrita S, Lachhman DS, Paramjit K, Mandeep SB, Balwinder KB *et al*. Prevalence and haemato-biochemical profile of A. marginale infection in dairy animals of Punjab (India), Asian Pacific J Tropical Medicine 2013, 139-144.
3. Aubry P and Geale DW. A review of bovine anaplasmosis. Transbound. Emerg. Dis., 2011; 58:1-30.
4. Doyle RL, Franca RT, Oliveira CB, Rezer JFP, Klafke GM, Martins JR *et al*. Cattle experimentally infected by Anaplasma marginale: Influence of splenectomy on disease pathogenesis, oxidative profile, and antioxidant status, Microbial Pathogenesis. 2016, 95:193-199
5. Khan A, Saeed K, Nasreen, Niaz S and Akhtar N. Prevalence of Anaplasmosis in Cows and Buffaloes of District Charsadda, Khyber Pakhtunkhwa, Pakistan, Global Veterinaria. 2016, 16:431-440
6. Radostits OM, Gay CC, Hinchcliff KW and Constable PD. Veterinary Medicine. A textbook of the diseases of Cattle, Horses, Sheep, Pigs and Goats 10th ed., Book power Saunders, London, New York, 2010, 1455-1459
7. Schalm OW, Weiss, Douglas J, Wardrop K, Jane. Veterinary Haematology. 6th edition Wiely – Blackwell publishing, U.S.A., 2010, 799-809
8. Tarek RAE, Mona AM, Wafaa AO, Azzas AG. Serological survey of A. marginale (Rickettsia) Antibiotic in animals by major surface protein 5 competitive Inhibition Enzyme Liked Immunosorbent Assay. Assoc. 2009; 50:83-85.
9. Vahid N, Parviz S. Molecular Detection of *Anaplasma bovis* in Cattle from Central Part of Iran. Veterinary Research Forum. 2010; 2:117-122.