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Detection of Anaplasma antibodies through SNAP4Dx plus and therapeutic management of canine anaplasmosis

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Abstract
Tick-borne gram negative intracellular parasites- *Anaplasma platys* and zoonotic *Anaplasma phagocytophilum* causes Canine anaplasmosis with very few established clinical signs like pyrexia, anorexia, lethargy, anaemia and haematological profile showing thrombocytopenia, reduced haematocrit and haemoglobin. A canine Anaplasma antibodies detected case in Tura, Meghalaya through SNAP 4Dx Plus lateral flow assay showed usual signs and blood profile indicative of anaplasmosis with additional clinical signs which were least documented such as leaky bilateral hindlimb oedema, eosinophilia and biochemical parameters like reduced blood urea and ALP. The animal was treated with imidocarb and combi drugs of doxycycline, clindamycin and metronidazole and subsequent improvement was observed.

Keywords: *Anaplasma platys*, *Anaplasma phagocytophilum*, SNAP 4Dx Plus, bilateral oedema, zoonotic, canine

Introduction
Canine anaplasmosis is caused by gram-negative intracellular bacterium of the family Anaplasmataceae and spreads through mainly Ixodid ticks as vectors (Kohn *et al*., 2008) [11]. At present, the genus *Anaplasma* comprises of seven valid species, *i.e*., *Anaplasma bovis*, *A. caudatum*, *A. centrale*, *A. marginale*, *A. ovis*, *A. phagocytophilum*, and *A. platys* (Dantas *et al*., 2017) [9]. As per most of the findings, two species have been identified as pathogenic in dogs; *A. platys* causing canine infectious cyclic thrombocytopenia, and *A. phagocytophilum*, which parasitizes neutrophils, causing granulocytic anaplasmosis in canines of many countries in the northern hemisphere. *A. phagocytophilum* is zoonotic in nature with wide host range and the host range includes dogs, cats, horses, ruminants, humans, carnivores, reptiles, birds, and rodents (Berzina *et al*., 2014; Atif *et al*., 2021) [3, 2]. Due to the rapid expansion of the zoogeographical ranges of many causative agents due to international travel and the increased mobility of dogs at both the national and international level to meet the demand for companion animals in modern society, vector-borne infections such as dirofilariosis, ehrlichiosis, anaplasmosis, and lyme borreliosis are being recognized as emerging and/or re-emerging problems in dogs and man (Borthakur *et al*., 2014) [4].
Till date, little is known about the clinical and laboratory results associated with canine anaplasmosis and also the implications of co-infections with other vector-borne pathogens (VBPs). Some of the common clinical observations associated with *A. platys* infection includes anorexia, weight loss, lethargy, lymphadenomegaly, hyperthermia and haemorrhage and less frequent signs such as musculo-skeletal disorders, cutaneous lesions, splenomegaly, dehydration and ocular inflammation have been reported. Moreover, haematological abnormalities like thrombocytopenia, anemia, leukocytosis and leucopenia have been documented (Bouzouraa *et al*., 2016) [5]. *A. phagocytophilum*, on the other hand, infects and survives within neutrophils by inhibiting critical neutrophil processes such as motility, phagocytosis, the oxidative burst mechanism, and neutrophil-endothelial cell contacts, as well as interfering with apoptosis. *A. phagocytophilum* causes an acute febrile illness in dogs that is characterized by lethargy and inappetence. Lameness, coughing, polydipsia, intermittent vomiting, and haemorrhages are fewer common symptoms (Carrade *et al*., 2009) [7].

Materials and Methods
A four-year-old male German shepherd dog was presented to Tura Vet's Clinic, Meghalaya

with signs of bilateral oedematous swellings on hind-limbs and pyrexia. Initially, it was treated symptomatically. The swellings did not subside after 7 days of therapy and the condition of the animal worsened with complicated signs like exudative discharge from the ruptured oedematous limbs, inappetence, pale mucous membrane and severe pyrexia (103.7º F). The dog had history of tick infestation so the animal was subjected to undergo haematological profile examination as well as serum biochemistry (Table 1). Haematological profile indicated reduced haematocrit (31.8%), anaemia (Hb-11.1g/dL), thrombocytopaenia (151 K/µL) and eosinophilia (2.55 K/µL). Serum biochemistry report revealed low ALP (<10 U/L) and blood urea (4.48 mg/dL). Further, the animal was screened for *Ehrlichia canis*, *Ehrlichia ewingii*, *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Anaplasma platys* antibodies using SNAP 4Dx Plus lateral flow assay test kit (IDEXX Laboratories, United States). The test results indicated the presence of antibodies of either *A. phagocytophilum* or *A. platys* or both.

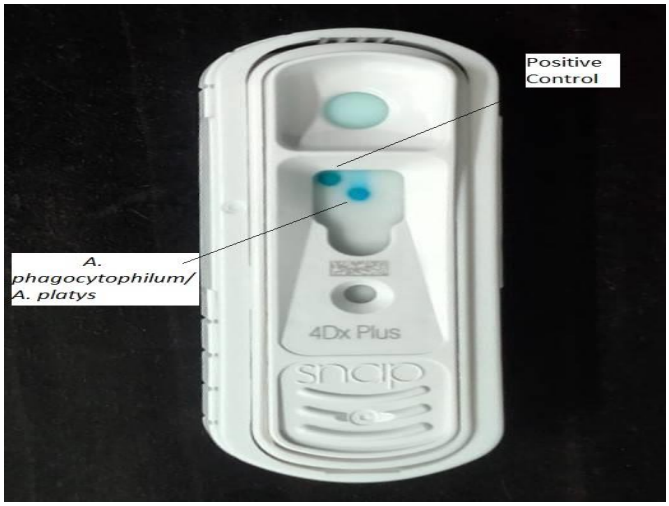

Fig 1: Detection of Anaplasma antibodies using IDEXX's SNAP 4Dx Plus

Table 1: Haemato-biochemical profile of the animal

Parameters	Observation
Haematocrit	31.8
Haemoglobin	11.1
MCHC	34.9
WBC	14.10
Granulocyte %	70.9
Lymphocyte & Monocyte %	29
Neutrophils	7.45
Granulocytes	10
Lym & Mono	4.1
Eosinophils	2.55
Platelets	151
Creatinine	0.79
Urea	4.48
BUN: Creatinine	6
ALT	36
AST	17
ALP	<10

Results and Discussion

The dog was administered Imidocarb injection @ 6.6 mg/kg intramuscularly (Checa *et al*., 2017) [8]. Concurrently, triple drug therapy using Doxycycline (@ 10 mg/kg), Clindamycin (@ 5.5 mg/kg) and Metronidazole (@ 15 mg/kg I/V, 25 mg/kg PO) which were found effective for haemoparasites was initiated intravenously for the first 3 days followed by oral dosing for 10 days. A second dose of Imidocard injection was given 2 weeks after the initial shot. The antibiotic therapy was supplemented with other supportive drugs which included gastroprotectants, haematinic syrup, hepatoprotectants and fluid therapy. Diuretics was also used to check the oedematous swelling of hind limbs. Almendros *et al*. (2020) [1] documented that treatment of haemoparasites with the above antibiotic combination proved to have given more than 87% success rate.

Pyrexia subsided 4 days after initiation of antibiotics and appetite was also regained. The leaky oedema started drying around 5 days post medication; however, it took nearly two weeks for the swollen limbs to return to its initial state. The animal recovered subsequently after this vigorous therapeutic protocol.

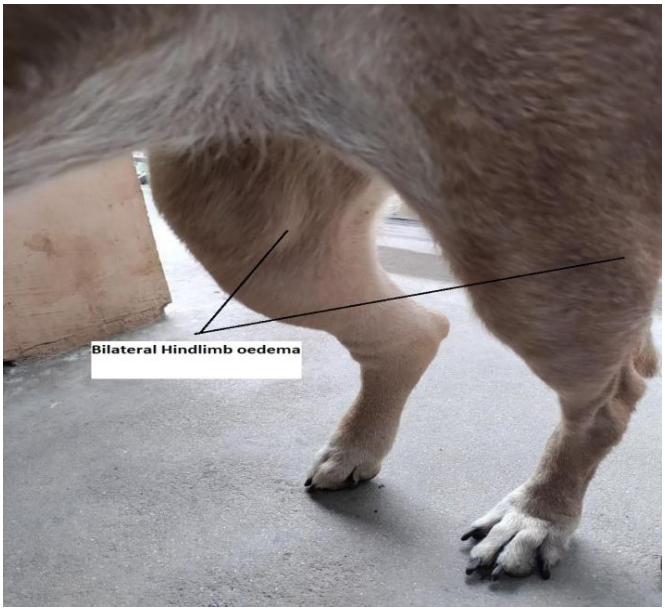

Fig 3: Bilateral oedema of hindlimbs

Fig 4: A typical oedematous spot

From established records, fever, anaemia, lethargy, anorexia, and weight loss are common findings in anaplasmosis. *A. platys* through its vector *Rhipicephallus sanguineus* (common brown dog tick) causes thrombocytopaenic anaplasmosis and occurred more commonly in dogs. On the other hand, *A. phagocytophilum* parasitizes mostly on neutrophils causing granulocytic anaplasmosis and also on eosinophils. As it is zoonotic with multiple host range, *A. phagocytophilum* has been reported to have caused oedema in limbs, prepuce, ventral abdominal wall in horses, llamas and alpacas as a result of thrombosis and perivascular accumulations (Pusterla *et al*., 2007) [12]. Oedematous cutaneous skin lesions, lameness and polyarthritis in dogs and humans have also been recorded (Cappiello and Oliva, 2006; Berzina *et al*., 2014) [6, 3]. In the serum biochemistry report, the reduction in urea and ALP might have been due to protein malnutrition which could be related to anorexia and stress of the animal (Davenport *et al*., 1994; Lakshmi *et al*., 2016) [10, 13]

The clinical and haemato-biochemical profile of this particular case shows signs which are specific to both *A. platys* as well as *A. phagocytophilum*. This might be a case of co-infection of both the species reporting for the first time in Meghalaya as well as in India as limited data are available till date. Moreover, similar reports of such cases are increasing in Meghalaya.

Conclusion

The increase in such cases is alarming to both animal and human health as these parasites are tick-borne and *A. phagocytophilum* is known to be zoonotic. Henceforth, a deeper investigation seems mandatory to prevent disease outbreak at human-animal interface at the earliest.

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