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Studies on the concomitant blood protozoan infections of an equine and its hemato-biochemical profiles on and after treatment

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Abstract

An equine with the clinical signs of urticaria was reported to the Veterinary Clinical complex, Apollo College of Veterinary Medicine, Jaipur. The clinical signs revealed by the horse were normal temperature, conjunctivitis, lymphnode swelling, abdominal respiration, loss of lusture of the skin. Based on the observations, blood smear examination and the hemato- biochemical parameters were carried out. The tests revealed the presence of *Babesia*, *Anaplasma sp* & *Trypanosome cruzei*. The equine was treated with diminazene aceturate as a single dose (7.0mg/kg body weight) and oxytetracycline (22 mg/kg body weight) for a period of 14 days along with supportive therapy. Blood samples were analyzed for the above parasites infection in the equine by blood smear examination and by hemato-biochemical parameters studies on and after the treatments. The clinical outcome of the equine from the above concomitant blood protozoan infections by treatments is discussed in this study.

Keywords: Concomitant blood protozoan infections, equine, *Babesia*, *Anaplasma*

Introduction

Hemoprotozoan diseases, especially babesiosis, anaplasmosis, theileriosis and trypanosomiasis are considered as some of the major diseases causing heavy losses in terms of production. Tick-borne diseases cause substantial losses to the livestock industry throughout the world by their decreased productivity (Ananda *et al.* 2009) ^[1] and increased cost for control measures. (Makala *et al.* 2003) ^[12]. Ticks as vectors is mostly responsible for the initiation of many hemoprotozoan diseases. A hot and humid climate is highly favorable for the survival of ticks. (Kohli *et al.* 2014) ^[9]. The agro-climatic conditions of the area are highly favorable for the growth and multiplication of the ticks. Ticks act as natural vectors of theileriosis, babesiosis, anaplasmosis. Prevalence of *B. bigemina*, *T. annulata*, *T. mutans*, *A. marginale* are reported in animals of India. (Amanda *et.al* 2009; Vahora *et al.* 2012) ^[30]. Muhammad Asif *et al.* (2019) ^[16] reported the prevalence of *Anaplasma marginale* and *Theilera annulata* in eqines in Southern Punjab; Pakisthan. Khadija Javed *et al.* (2014) ^[8] reported mixed infection of tick borne hemoprotozoan diseases *Babesia*, *Anaplasma Sp* & *Theileria* organisms in equine population in around Lahore, Pakistan. Maria Fernanda Posada-Guzman (2015) ^[13] reported a combined infection status of *Babesia caballi* and *Theileria equi* in equines in Costa Rica. Selmi, *et al.* (2019) ^[26] reported natural infections with *Babesia*, *Trypanosoma*, & *Anaplasma sp* in military livestock, Tunisia and reported 17.0% horses were infected with *Anaplasma sp*. The severity of the infection determined by the strain of the involved trypanosome and host (Anosa 1988) ^[2]. The tick-borne diseases are dangerous to the health of livestock and human. The spectrum of tick-borne diseases infecting humans and domestic animals is on the rise giving concern to veterinarians and physicians. (Dantas-Torres F., *et al.* 2012) ^[3]. Hematological biochemical parameters provide precious information about the severity of the infection and diagnosis. (Odedara A.B *et al.* 2021) ^[19]. In this study the clinical manifestations experienced by the infected equine with *Babesia*, *Anaplasma* & *Theileria* organisms and the alterations occurred in the hemato-biochemical parameters were studied before and after treatments for better management of the infected horse.

Materials and Methods

Case studies

Clinical examination, blood smear examination and hemato-biochemical studies and the treatments were carried out for the infected equine as per standard procedures before and after the treatment.

Blood parasites identification of the infected equine was carried out as stated below.

Babesia equi was identified for the presence of merozoites in the RBCs as two or four in a Maltese cross formation and are seen as pyriform as suggested by Vial and Gorenflot, (2006) [31] and it was found in a single erythrocyte mainly in pairs forming an acute angle. (Edwards's *et.al* 2005) [4]. *Trypanosoma cruzi* was confirmed by direct parasitological examination to diagnose trypomastigotes of *Trypanosoma cruzi* by using peripheral blood smear examination by staining methods as suggested by Western College of Veterinary Medicine (2021) [7], Saskatchewan, France. *Anaplasma* Sp. was confirmed by its round, purple thinner, smaller inclusions, located in marginal position of the erythrocytes as suggested by Selmi *et al.* (2019) [26].

Treatment regimen followed and its outcome

The equine was treated with diminazene aceturate as a single dose (7.0mg/kg body weight) for trypanosome and *Babesia* infections and oxytetracycline (22 mg/kg body weight) for a period of 14 days along with supportive therapy as suggested by Morrow and Sommardhl, (2014) [15]. Blood smear examination of the horse was carried out after a period of 30 days of treatment which revealed *B. equi* residual organisms when other blood protozoans were found negative.

Results and discussion

Clinical manifestations of the infected equine

Hailer *et al.* (1997) [6] reported varying degree of anorexia, elevated or normal temperature, increased heart respiratory and pulse rates, colic, constipation followed by diarrhea. Rothschild and Knowles (2007) [22] reported that equids with sub-acute piroplasmiasis may display anorexia, lethargy, elevated or normal temperature, weight loss, anemia, limb edema, increased heart respiratory and pulse rates. Zobba *et al.* (2008) [32] & McFarland, (2016) [14] reported mild to general weakness, depression, rapid or shallow breathing, weight loss, fever, anorexia, anemia, elevated pulse and respiratory rate, congested mucus membrane, icterus, colic, edema of distal limbs, eyelid, incoordination and abortion in pregnant mares in infected equine. In this study the clinical signs revealed by the horse were temperature ranged between 99-101*, pulse 32-48, respiration 22-33, heart rate, 38-48, conjunctivitis, lymphadenopathy, abdominal respiration, loss of luster of the skin, and laminitis

Blood smear examination of the infected equine.

Blood smear examination of this equine revealed the positivity of the blood parasites *i.e.* *Trypanosoma cruzi*, *Babesia equi*, & *Anaplasma* Sp. before treatment.



Fig 1: Trypomastigote of *T. cruzi*



Fig 2: *Anaplasma* sp. & *T. cruzi*

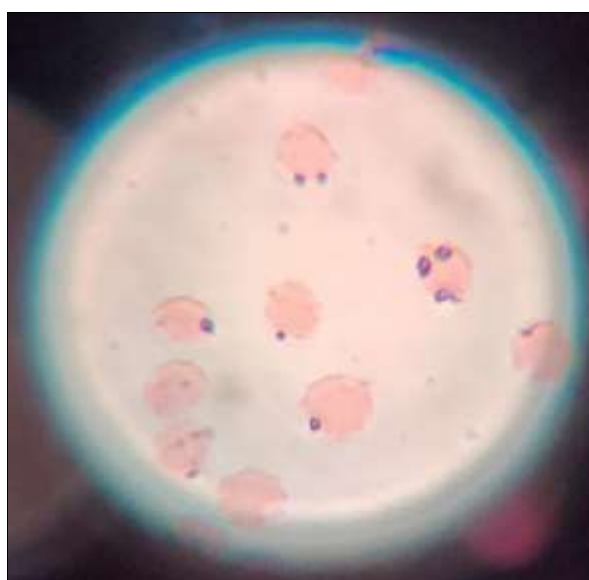


Fig 3: *B. equi* & *Anaplasma* Sp.

Fig 4: *B. equi*

Rubino *et al.* (2006) [23] reported 34.9% prevalence of hemoprotozoans in horses. Nagore *et al.* (2004) [17] reported 37.5% combined infection status in equines due to Theileriosis and Babesiosis. Shehzad *et al.* (2003) [27] reported 27.27% mixed infection due to *Babesia* Sp in equines. Khadiji Javed *et al.* (2014) [8] reported 10.84% mixed infection due to Anaplasmosis and Babesiosis in equines in around Lahore. Nimako-Boateng, (2022) [18] reported 5% prevalence of *Babesia caballi* and *Trypanosoma equiperdum* and its effects on blood parameters of horses in the Ashanti region of Ghana. In this study also the concomitant infection of the above blood parasites *i.e.* *Anaplasma* Sp, *Babesia equi* & *Trypanosoma cruzi* were observed in the infected equine blood. (Fig.1- Trypomastigote of *Trypanosoma cruzi* Fig.2- *Anaplasma* Sp & Trypomastigote of *T. cruzi* and Fig.3- *B. equi* & *Anaplasma* Sp Fig.4- *B. equi*).

Table 1: Hemato-biochemical parameter studies of the infected equine.

Parameters	Equine treatment		Normal values
	Before	After	
Hemogram			
Haemoglobin (g/dl)	9.00	13.0	10.1-16.1
Hematogrit (%)	26.7	38.2	27-43
PCV (%)	-		
TEC (10 ⁶ ul)	4.48	6.31	6.0-10.4
MCV (fl)	59.7	60.6	37-59
MCH (pg)	12.3	15.2	13.7-18.2
MCHC	30.1	36.0	35.3-39.3.
Platelets (10 ⁵ ul) (thou/mm ³)	1,64,000	1,54,000	117-256(thou/mm ³)
TLC (WBC) (ul)	8,400	12100	5.6-12.1 (thou/mm ³)
Neutrophils (%)	50.0	68	52-57
Lymphocytes (%)	40.0	24	21.0-42.0
Monocytes (%)	06	06	3-6.0
Eosinophil (%)	04	02	2-7.0
Basophils (%)	0.0		0-2.0
Biochemical tests	Liver function tests		
Total protein	6.24	7.01	5.6-7.6
Albumin(g/dl)	2.50	3.25	2.6-4.0
Globulin(g/dl)	3.74	2.98	2.6-4.1
A/G ratio	0.66	0.30	-
AST (ul)	198.01	183.00	160-412
ALT (ul)	40.13	18.71	2.7-21
Alkaline phosphatase	265.11	224.31	70-227
Bilirubin direct (g/dl)	0.16	0.20	0.1-0.4
Bilirubin in-direct(g/dl)	1.24	0.66	0-0.3
Bilirubin total	2.40	0.86	0.1-6.2
Biochemical tests	Kidney function tests		
Blood urea mg/dl		0.21	0.1-0.6
BUN (mg/dl)	20.14	23.17	11-27
Creatinine mg/dl	1.30	0.96	0.4-2.2
Phosphorus mg/dl	2.45	3.30	1.5-4.7
Sodium mmol/L	139.4	130.9	128-142
Potassium mmol/L	2.70	3.91	2.9-4.6
Chloride mmol/L	104.9	103.8	98-109

Rashid *et al.* (2009) [21] and Rubino *et al.* (2006) [23] reported a decrease in TEC and Hb, but slight increase in TLC values. Kumar *et al.* (2007) [10] reported decreased Hb values in infected horses than from the values of healthy equines. Khadiji Javed *et al.* (2014) [8] reported remarkable increase in TLC, slightly increased TEC values, & decreased Hb level, while PCV remained in the normal range in horses. Shehzad *et al.* (2003) [27] described that the hematology of hemoparasitic equines decrease in total RBCs, total leucocyte count, Hb estimation, PCV, neutrophils and basophils in the infected horses. Nimako-Boateng *et.al* (2022) [18] reported low values of hematocrit, MCH, MCHC & high values of MCV indicating macrocytic hypochromic anemia in equine

population in the combined infection of *Babesia caballi* and *Trypanosoma equiperdum*. Osman, S.A. (2017) [20] reported alterations in hematological parameters due to supportive mechanisms of parasitaemia due to increased level of malondialdehyde ions in the plasma that alters the RBC biochemical properties.

Scoles and Ueti (2015) [25] observed mixed infection status of the equines were common in endemic areas and it was observed by the author that persistent infection of *B. equi* is due to sequestration of the organisms and immune evasion strategies (Ueti *et al.* 2012) [29]. Schein 1988; Rothschild & Knowles (2007) [22, 24] explained that the equine immune response is unable to eliminate *B. equi*, and infected horses

are reported to remain infected for life. Ganesan (2023) ^[5] reported the above observation in equines piroplasmiasis. Mahmoud *et al.* (2016) ^[11] reported decreased Hb values due to destruction of RBCs. Elevation in the bilirubin content (direct) indicate the breakdown of Hb and diagnostic marker of liver and blood disorders. Zobba *et al.* (2008) reported significant increase in ALT due to damage of erythrocytes in the infected equine. Odedara, A. B. *et al.* (2021) ^[19] studied the hemato-biochemical profile of haemo-protozoan infected horses, which showed significant decrease in Hb, PCV, TEC, MCHC, platelet count, and lymphocyte % with increase in TLC and neutrophils. Further it was reported that the blood glucose, total protein, albumin, AST & BUN level increased in this infected equine due to imbalance between oxidants and antioxidants those played a central role in pathological conditions associated with hemoprotozoan infections. Muhammad Asif *et al.* (2019) ^[16] reported significant increase in AST & ALT with *T. annulata* and *A. marginale* combined infection in equines in Pakistan. Hailer *et al.* (1997) ^[6] reported variations in biochemical changes occurs due to wide range of factors such as nutrition, weather, and hydration status, presence of other infectious and non-infectious diseases and the general health conditions of the animals. In this study the Hb, hematocrit value, total RBC count, MCH, MCHC values were in decreased levels and MCV, bilirubin (indirect), SGPT (ALT) values were in increased levels before treatment and returned to normal after treatment (Table1).

Conclusions

An equine with the multiple infections of *Babesia equi*, *Anaplasma Sp.*, & *Trypanosome cruzi* was diagnosed and treated successfully. The clinical stress experienced by the equine due to the above concomitant infections and the hemato-biochemical changes occurred during this period before and after the treatment indicates the use of hemato-biochemical studies for better management practices. A detailed study for the prevalence of the concomitant infections of the blood parasites and the involvement of the causative agents in equine population needed for better care of the equine population.

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Author's Contribution

Not available.

Conflict of Interest

Not available.

Financial Support

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