CASE REPORT

ANAPLASMOSIS AND THEILERIOSIS IN FRIESIAN CROSS CATTLE IN KELANTAN

WAN NORUL HUDA, W. A. W.*, WAN NURULIYANA, W. A. AND ZAINANI, H.

Eastern Zone Veterinary Laboratory, Kota Bharu, Kelantan
** Corresponding author:* shadowfax_cruz@yahoo.co.uk

ABSTRACT. *Anaplasma marginale* is a rickettsial parasite that causes progressive anemia in ruminants leading to huge economic losses. This is a diagnostic case of rickettsial infection, anaplasmosis, and theileriosis in Friesian cross cattle, which cause death and high economic loss to the farm in Pulai Chondong District, Kelantan. The case is diagnosed as jaundice due to *Anaplasma* spp. and *Theileria* spp. based upon postmortem finding, case history, and detection of blood parasites through diagnostic test by thin blood smear as well as May Grunwald Giemsa (MGG) staining technique. Hence, a greater understanding of the hemoparasitic infection and its preventive management in animals would help reduces the economic losses of the farm. A herd of Friesian Cross Dairy Cattle in Machang, Kelantan had a history of high mortality of 50 % with the herd showing signs of jaundice and drop in milk production. Blood samples were taken from the five remainder animals on the farm as well as postmortem done on one carcass. Postmortem of the carcass revealed generalized yellowish discoloration of body fats and internal organs with dark brown urine in the bladder and oedematous lungs. Klebsiella pneumonia was isolated from the kidneys. Histopathology examination revealed fibrinous pneumonia, pulmonary oedema and haemorrahge, severe haemorrhage and haemosiderosis of hepatocytes and spleen, and interstitial hemorrhage of the nephrocytes. Blood analysis revealed that the herd suffering from anaemia and have elevated levels of liver enzymes. However, the elevated levels do not indicate whether the herd was suffering from prehepatic, hepatic, or post hepatic jaundice. Thin blood smears with May Grunwald Giemsa staining technique revealed Anaplasma spp. and Theileria spp. under 100 x magnification. Thus, the final diagnosis of the herd was anaplasmosis and theileriosis. Hence, greater understanding of the haemoparasitic infection and its preventive management in animals would help reduces the economic losses of the farm.

Keywords: jaundice, anaplasmosis, theileriosis, hemoparasitic infection, economic losses

INTRODUCTION

Anaplasmosis is an infectious blood disease in cattle caused by the rickettsial parasites, *Anaplasma marginale*, and *Anaplasma centrale*.

This situation is also known as yellow fever. This rickettsial parasites infects red blood cells and causes severe anemia in cattle. It is most commonly spread by ticks. Cattle, buffalo, goat, sheep, and some wild ruminants could be infected with anaplasmosis but cattles seem to be more susceptible to Anaplasma infection than the buffaloes (Rajput *et al.*, 2005). Anaplasmosis is progressive hemolytic anemia associated with jaundice, fever, hyperexcitability, abortions, decreased milk production, and in some extreme cases sudden death (Richey *et al.*, 1990; Radostitis *et al.*, 2000). *Anaplasma* is transmitted by various species of ticks of at least 20 species (Marchette *et al*., 1982; Kocan *et al*., 2004) such as Rhipicephalus spp., *Hyalomma spp., Boophilus spp., Demacentor spp.*, and *Ixodes*

spp. (Jongejan *et al.*, 2004). Amongst all these, *Boophilus microplus* is found to be the major transmitting agent (Aubrey *et al*., 2011). There are also alternative means of spreading of these diseases through mechanical transmission by biting flies or blood-contaminated fomites.

Theileriosis is also an infectious blood disease caused by *Theileria* spp. which is an obligate intracellular protozoan parasite that infect both wild and domestic Bovidae. Small ruminants can be infected too with some species. The transmission is by ixodid ticks, which have complex life cycles in both invertebrate and vertebrate hosts. The two most pathogenic and economically important of *Theileria* spp. that infect cattle are T. *annulata* and T. *parva*.

For diagnosis of bovine anaplasmosis and theileriosis from clinical samples, classical Giemsa-stained thin blood smear (GSTBS) method is a gold standard test for easy, early, and economic detection of parasites. Administration of tetracycline antibiotics is the effective treatment for this disease, along with other supportive medication such as parenteral hematinics, vitamin C, and liver extract to ensure complete and smooth recovery (Kumar *et al*., 2015)

This case report details anaplasmosis and theileriosis diagnosed from a herd of dairy cattle in Machang, Kelantan, which resulted in high mortality and economic loss.

CASE REPORT

Postmortem was done on a carcass with a history of 50 % mortality rate in the dairy farm in Machang, Kelantan. The organs samples are collected for Bacteriology and Histopathology. Besides that, blood was also collected in EDTA and plain tube from cattle, which were the remainder of the herd and sent to the Eastern Zone Veterinary Laboratory (Kelantan) for complete blood count and serum biochemistry. Thin blood smears were prepared promptly after blood collection. The blood smears were labelled, air-dried, stained with May Grunwald Giemsa, and then examined microscopically to identify the presence of *Theileria* spp., *Anaplasma* spp., and *Babesia* spp. in the erythrocytes. The smears examination was performed at 100× magnification using compound microscope. Based on that, the parasites were identified as described by OIE (OIE Terrestrial Manual, 2008).

FINDINGS

Upon postmortem there was generalized yellowish discoloration of body fats and internal organs. The urine was dark brown which is due to the presence of bilirubin. The lung was edematous (pulmonary edema) (Figure 1 to 3). *Klebsiella pneumonia* was isolated from the kidneys. Histopathology revealed fibrinous pneumonia, pulmonary edema and hemorrhage, severe hemorrhage and hemosiderosis of hepatocytes and spleen, and interstitial hemorrhage of the nephrocytes (Figure 4-6).

All five cattle were found positive for *Anaplasma* spp. and *Theileria* spp. infection based on May Grunwald Giemsa staining technique. *Anaplasma* spp. in the blood smear was identified and observed as rounded and dense with intra-erythrocytic bodies situated on or near the margin of the erythrocytes (as shown in Figure 7 & 8). *Theileria* spp. is identified as signet ring-shaped or comma-shaped piroplasm inside the erythrocytes (see Figure 7 & 8). The result for blood count analysis revealed normal value within the range except for RBC, Hemoglobin and PCV, which indicate that four out of five animals were anemic (Table 1).

Figure 1. Yellowish discolouration of body fat and internal organs

Figure 2. Yellowish discolouration of body fat and internal organs

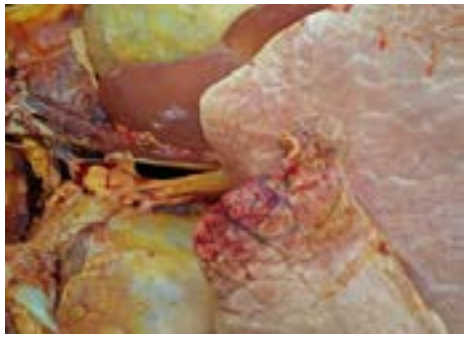

Figure 3. Yellowish discolouration of liver, lung, and heart

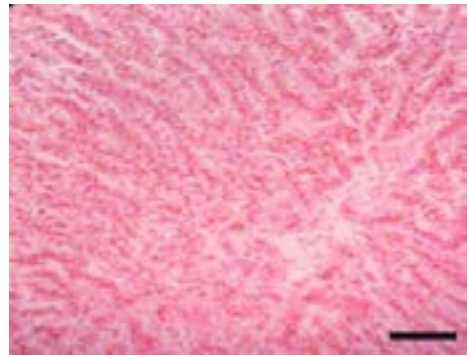

Figure 4. Severe hemorrhage of hepatocytes. H&E, x 200 magnification

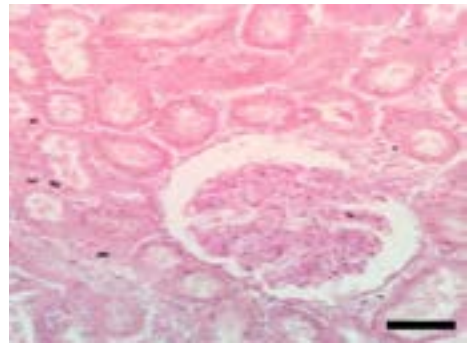

Figure 5. Interstitial hemorrhage of nephrocytes. H&E, x 400 magnification

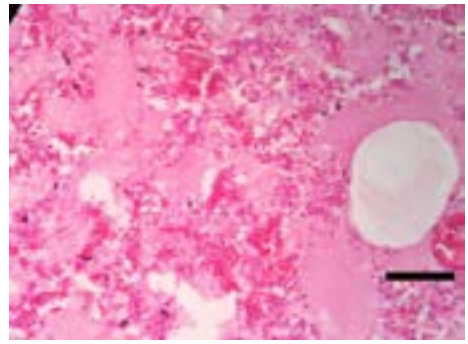

Figure 6. Fibrinous pneumonia, pulmonary edema and severe haemorrhage. H&E, x 400 magnification

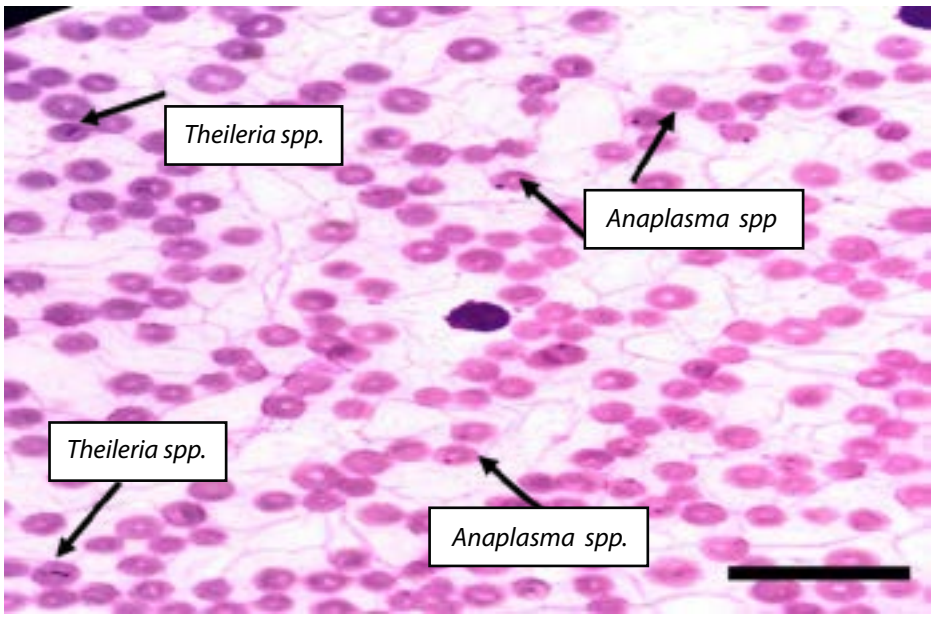

Figure 7. *Anaplasma* spp. and *Theileria* spp. detected in the erythrocytes. MGG Quick stain,1000 X magnification

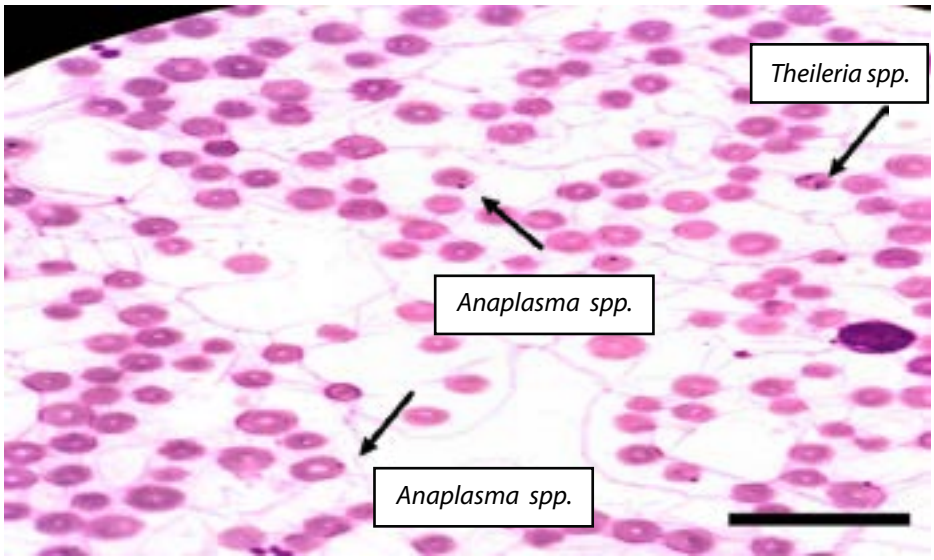

Figure 8. *Anaplasma* spp. and *Theileria* spp. detected in the erythrocytes. MGG Quick Stain, 1000 X magnification

Blood count analysis revealed four out of five animals were anaemic with elevated values of RBC, Haemoglobin and PCV (Table 1). Serum biochemistry analysis focused mainly on liver enzymes as the postmortem results indicated the herd was suffering from jaundice. The liver enzymes tested are as shown in Table 2. Results showed a slight increase of Alanine Phosphatase (ALP) in three cattle (ID No C, D, E). High levels of Alanine Phosphatase (ALP) may indicate liver inflammation, blockage of the bile ducts, or bone disease. Total Bilirubin is high in cattle E may indicate that the liver is not functioning properly. Cattle A showed high level Aspartate Aminotransferase (AST) while cattle C and D slightly lower level of Aspartate Aminotransferase (AST), which may indicate a problem with the liver or muscles. The Lactate Dehydrogenase (LDH) levels were high for all cattle. It may indicate some form of tissue damage (see Table 2). However, serum biochemistry result obtained in this case is significant in indicating whether the herd is suffering from pre hepatic, intra hepatic or post hepatic jaundice.

Table 1. Blood count analysis

ID No / Blood Parameter	A	B	C	D	E	Blood Parameter Reference
RBC	5.05	3.15	1.73	2.39	2.79	5-10
PCV	24.7	16.7	14.9	14	13.2	24-46
HB	8.9	5.9	4.9	5.1	5.5	8-15
WBC	12.9	16.5	13.9	11.4	13.9	4-12
ID No / Blood Parameter	**A**	**B**	**C**	**D**	**E**	**Blood Parasite Reference**
Anaplasma spp.	Positive	Positive	Positive	Positive	Positive	Positive/negative
Theileria spp.	Positive	Positive	Positive	Positive	Positive	Positive/negative

Table 2. Serum biochemistry analysis

ID No / Biochemical Parameter	A	B	C	D	E	Biochemical Parameter Reference
Total Bilirubin	<8.55	<8.55	13.6	<8.55	38	1.7-27.2
Aspartate Aminotransferase (AST)	120	56.9	48.9	44.6	80.7	50-100
Alkaline Phosphatase (ALP)	72	82.6	107	112	118	10-100
Total Protein	12.9	16.5	13.9	11.4	13.9	4-12
Gamma-Glutamyltransferase (GGT)	67	70	70	60	68	55-75
Lactate Dehydrogenase (LDH)	2208	1493	2240	1708	2432	692-1445

DISCUSSION

The cause of jaundice and death of the cattle is due to prehepatic jaundice which is due to blood parasites. The process of jaundice occurs when the red blood cells are broken down by the blood parasites. This process releases hemoglobin which is then converted into bilirubin. When there is too much bilirubin to process in the liver, it will cause it to overflow and leak into body tissues.

In this case report, the diagnosis of anaplasmosis and theileriosis in cattle is based on the tentative case history and microscopic examination for confirmation in clinical cases using thin blood smear and May Grunwald Giemsa staining method. This method gives a clear picture of the blood parasite's morphology. However, recent molecular technology is also important in the diagnosis of hemoparasitic infection in term of accuracy, but they are costlier, time-consuming, and require expertise which limits their use (Kumar *et al*., 2015).

Disease prevalence increases due to many various factors. Hot and humid climatic conditions, restricted movement of animals due to confinement as well as because of tick breeding season resulting in an abrupt increase in *Anaplasma* and *Theileria* infection. They were acutely affected (Kumar *et al*., 2015). Cattles are at risk of infection when they move to new areas where infected ticks are present. However, there were no ticks observed either during postmortem of the carcass nor from the remainder of the herd. Based on that, we can rule out that the infected ticks are from the farm.

Infected animals can spread the infection when transported to new areas and spread the disease to uninfected animals. From the history, the cattle were imported from Australia, entered Malaysia and being isolated at the other station before being transported to the farm. The cattle entered the farm for about almost a month before started showing the symptoms and death with 50% mortality. So, we can predict that the cattle were already being infected at the native land/isolation area and later spread the infection to the uninfected animals when transported to this farm. As this study did not find any ticks, and could only found flies, hence they were probably spread by other mechanical transmission such as biting flies/fomites.

Animals found positive for anaplasmosis were treated with oxytetracycline intravenously (10 mg/kg) daily for 3 days. Response to treatment was assessed on the 7th day post-treatment by repeated blood sampling. They responded well to the treatment given with no symptom of jaundice observed and no death reported after the treatment. Clinical picture after 1 week of treatment showed improvement in appetite. The mucous membrane was also light in color. Repeated blood sampling results were negative for blood parasites. Deticking programs were also done routinely in the farm to prevent the tick infestation in the herd.

CONCLUSION

Anaplasmosis and theileriosis are important disease that cause death, reduce milk production, and cause high economic loss to the farm. A greater understanding of hemoparasitic infection and its preventive management in animals would help reduce the economic losses of the farm.

REFERENCES

1. Aubry P, Geale D.W. (2011). A review of bovine anaplasmosis. Transbound. Emerg. Dis., 58(1), 1–30.
2. Jongejan F, Uilenberg G. (2004). The global importance of ticks. Parasitology, 129, S3–S14.
3. Kocan K.M, De La Fuente J, Blouin E.F, Garica-Garcia J.C. (2004). *Anaplasma marginale* (Rickettsiales:Anaplasmataceae):Recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology, 129, 285–300.
4. Kumar T, Sindhu N, Charaya G, Kumar A, Kumar P, Chandratere G, Agnihotri D, Khurana R. (2015). Emerging status of anaplasmosis in cattle in Hisar. Vet World, 8(6), 768-71.
5. Marchette N, Stiller D. (1982). Ecological relationships and evolution in the rickettsiae. The *Anaplasmataceae Bartonellaceae and Rochalimaea Quintana*, 98–106.
6. OIE. Terrestrial Manual. (2008). Bovine anaplasmosis. France: OIE, Paris.
7. Rajput Z.I, Hu S.H, Arijo A.G, Habib M, Khalid M.J. (2005). Comparative study of anaplasma parasites in tick carrying buffaloes and cattle. J. Zhejiang. Univ. Sci., 6, 1057–1062.
8. Richey E. J, Palmer G.H. (1990). Bovine anaplasmosis. Compend. Contin. Educ. Pract. Vet., 12, 1661–1668
9. Radostitis O.M, Gay C.C, Blood D.C, Hinchliff K.W. (2000). A textbook of the diseases of cattle, sheep, goats and horses. Veterinary Medicine, 9, 1261–1265.