Research Article

A Case Report of Equine Piroplasmosis in a Postpartum Indigenous Pony Mare in Malaysia

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Abstract | We report a case of Equine Piroplasmosis in a postpartum mare in Kelantan, Malaysia. A 15-year-old Malaysian Indigenous Pony mare was referred to a veterinary clinic after two months of losing weight after giving birth to a foal that died 24 hours later. Physical examination revealed a pale mucous membrane, petechial haemorrhage in the vulva and hemoglobinuria, indicating severe destruction of red blood cells as typically seen in case of blood parasite infestation. Blood smear stained with Giemsa confirmed the presence of high parasitemia of *Theileria sp*. A Polymerase Chain Reaction (PCR) revealed that the infection was caused by *Theileria equi.* Sequencing analysis was performed, and the sequence was deposited in GenBank with accession number PP195844. Phylogenetic analysis was then conducted to understand the global transmission and pattern of *T. equi* infection among horses worldwide. The phylogenetic analysis of *T.equi* from this case indicated 100% similarity to *T. equi* isolated in South Korea , suggesting a close epidemiological link between the isolates. The epidemiological link needs to be further explored to provide an effective plan in controlling disease transmission. Equine Piroplasmosis can cause death especially in immunosuppressed horses, thus breeding horses need to be screened for piroplasmosis before mating and parturition to avoid this life-threatening events. It can be done by taking the blood and submitting for a blood smear to detect the presence of hemoparasite before stressful events such as breeding and parturition occurred. Early detection of the disease can improve the prognosis of cases and increase the survival rate in an infected horse.

Keywords | Piroplasmosis, Tick-borne disease, *Theileria equi,* Phylogenetic, Diagnosis, Blood protozoa

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INTRODUCTION

Equine Piroplasmosis (EP) is a tick-borne disease caused by the protozoa from the phylum of Apicomplexa, class of Aconoidasida, and order of Piroplasmida. It is listed as one of the notifiable diseases by the World Organization for Animal Health (OIE). It consists of two main families that are responsible for EP which are Babesiidae and Theileridae. The most common species infecting horses are *Babesia caballi* and *Theileria equi*. They are transmitted by 14 Ixodid tick species, including *Hyalomma, Rhipicephalus, Dermacentor, Amblyomma, Haemaphysalis and Ixodes* (Onyiche *et al*., 2019; Romiti *et al*., 2020; Scoles and Ueti, 2013; Rocafort-Ferrer *et al*., 2022).

Tick transmission can occur via intrastadial, transstadial, or transovarial, with transovarial transmission being most common in *B. caballi* infection (Tamzali, 2013). The parasite also can be transmitted through iatrogenic transmission where it occurs when an uninfected blood is accidentally mixed with infected blood through contaminated needles or blood transfusions (Wise *et al*., 2013). Other than that, transplacental transmission of *T. equi* from a carrier mare to fetuses had been reported, causing neonatal piroplasmosis or abortion in horses and mules (Allsopp *et al*., 2007; Chhabra *et al*., 2012; Georges *et al*., 2011; Sudan *et al*., 2015; Sant *et al*., 2016; Françoso *et al*., 2018). Transplacental transmission has been conclusively demonstrated for *T. equi*, supported by serological, microscopic, and molecular findings in foals across several countries, whereas evidence for *B. caballi* remains inconclusive despite occasional DNA detection (Phipps and Otter, 2004; Allsopp *et al*., 2007; Kumar *et al*., 2008; Georges *et al*., 2011; Chhabra *et al*., 2012; Sudan *et al*., 2015; Sant *et al*., 2016; de Sousa *et al*., 2017; Oliveira *et al*., 2019; Françoso *et al*., 2018; Bartolomé del Pino *et al*., 2023; Hermans *et al*., 2025). Additionally, the recent identification of *T. equi* DNA in colostrum suggested a possible alternative transmission route beyond placental transfer (Hermans *et al*., 2025).

Equine Piroplasmosis (EP) mostly infects equidae family groups such as horses, zebras, donkeys and mules (Rothschild, 2013). Other than that, current epidemiological studies also revealed infection of *T. equi* and *B. caballi* in camels (Qablan *et al*., 2012; Jasim *et al*., 2015; Bahrami *et al*., 2017), dogs (Rosa *et al*., 2014; Fritz, 2010; Hegab *et al*., 2023), tapir (Da Silveira *et al*., 2017; de Souza Gonçalves *et al*., 2020) and subclinically detected in Black Rhinoceros (Zimmermann *et al*., 2021).The capacity of these protozoa to infect multiple host species is of particular concern in Malaysia, where multispecies rearing is a common practice among horse owners. Additionally, the proximity of equine facilities to forested areas increases the risk of exposure to wildlife that may serve as reservoirs for vector-borne transmission (Naama *et al*., 2021).

Equine piroplasmosis imposes considerable economic burdens on horse owners due to increased treatment expenses, diminished reproductive efficiency, and pregnancy losses. Infections with *T. equi* of greater concern, as affected horses can remain lifelong carriers, thereby restricting their participation in international events where seropositive animals are prohibited (Aziz and Al-Barwary, 2018). Clinically, the disease may manifest in peracute, acute, subacute, or chronic forms, with the severity and presentation varying depending on the progression of infection. Peracute EP is rare and causes sudden death in adults and neonatal foals, although some infected foals are born without any sign of illness (Allsopp *et al*., 2007). Acute EP has a poorer prognosis and is more severe in stress-induced and immunocompromised horses. The symptoms include fatigue, anorexia, peripheral edema, high fevers, tachypnea, tachycardia, petechial, and ecchymosis hemorrhage of mucous membrane (Tamzali, 2013). In severe cases, the digestive, hepato-biliary and cardiovascular system may get involved, leading to signs such as colic, impaction, constipation, diarrhea (Wise *et al*., 2013; Tamzali, 2013), hemoglobinuria, bilirubinuria, jaundice (Rothschild, 2013) weak and arrhythmic arterial pulse and diastolic murmur (Diana *et al*., 2007; Aida *et al*., 2023). Subacute EP infection has a similar presentation but may be less severe (Zobba *et al*., 2008). Chronic EP infection is difficult to suspect due to non-specific signs, such as loss of appetite, performance reduction, weight loss, and poor body condition (Rothschild, 2013).

Equine piroplasmosis (EP) has been reported in 41 countries, predominantly in South and North America, South and West Africa, Europe, and Asia (WAHIS, 2025). The prevalence of EP varies considerably across regions, corresponding closely to the distribution of competent tick vectors (Onyiche *et al*., 2019). In Asia, low molecular prevalence rates were observed in Indonesia, with 8.5% for *T. equi* and 2.1% for *B. caballi* (Nugraha *et al*., 2018), while serological surveys in Thailand reported prevalence rates of 5.4% and 2.5% for *T. equi* and *B. caballi*, respectively (Kamyingkird *et al*., 2014). In contrast, substantially higher prevalence rates were recorded in Mongolia (78.9%) and India (62.7%) (Otgensuren *et al*., 2024; Mavadiya *et al*., 2023).

In Malaysia, several reports published by the Department of Veterinary Services (DVS) based on their national surveillance program, have documented a low prevalence of equine hemoprotozoan infections in Malaysia (Naama *et al*., 2021; Zawida *et al*., 2018; Chandrawathani *et al*., 1998). However, the surveillance relied solely on microscopic examination of blood smears, a method with limited sensitivity, which may underestimate the true extent of the disease in the country. In addition, a study on equine piroplasmosis using microscopic, molecular, and serological methods was conducted by Al-Sarhan (2017), but it was limited to Kelantan, a state in Malaysia and therefore did not represent the national situation. The first clinical case of EP in Malaysia was reported by Al-obaidi *et al*. (2015), highlighting the emergence of the disease as a potential threat to the Malaysian equine industry. Lack of study has been published on the phylogenetic analysis of equine piroplasmosis (EP) in Malaysia, leaving the origin and geographical distribution of the disease uncharacterized. Phylogenetic analysis of piroplasmosis agents is essential for tracing the source of infection, thereby facilitating the development of targeted preventive strategies to control its spread. This study reported the phylogenetic characterization of *T. equi* isolated from naturally infected indigenous pony horses in Malaysia.

Clinical Report

Case history: A 15-year-old Malaysian Indigenous Pony mare was presented to University Malaysia Kelantan Veterinary Teaching Hospital (HPV UMK) with complaints of persistent diarrhea and loss of weight for the past 2 months. History taking revealed the mare was purchased from the local seller last 5 months earlier and it was in good health. However, the mare started to have diarrhea and decrease in appetite in the last 2 months after giving birth to a full-term foal. The foal died 24 hours postpartum after showing signs of weakness, lethargic, unable to stand and refused to suckle despite getting assistance from the owner. The mare was previously treated symptomatically by a local private veterinarian with Marbofloxacin, Oxytetracycline, Flunixin Meglumine and multiple fluid therapy sessions with 0.9% Sodium Chloride (0.9% NaCl) also done within this 2 months duration, however the clinical signs persist. No diagnostic procedures were conducted initially; thus, the condition was managed according to its symptoms. The mare was not vaccinated against any disease before and after being purchased. However, it was dewormed with Ivermectin after being purchased. There was no tick present on the mare and other horses in the same stable.

Clinical presentation: Upon presentation at HPVUMK, the mare appeared dull but was still responsive to the stimuli. General observation revealed a rough and dull hair coat with a body score of 1/5, characterized by a prominent rib cage and pelvic bone indicating emaciation (Figure 1A). No ticks were observed on the skin, but there was evidence of persistent watery diarrhea characterized by the presence of fecal stain around its anus and tail region (Figure 1B). The feces were observed and scored as 4/4 based on its malformation and the presence of watery diarrhea. The physical examination revealed a heart rate of 42 beats/minute (normal range: 28 - 40 beats/minutes), a respiratory rate of 26 breaths/minute (normal range: 10 - 14 breaths/minutes), and a rectal temperature of 35.7 °C (normal range: 37.3 °C - 38.2 °C). The mare was hypothermic with tachypnea and slight tachycardia. The mare's capillary refill time (CRT) was at 3 seconds (normal CRT: <2 seconds) while the dehydration status was 10% (normal dehydration status: < 5%) using indicators from the delay in the skin tent, dryness in mucous membrane and sunken of eyeballs. The oral mucous membrane appeared pale (Figure 1C), while the vulvar mucosa indicated the presence of petechial hemorrhage on its mucosa (Figure 1D). Muscle weakness in both hindlimbs was detected upon gait examination. There was also the presence of a reddish color of urine observed when the horse urinated. Blood was taken from the jugular vein in both plain and EDTA tubes for thin blood smear examination, complete blood count, serum biochemistry analysis, and Polymerase Chain Reaction (PCR). The feces were taken for bacteriology and parasitology, and urine was sent for urinalysis. At this point in time, the differential diagnosis for this case was highly suggestive of hemo- and endoparasite infestation based on the indication from the mucous membrane and urine which showed evidence of RBC lysis in the blood circulation. It was supported with evidence of poor body condition and gastrointestinal problem which is commonly seen in both hemo- and endoparasite infestation.

Figure 1: Clinical manifestations observed in horses infected with hemoprotozoan parasites. (A) Severe emaciation with a body condition score of 1/5. (B) Fecal staining around the perineal region, indicative of diarrhea. (C) Pallor of the oral mucous membrane (highlighted by a black circle). (D) Petechial hemorrhages on the vulvar mucous membrane (indicated by a black arrow).

Diagnosis

Clinical pathology: The blood samples were collected by jugular venipuncture at presentation. The blood samples were placed in two 10-mL tubes. One tube contained Ethylenediaminetetraacetic acid (EDTA) at a concentration of 1 mg/mL as an anticoagulant; the second tube contained no anticoagulant. Sera were separated from the second tube and stored at -20°C until required for examination. The hematology and serum biochemistry results were listed in Table 1.

The hematological results demonstrate severe macrocytic hypochromic anemia characterized by low hematocrit (HCT), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), while high in mean corpuscular volume (MCV). Other than that, there was also the presence of moderate leukocytosis and lymphocytosis, and monocytosis, results of increased inflammatory responses and enhanced mononuclear phagocytic activities to engulf the senescent and dead erythrocytes due to extensive extravascular and intravascular hemolysis.

Table 1: Hematologic and serum biochemistry result of the mare infected with *Theileria equi*.

PARAMETER	RESULT	REFERENCE
Hb (g/dl)	7.0	11.0-17.0
HCT (%)	15.6	35.0-55.0
MCV (μm^3)	140.0	34.0-58.0
MCH (pg)	18.6	26.0-34.0
MCHC (g/dl)	16.1	31.0-35.5
RDW (%)	14.2	10.0-16.0
WBC ($10^3/\mu L$)	31.9	4.0-12.0
Lymphocyte ($10^3/\mu L$)	17.0	1.5-5.5
Monocyte ($10^3/\mu L$)	1.0	0.1-0.8
Granulocyte ($10^3/\mu L$)	14.2	2.0-8.0
Albumin (g/dL)	2.7	1.9-3.2
Alanine transaminase (μ/l)	148	5-50
Blood urea nitrogen (mg/dL)	45	10-25
Creatinine (mg/dL)	5.6	0.8-2.2
Phosphate (mg/dL)	10.5	1.8-5.6
Total protein (g/dL)	9.7	5.6-7.9
Globulin (g/dL)	7.0	2.4-4.7

Serum biochemistry results demonstrate moderate elevation of alanine transaminase (ALT) suggestive liver injury secondary to oxidative stress from the immune response against the parasite. Other than that, a moderate increase in blood urea nitrogen (BUN) and creatinine indicated azotemia, suggestive of kidney dysfunction. Furthermore, the phosphate (PHOS), total protein (TP), globulin (GLOB) and albumin (ALB) levels were also increased, indicating presence of active infection and inflammation together with dehydration occurred due to diarrhea and lack of appetite.

Parasitology: Fecal and blood samples were collected to screen for endo and hemoparasites. Ten grams of fecal samples were sent to Parasitology Lab and examined using a modified McMaster and sedimentation method to count for the nematodes and trematodes eggs, respectively. Test results were negative for both tests. Five (5) mL blood aliquot with EDTA was also submitted for hemoparasite examination. A thin blood smear was performed and stained with 10% Giemsa stain. The blood smear revealed the presence of trophozoites appearing as oval, round, and spindle shapes, measuring approximately 2-3 µm in the erythrocytes, suspected as *T. equi*. (Figure 2A). The trophozoites can also be seen in the neutrophil (Figure 2B). Both fecal and blood samples were processed according to the guidelines provided by Manual on Parasitology (Chandrawathani *et al*., 2019).

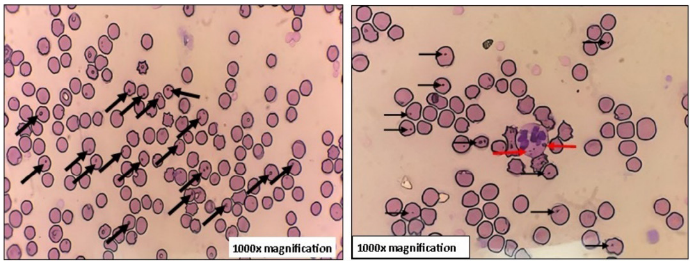

Figure 2: Microscopic examination of Giemsa-stained blood smears showing intraerythrocytic and intraleukocytic stages suspected as *Theileria equi*. (A) Presence of trophozoites within erythrocytes (indicated by a black arrow). (B) Trophozoites observed in both erythrocytes (black arrows) and leukocytes (red arrow).

Urinalysis

Fifty (50) ml urine was collected by manual catch during urination into a urine specimen container and submitted to Clinical Pathology Lab for physical, chemical and microscopic urinalysis. Physical urinalysis revealed reddish to brownish colour with cloudy turbidity. Chemical urinalysis using Dipstick test revealed positive in nitrite and leukocyte esterase, suggestive of urinary tract infection. There was also the presence of glucose and urobilinogen indicating kidney and liver injury. Erythrocytes and hemoglobin were also detected in the urine, indicating intravascular hemolysis caused by the *T. equi*. The result of urinalysis was presented in Table 2.

Table 2: Urinalysis result of the mare infected with *Theileria equi*.

PARAMETER	RESULT	REFERENCE
pH	8	5.5-8.0
Nitrites	Positive (+2)	Negative
Protein	Negative	Negative
Glucose	Positive (+4)	Negative
Ketone	Negative	Negative
Urobilinogen	Positive (+4)	Negative
Bilirubin	Negative	Negative
Erythrocyte	Positive (+4)	Negative
Hemoglobin	Positive (+4)	Negative

Links Researchers

Bacteriology

Rectal swabs were submitted to the Bacteriology Lab for bacterial isolation and identification. It was cultured in blood and Mac Conkey agar at 37°C for 24 hours. The swab culture showed growth of mucoid, large, grayish colony on blood agar, but none was growth in Mac Conkey agar. The organism was identified as *Enterobacter sp.* based on the biochemical test. *Enterobacter sp.* was considered as opportunistic bacteria in horses and is mostly found in the intestine and environment such as from soil and sewage (Uchida-Fujii *et al*., 2020).

Table 3: The protocols for each primer used to detect *T. evansi, B. caballi and T. equi* for this study.

Agent	Primer name	Fragment size (bp)	Initial denaturation	Denaturation and annealing extension	Cycles	Final extension	Target gene
T. evansi	Kin 1	540	95°C / 3 minutes	95°C / 45 seconds	35	72°C / 10 minutes	ITSI rDNA
	Kin 2						
B. caballi	TMB-F	650					SSU rRNA
	BC_R						
T. equi	TMB-F	360					
	EQUI_R						

Molecular Biology

A 5 ml of whole blood coated in an EDTA tube was processed for Polymerase Chain Reaction (PCR) to test for *Trypanosoma evansi, Babesia caballi* and *Theileria equi* using different sets of primer as mentioned in Table 3. The DNA was extracted from 300 µl of whole blood using PrimeWay Genomic DNA Extraction Kit according to manufacturer instructions. A total of 25 µl mixture of PCR reaction was prepared, which included 12.5 µl of commercial Master mix (PCRBIO Taq Mix Red), 5.5 µl of nuclease free water, 1 µl each of forward and reverse primer and 5 µl of genomic DNA. Each mixture was submitted for amplification following the protocol outlined in Table 3. The product of amplification was visualized on 1.5% agarose gel stained with Midori green dye using Gel DocTM EZ imager (BIO RAD, USA). The mare was tested PCR positive for *T. equi* but negative for *B. caballi* and *T. evansi*. Thus, the definitive diagnosis for this case is Equine Piroplasmosis caused by *T. equi*. The amplified product of PCR mixture was sequenced in both directions. The result from the sequence was compared with the available sequence in the GenBank of the National Centre for Biotechnological Information (NCBI - www.ncbi.nim.nih.gov) using the Basic Local Alignment Search Tool (BLAST) programme. The nucleotide sequence showed 100% identity and query cover for *Theileria equi*. The sequence was deposited in GenBank with accession number PP195844. A phylogenetic analysis was performed with the Maximum Likelihood technique using the MEGA X program (Kumar *et al*., 2018) based on the model General Time Reversible. It contained eleven nucleotide sequences: the one acquired for this work, nine sequences of *T. equi* (known genotype) received from GenBank®, and one outgroup sequence of *T. annulata*. A discrete MUSCLE technique was used to align the sequences. The cut off point was set at 50% to further investigate the significance of the divergence.

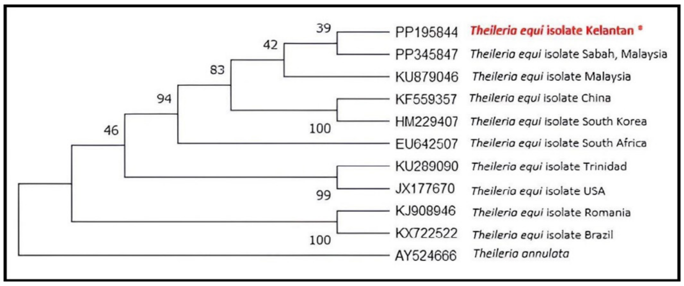

Figure 3: Phylogenetic analysis based on the 18S SSU rRNA gene of *Theileria equi* isolate from the present study (indicated by *). The tree includes representative reference sequences of *Theileria equi*, with *Theileria annulata* used as the outgroup. Multiple sequence alignment was performed using ClustalW, and the phylogenetic tree was constructed using the Maximum Likelihood method.

As indicated in Figure 3, all the sequences were clustered within the *T. equi* clade, except for the outgroup sequence which in *T. annulata* clade, indicating genetic uniformity in the population. It showed 100% similarity with isolate from South Korea (HM229407), 94% with isolate from South Africa (EU642507) and 83% with isolate from China (KF559357). Other than that, three isolates from Malaysia, including one from this case showed genetic variation among isolates indicating genetic diversity of *T. equi* in Malaysia. Difference in breeds, location and years of sample collected may influence the diversity in *T. equi* isolates in Malaysia.

Treatment

On the day of the presentation, only Kaolin Pectin, 4 ml/kg, twice a day, per orally, was given to the mare to encounter the diarrhea. It used to harden the stool and absorb any toxin release from the chronic diarrhea. The mare was already treated by a private vet a day before our visit. The vet had administered 0.9% sodium chloride (INFUSOL®, NS) dosed at 20 ml kg−1 h−1, intravenously to correct hydration status. It also had been injected with two types of antibiotics, which were Marbofloxacin, 2 mg/kg, and Oxytetracycline, 6.6mg/kg. Marbofloxacin was given as a broad-spectrum, bactericidal antibiotic aimed to treat both gram-positive and gram- negative bacterial infections, while Oxytetracycline was given to treat hemoparasite infestation, which was suspected based on the clinical presentation. Other than that, 20 ml of multivitamins (Vitavet) was given as a vitamin supplement to support the body system and stimulate appetite, and ivermectin, 0.2mg/kg was injected intramuscularly, and at the same time, to stabilize the mare's condition. Once the confirmatory diagnosis of Equine Piroplasmosis was reached, Diminazene Aceturate (Barenil®) was planned to be administered (3.5 mg/kg), intramuscularly. However, the mare was found dead by the owner on the day of the planned second visit. No post-mortem was conducted, and the carcass was disposed of by the owner. Equine Piroplasmosis is a notifiable disease in Malaysia; thus, veterinary authorities were promptly informed of this case.

RESULTS AND DISCUSSION

In this case, EP was diagnosed based on the clinical signs and laboratory findings. Pale mucous membrane and petechial hemorrhage in the vulva accompanied with hemoglobinuria seen in this horse that put blood parasite infestation as one of the differential diagnoses. *T. equi* and *B. caballi* will invade the bloodstream and replicate within the erythrocytes, leading to the accumulation of oxidative ions (Gopalakrishnan *et al*., 2015), which will further alter the cellular membrane by disrupting its osmotic fragility, causing hemolysis (Harvey, 1997). Hemolysis of RBC will reduce the number of circulating RBC in the bloodstream which contributes to the pale appearance of mucous membrane, as seen in this case. It was also reflected in a blood analysis which showed severe macrocytic hypochromic anemia similar to what was described by Maiti *et al*. (2021), Mahmoud *et al*. (2016), Al-Obaidi *et al*. (2016).

The lysis of RBC releases hemoglobin into the bloodstream, which will be processed to form its byproducts such as bilirubin. It will be sent to the liver to be eliminated from the body through feces, while the excess hemoglobin will be filtered by the kidneys. In severe cases where massive hemolysis occurs, the overflow of hemoglobin in the bloodstream will burden the kidney to filter the hemoglobin effectively, leading to kidney dysfunction. A marked increase in kidney parameters such as BUN and creatinine in this case was evidence of kidney dysfunction. This condition will lead to the escape of hemoglobin into the urine, known as hemoglobinuria, which can be confirmed by urinalysis. Other than that, massive hemolysis will also cause the excessive accumulation of bilirubin in the liver, which will then develop signs such as jaundice. In this case, jaundice was not seen yet; however, the liver parameter such as ALT had been significantly increased, indicating the early signs of liver injury.

Lysis of RBC is also speculated to cause coagulopathy in horses. The mechanism is still, inconclusive; however, it may be related to overproduction of inflammatory mediators that triggers the host defense mechanism and inflammatory reaction by promoting the coagulation causing hypercoagulability, systemic inflammatory response syndrome, and subsequent multiorgan system dysfunction as seen in canine babesiosis (Barić Rafaj *et al*., 2013). It is supported by the blood analysis which shows marked increase in leukocyte including neutrophils and lymphocyte count indicating severe infection which stimulated high amounts of inflammatory mediators and causing coagulopathy which was reflected in low amounts of platelets in blood analysis. Petechiation of mucous membrane as seen in this case was one of the signs seen as a result of coagulation disorder caused by the parasites.

Based on the clinical signs and laboratory diagnosis, it was consistent with the diagnosis of equine piroplasmosis. Pale mucous membranes, petechial hemorrhages, and hemoglobinuria were attributed to severe hemolysis caused by intraerythrocytic replication of *T. equi*, as confirmed by macrocytic hypochromic anemia on blood analysis. Other than that, elevated kidney and liver parameters indicated secondary renal and early hepatic dysfunction due to the accumulation of hemoglobin and bilirubin. Leukocytosis, thrombocytopenia, and mucosal petechiation shown in this case suggested the presence of inflammatory-mediated coagulopathy. The signs and laboratory analysis reflected a systemic parasitic infection leading to multisystem involvement, highlighting the need for early diagnosis and intervention.

All these signs of EP were seen more obviously after the mare delivered its foal that died 24 hours after the parturition. Thus, it is speculated that the mare was a carrier for the EP and the parasites flare up in response to the parturition. A carrier horse may have EP without any obvious clinical signs, but a flare-up of the infection and impairment of the immune system may relapse following stressful events (Ali *et al*., 1996). It has the risk of turning overt infection into clinical disease following a stressful event (Hailat *et al*., 1997). Pregnancy in carrier mares can result in abortion, miscarriage, stillbirth, or neonatal EP via intrauterine infection (De Waal, 1992; Lewis *et al*., 1999; Allsopp *et al*., 2007). In this case, the foal was successfully delivered at full term; however, its condition deteriorated after delivery, and it died 24 hours postpartum. No postmortem had been conducted on the foal; however, it can be speculated that the foal died due to neonatal piroplasmosis based on the mare's diagnosis and information from the owner regarding the signs shown by the foal before its death. The signs of neonatal piroplasmosis are similar to the infection of EP in adult horses, but it is accompanied by other diverse and nonspecific signs such as weakness at delivery, which make the foal unable to stand properly for suckling (Rothschild, 2013), although some infected foals are born without any signs of illness (Allsopp *et al*., 2007). The amount of parasite load across the placenta during pregnancy, the virulence profile of the strain, and the immunological status of the foal or mare may contribute to the severity of the infection (Levi *et al*., 2018).

Equine Piroplasmosis can be diagnosed by microscopic examination of thin blood smear stained with 10% Giemsa stain. This method had low sensitivity in carrier horses as it was only detected in a high burden of parasitemia. *B. caballi* and *T. equi* can be distinguished through blood smear by observing the shape of the merozoites. Merozoites of *B. caballi* often form pairs and join at their posterior end in two pyriform shaped, while *T. equi* form "Maltese cross" formation from four pyriform shaped (de Waal, 1992). It is difficult to differentiate both parasites from trophozoites appearance as both of them appear similar as round, oval or elliptical in up to 3 μm in diameter (Rothchild, 2013) inside the erythrocytes for both parasites, while inside lymphocytes for *T. equi* (Mehlhorn and Schein, 1998). Although the presence of trophozoites in both erythrocytes and lymphocytes in this case suggested a *T. equi* infection, it is not entirely ruled out that there may be a mixed infection with *B. caballi,* necessitating further molecular identification using PCR. Polymerase Chain Reaction (PCR) was more reliable for clinical diagnosis as it will detect the antigen which reflects current exposure compared to other serological diagnosis which detected antibodies resulting from current or previous exposure. In this case, PCR analysis targeting SSU rRNA confirmed that the causative agent was *T. equi*.

The phylogenetic analysis showed 100% similarity with isolate found in South Korea which was previously deposited in GenBank under accession numbers HM229407. The epidemiological link may not be 100% accurate due to usage of partial sequence from this study, however; we can conclude that there is a similarity between the isolates from Malaysia and South Korea, potentially; due to shared ancestral genes of *T. equi.* The animal movement inter-country due to the importation of live horses Seo *et al*. (2013) and participation in international equestrian competitions may be the possible reasons for the migration of piroplasm transmission. In 2023, Aida *et al*. (2023) reported a clinical case of equine piroplasmosis from participating horses in equestrian competition that shows the possibilities of infection even though the horse had been screened to be sero-negative for EP upon arrival. Thus, close monitoring of animal health during quarantine is very crucial to control disease transmission worldwide.

In summary, this case reflects the hidden risks posed by carrier horses, where EP can silently persist and then flare into severe, life-threatening disease under stressful conditions such as parturition. The mare's progression from subclinical infection to systemic illness, and the suspected neonatal loss, highlight the profound impact EP can have not only on individual animals but also on breeding outcomes. The confirmation of *T. equi* infection through PCR and the genetic link to strains from South Korea emphasize the role of international horse movement to silently facilitate disease transmission. This case serves as a reminder that even clinically healthy horses may harbor significant pathogens, reinforcing the need for vigilant screening, long-term monitoring, and a deeper understanding of stress-induced disease reactivation to ensure the health of horses was protected.

CONCLUSIONS AND RECOMMENDATIONS

Equine Piroplasmosis can be a life-threatening event in immunosuppressed horses. Screening for the disease before mating and parturition is necessary to prevent the disease transmission and promote an early detection of the disease before they are exposed to the immunosuppressed condition. Treatment of piroplasmosis in chronically infected horses is not promising, thus early detection and prompt treatment are necessary to improve the prognosis of the infected horses.

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NOVELTY STATEMENTS

This report documents the first molecular and phylogenetic confirmation of Theileria equi infection in a postpartum mare in Kelantan, Malaysia. The isolate demonstrated 100% genetic similarity to a South Korean strain, suggesting a possible transboundary epidemiological connection. The case highlights the clinical impact of T. equi infection in immunocompromised horses and emphasizes the need for routine pre-breeding and pre-parturition screening to prevent disease-associated reproductive losses. These findings contribute novel insights into regional transmission dynamics and support improved control strategies for equine piroplasmosis in Southeast Asia.

AUTHOR'S CONTRIBUTIONS

Nur Zul Izzati Mohd Rajdi: Methodology, Investigation, Writing-original draft.

Mimi Armiladiana Mohamad: Investigation, Resources.
Rumaizi Shaari: Investigation, Writing-review and editing.
Mohd Farhan Hanif Reduan: Formal analysis, Writing-original draft.
Mohammad Sabri Abdul Rahman: Methodology, Formal analysis, Writing-review and editing. Mohammad Dauda Goni: Writing-review and editing.
Li Peng Tan: Formal analysis, Writing-original draft, Methodology.

Conflict of Interest

The authors declare that the research was conducted in the absence of any potential conflict of interest.

REFERENCE

Aida H, Foreman JH, Ochi A, Takizawa Y, Yamanaka T (2023). A case of equine piroplasmosis in the Tokyo 2020 Olympic Games. J. Equine Sci., 34(3): 93–99. https://doi.org/10.1294/jes.32.93

Ali S, Sugimoto C, Onuma M (1996). Equine piroplasmosis. J. Equine Sci., 7(4): 67–77. https://doi.org/10.1294/jes.7.67

Allsopp MTEP, Lewis BD, Penzhorn BL (2007). Molecular evidence for transplacental transmission of *Theileria equi*from carrier mares to their apparently healthy foals. Vet. Parasitol., 148(2): 130–136. https://doi.org/10.1016/j.vetpar.2007.05.017

Al-Obaidi QT, Al-Sultan II, Arshad MM, Mohd Azam KGK, Mimi AM (2015). Clinical case of acute equine piroplasmosis in a Malaysian mare. Res. Opin. Anim. Vet. Sci., 5(6): 270–274.

Al-Obaidi QT, Mohd Mokhtar A, Al-Sultan II, Azlinda AB, Mohd Azam KGK (2016). Equine piroplasmosis in Kelantan, Malaysia: clinico-hemato-biochemical alterations in subclinically and clinically infected equids. Trop. Biomed., 33(4): 619–631.

Alsarhan QTS (2017). Prevalence, risk factors and genetic diversity of equine piroplasmosis in Kelantan, Malaysia. PhD thesis, Universiti Malaysia Kelantan, Kelantan, Malaysia.

Aziz KJ, Al-Barwary LTO (2019). Epidemiological study of equine piroplasmosis (*Theileria equi* and *Babesia caballi*) by microscopic examination and competitive-ELISA in Erbil Province, North-Iraq. Iran. J. Parasitol., 14(3): 404–412.

Bahrami S, Tabandeh MR, Tafreshi ARG (2017). Prevalence and molecular identification of piroplasmids in Iranian dromedaries (*Camelus dromedarius*). J. Zoo Wildl. Med., 48(4): 1026–1030. https://doi.org/10.1638/2016-0233.1

Barić Rafaj R, Kuleš J, Selanec J, Vrkić N, Zovko V, Zupančič M, Trampuš Bakija A, Matijatko V, Crnogaj M, Mrljak V (2013). Markers of coagulation activation, endothelial stimulation, and inflammation in dogs with babesiosis. J. Vet. Int. Med., 27(5): 1172–1178. https://doi.org/10.1111/jvim.12146

Bartolomé del Pino LE, Meana A, Zini M, Cersini A (2023). Evidence of transplacental transmission of equine piroplasms *Theileria equi* and *Babesia caballi* in an Italian breed mare. Folia Parasitol., 70: 005. https://doi.org/10.14411/fp.2023.005

Chandrawathani P, Yusof M, Slamah B, Rohaya MA (1998). A survey on equine piroplasmosis in selected areas in Peninsular Malaysia. J. Vet. Malays., 10(1): 25–26.

Chhabra S, Ranjan R, Uppal SK, Singla LD (2012). Transplacental transmission of *Babesia equi* (*Theileria equi*) from carrier mares to foals. J. Parasit. Dis., 36: 31–33. https://doi.org/10.1007/s12639-011-0072-1

Da Silveira AW, De Oliveira GG, Menezes Santos L, da Silva Azuaga LB, Macedo Coutinho CR, Echeverria JT, Antunes TR, do Nascimento Ramos CA, Izabel de Souza A (2017). Natural infection of the South American tapir (*Tapirus terrestris*) by *Theileria equi*. J. Wildl. Dis., 53(2): 411–413. https://doi.org/10.7589/2016-06-149

de Sousa SH, Paludo GR, Freschi CR, Machado RZ, de Castro MB (2017). Theileria equi infection causing abortion in a mare in Brazil. Vet. Parasitol.: Reg. Stud. Rep., 8: 113–116. https://doi.org/10.1016/j.vprsr.2017.03.008

de Souza Gonçalves T, Barros FDNL, Inoue LS, de Farias DM, dos Santos Lima J, Nobre AV, Aidar ESA, Diniz RRF, Gering AP, Scofield A (2020). Natural *Theileria equi* infection in captive *Tapirus terrestris* (Perissodactyla: Tapiridae) in the Brazilian Amazon. Ticks Tick Borne Dis., 11(4): 101452. https://doi.org/10.1016/j.ttbdis.2020.101452

De Waal DT (1992). Equine piroplasmosis: a review. Br. Vet. J., 148(1): 6–14. https://doi.org/10.1016/0007-1935(92)90061-5

Diana A, Guglielmini C, Candini D, Pietra M, Cipone M (2007). Cardiac arrhythmias associated with piroplasmosis in the horse: A case report. Vet. J., 174(1): 193–195. https://doi.org/10.1016/j.tvjl.2006.04.003

Chandrawathani, P., Premaalatha, B., Omar, J., Mamat, Z. C. (2019). Manual on parasitology. Department of Veterinary Services Malaysia.

Françoso R, Riccio AV, Fernandes CB, Alonso MA, Belli CB (2018). Transplacental transmission of *Theileria equi* in mules: Should we worry? Vet. Parasitol., 264: 39–41. https://doi.org/10.1016/j.vetpar.2018.10.017

Fritz D (2010). A PCR study of piroplasms in 166 dogs and 111 horses in France (March 2006 to March 2008). Parasitol. Res., 106(6): 1339–1342. https://doi.org/10.1007/s00436-010-1804-3

Georges KC, Ezeokoli CD, Sparagano O, Pargass I, Campbell M, D'Abadie R, Yabsley MJ (2011). A case of transplacental transmission of *Theileria equi* in a foal in Trinidad. Vet. Parasitol., 175(3-4): 363–366. https://doi.org/10.1016/j.vetpar.2010.10.019

Gopalakrishnan A, Maji C, Dahiya RK, Suthar A, Kumar R, Kumar S (2015). Oxidative damage inflicted by *Theileria equi* on horse erythrocytes when cultured in vitro by microaerophilous stationary phase technique. J. Equine Vet. Sci., 35(9): 763–767. https://doi.org/10.1016/j.jevs.2015.07.020

Hailat NQ, Lafi SQ, Al-Darraji AM, Al-Ani FK (1997). Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. Vet. Parasitol., 69(1-2): 1–8. https://doi.org/10.1016/S0304-4017(96)01100-4

Harvey JW (1997). The erythrocyte: physiology, metabolism, and biochemical disorders. In: Clinical Biochemistry of Domestic Animals, 157–203. https://doi.org/10.1016/

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B978-012396305-5/50008-7
Hegab AA, Fahmy MM, Omar HM, Ghattas SG, Mahmoud NE, Abuowarda M (2023). Occurrence and genotyping of *Theileria equi* in dogs and associated ticks in Egypt. Med. Vet. Entomol., 37(2): 252–262. https://doi.org/10.1111/mve.12627
Hermans LM, Bonsergent C, Josson A, Rocafort-Ferrer G, le Guyader M, Pessey SA, Leblond A, Malandrin L (2025). Evaluation of *Theileria equi* vertical transmission rate and routes in a cohort of asymptomatic mares and their foals. Ticks Tick Borne Dis., 16(1): 102432. https://doi.org/10.1016/j.ttbdis.2024.102432
Jasim HJ, Azzal GY, Othman RM (2015). Conventional and molecular detection of *Babesia caballi* and *Theileria equi*parasites in infected camels in south of Iraq. Basrah J. Vet. Res., 14(2): 110–121.
Kamyingkird K, Yangtara S, Desquesnes M, Cao S, Moumouni A, Jittapalapong S, Nimsupan B, Terkawi MA, Masatani T, Nishikawa Y, Igarashi I (2014). Seroprevalence of *Babesia caballi* and *Theileria equi* in horses and mules from Northern Thailand. J. Protozool. Res., 24(1-2): 11–17.
Kumar S, Kumar R, Gupta AK, Dwivedi SK (2008). Passive transfer of *Theileria equi* antibodies to neonate foals of immune tolerant mares. Vet. Parasitol., 151(1): 80–85. https://doi.org/10.1016/j.vetpar.2007.10.001
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol., 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
Levi MM, Tirosh-Levy S, Dahan R, Berlin D, Steinman A, Edery N, Savitski I, Lebovich B, Knowles D, Suarez CE, Baneth G (2018). First detection of diffuse and cerebral *Theileria equi* infection in neonatal filly. J. Equine Vet. Sci., 60: 23–28. https://doi.org/10.1016/j.jevs.2017.10.016
Lewis BD, Penzhorn BL, Volkmann DH (1999). Could treatment of pregnant mares prevent abortions due to equine piroplasmosis?: research communication. J. S. Afr. Vet. Assoc., 70(2): 90–91. https://doi.org/10.4102/jsava.v70i2.760
Mahmoud MS, El-Ezz NTA, Abdel-Shafy S, Nassar SA, El Namaky AH, Khalil WK, Knowles D, Kappmeyer L, Silva MG, Suarez CE (2016). Assessment of *Theileria equi* and *Babesia caballi* infections in equine populations in Egypt by molecular, serological and hematological approaches. Parasites Vectors, 9: 1–10. https://doi.org/10.1186/s13071-016-1539-9
Maiti SK, Ratre HK, Poyam MR, Hota A, Raghuvanshi PDS (2021). A case report on equine piroplasmosis in a Thoroughbred horse from Durg, Chhattisgarh and its therapeutic management. Haryana Veterin., 60(1): 5–7.
Mavadiya SV, Patel RM, Mehta SA, Vagh AA, Kalyani IH, Solanki JB, Dangar NS, Patel DR (2023). Prevalence of *Theileria equi* and *Babesia caballi* infections in horses in South Gujarat, India. Indian J. Anim. Res., 57(10): 1389–1392.
Mehlhorn H, Schein E (1998). Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi* Mehlhorn, Schein (1998). Parasitol. Res., 84: 467–475. https://doi.org/10.1016/S1383-5769(98)80349-8
Naama A, Rohaya MA, Tuba Thabitah AT, Nadiah H, Sock KK, Faizah ZN, Wan Norulhuda WAW, Hanim MF, Masrin A, Chandrawathani P (2021). Common equine parasites diagnosed in Peninsular Malaysia from the year 2014 to 2018. Malays. J. Vet. Res., 12(2): 29–38.
Nugraha AB, Cahyaningsih U, Amrozi A, Ridwan Y, Agungpriyono S, Taher DM, Guswanto A, Gantuya S, Tayebwa DS, Tuvshintulga B, Sivakumar T (2018). Serological and molecular prevalence of equine piroplasmosis in Western Java, Indonesia. Vet. Parasitol.: Reg. Stud. Rep., 14: 1–6. https://doi.org/10.1016/j.vprsr.2018.07.009
Oliveira AR, Pinheiro GRG, Souza TD, Flecher MC, Santos RL (2019). Abortion in association with transplacental *Theileria equi* infection in a mare from the State of Espírito Santo, southeast Brazil: case report. Arq. Bras. Med. Vet. Zootec., 71: 369–373. https://doi.org/10.1590/1678-4162-10696
Onyiche TE, Suganuma K, Igarashi I, Yokoyama N, Xuan X, Thekisoe O (2019). A review on equine piroplasmosis: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. Int. J. Environ. Res. Public Health, 16(10): 1736. https://doi.org/10.3390/ijerph16101736
Otgonsuren D, Amgalanbaatar T, Narantsatsral S, Enkhtaivan B, Munkhgerel D, Zoljargal M, Davkharbayar B, Myagmarsuren P, Battur B, Battsetseg B, Sivakumar T (2024). Epidemiology and genetic diversity of *Theileria equi* and *Babesia caballi* in Mongolian horses. Infect. Genet. Evol., 119: 105571. https://doi.org/10.1016/j.meegid.2024.105571
Phipps LP, Otter A (2004). Transplacental transmission of *Theileria equi* in two foals born and reared in the United Kingdom. Vet. Rec., 154(13): 406–408. https://doi.org/10.1136/vr.154.13.406
Rocafort-Ferrer G, Leblond A, Joulié A, René-Martellet M, Sandoz A, Poux V, Pradier S, Barry S, Vial L, Legrand L (2022). Molecular assessment of *Theileria equi* and *Babesia caballi* prevalence in horses and ticks on horses in southeastern France. Parasitol. Res., 121(3): 999–1008. https://doi.org/10.1007/s00436-022-07441-7
Rosa CT, Pazzi P, Nagel S, McClure V, Christie J, Troskie M, Dvir E (2014). Theileriosis in six dogs in South Africa and its potential clinical significance: clinical communication. J. S. Afr. Vet. Assoc., 85(1): 1–7. https://doi.org/10.4102/jsava.v85i1.1114
Rothschild CM (2013). Equine piroplasmosis. J. Equine Vet. Sci., 33(7): 497–508. https://doi.org/10.1016/j.jevs.2013.03.189
Romiti F, Magliano A, Antognetti V, Manna G, Cersini A, Scicluna MT, De Liberato C (2020). Investigation of Ixodid ticks as vectors of *Babesia caballi* and *Theileria equi* (Protozoa: Apicomplexa) in central Italy. J. Vector Ecol., 45(1): 25–31. https://doi.org/10.1111/jvec.12370
Sant C, d'Abadie R, Pargass I, Basu AK, Asgarali Z, Charles RA, Georges KC (2016). Prospective study investigating transplacental transmission of equine piroplasmosis in thoroughbred foals in Trinidad. Vet. Parasitol., 226: 132–137 https://doi.org/10.1016/j.vetpar.2016.07.008.
Scoles GA, Ueti MW (2013). *Amblyomma cajennense* is an intrastadial biological vector of *Theileria equi*. Parasites Vectors, 6: 1–9. https://doi.org/10.1186/1756-3305-6-306
Seo MG, Yun SH, Choi SK, Cho GJ, Park YS, Cho KH, Kwon OD, Kwak D (2013). Molecular and phylogenetic analysis of equine piroplasms in the Republic of Korea. Res. Vet. Sci., 94(3): 579–583. https://doi.org/10.1016/j.

rvsc.2013.01.014

Sudan V, Jaiswal AK, Srivastava A, Saxena A, Shanker D (2015). A rare clinical presentation of transplacental transmission and subsequent abortion by *Babesia (Theileria) equi* in a mare. J. Parasit. Dis., 39(2): 336–338. https://doi.org/10.1007/s12639-013-0337-y

Tamzali Y (2013). Equine piroplasmosis: An updated review. Equine Vet. Educ., 25(11): 590–598. https://doi.org/10.1111/eve.12070

Uchida-Fujii E, Niwa H, Kinoshita Y, Katayama Y, Nukada T (2020). Molecular phylogenetic and matrix-assisted laser desorption ionization time-of-flight mass spectrometry identification of isolates from horses identified as *Enterobacter cloacae* by biochemical identification. J. Equine Sci., 31(3): 49–55. https://doi.org/10.1294/jes.31.49

Qablan MA, Sloboda M, Jirků M, Oborník M, Dwairi S, Amr ZS, Hořín P, Lukeš J, Modrý D (2012). Quest for the piroplasms in camels: identification of *Theileria equi* and *Babesia caballi* in Jordanian dromedaries by PCR. Vet. Parasitol., 186(3-4): 456–460. https://doi.org/10.1016/j.vetpar.2011.11.070

Wise LN, Kappmeyer LS, Mealey RH, Knowles DP (2013). Review of equine piroplasmosis. J. Vet. Intern. Med., 27(6): 1334–1346. https://doi.org/10.1111/jvim.12168

World Animal Health Information System (WAHIS) (2025).

Zawida Z, Chandrawathani P, Nurulaini R, Khor SK, Adnan M, Premaalatha B, Zaini CM, Jamnah O, Azizah D, Norlida A, Suhaila SN (2010). Prevalence of protozoan diseases in local horses in Peninsular Malaysia. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 31(1 and 2): 48–50.

Zimmermann DE, Penzhorn BL, Vorster I, Troskie M, Oosthuizen MC (2021). Babesia bicornis, Theileria bicornis and Theileria equi in metapopulations of two black rhinoceros (Diceros bicornis) subspecies in South Africa and their potential impact on conservation. Ticks and Tick-borne Dis., 12(2): 101635. https://doi.org/10.1016/j.ttbdis.2020.101635

Zobba R, Ardu M, Niccolini S, Chessa B, Manna L, Cocco R, Parpaglia MLP (2008). Clinical and laboratory findings in equine piroplasmosis. J. Equine Vet. Sci., 28(5): 301–308. https://doi.org/10.1016/j.jevs.2008.03.005