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A clinical and diagnostic study of *Mycoplasma wenyonii* and *Haemobartonella bovis* infections in cattle of Mosul City, Iraq

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Abstract

Eleven local cows (2-6 years old), five calves (1 day–6 months) and two bulls from different farms were admitted to the Veterinary Teaching Hospital at the College of Veterinary Medicine, University of Mosul, Iraq for evaluation of poor appetite, fever, tachycardia, tachypnea, exhaustion, udder edema and anemia. Blood smear examination revealed an infection with coccoid shaped *Mycoplasma wenyonii,* seen on the surface of erythrocytes in the numbers ranging from 1 to 50, and large numbers in the background of blood smears. A few chain-shaped *Haemobartonella bovis* on the periphery of erythrocytes were also detected. Hematological examination of all infected animals showed a decrease in Hb, Hct, RBCs and platelates count, with significant increase of WBC counts, neutropenia, lymphopenia and monocytosis. The treated animals were recovered within three days following intramuscular injection of Imidocarb dipropionate (4 mg/kg body weight). This study reports the presence of clinical infections with *M. wenyonii* and *Haemobartonella bovis*, for the first time in Iraq and may form the basis in further studies.

Key words: Cattle, *Haemobartonella bovis*, *Mycoplasma wenyonii*

Introduction

Epierythrocytic parasites previously known as *Eperythrozoon* and *Haemobartonellan* have been reclassified to the genus *Mycoplasma* (Wiss and Jane, 2010). However, a recent study has confirmed that *Mycoplasma suis* can invade erythrocytes, which may protect them from the host immune responses and antimicrobial treatment (Groebel et al., 2009). Hemoplasma infection has gained increasing public health significance because of its potential for cross transmission from animals to humans (Yang et al., 2000; Yuan et al., 2009).

Eperythrozoon wenyonii (Mycoplsma wenyonii) has been recently reclassified with the genus *Mycoplasma* on the basis of 16S rRNA analysis (Neimark and Kocan, 1997; Neimark et al., 2001). Scanning electron microscopic analysis of *M. wenyonii* showed deformed erythrocytes with invaginations and presence of either rod or coccid shaped organisms embedded in the membrane of erythrocytes (Neimark et al., 2001). *Mycoplasma* infections are common in cattle worldwide. The infected cattle rarely die, but on occasionally shows acute clinical signs, swollen teats, edema of the distal portion of the hind limbs, transient fever, lymphadenopathy, rough coat, dramatically decreased milk production, subsequent infertility and weight loss (Smith et al., 1990). In addition, scrotal and hind limb edema, tachycardia, pyrexia and infertility were reported in bull in the United States in a chronic condition (Montes et al., 1994). Adverse environmental conditions and nutritional mismanagement are important factors predisposing the development of clinical eperythrozoonosis.

The present study describes the clinical, hematological and light microscopic features of *M. wenyonii* and *H. bovis* in naturally infected cattle.

Materials and Methods

Eleven local cows aged 2 to 6 years, five calves aged 1 day to 6 months and two bulls two years old from different farms were admitted to the Veterinary Teaching Hospital at the College of Veterinary Medicine, University of Mosul, Iraq, for evaluation of poor appetite, fever, tachycardia, tachypnea,

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emaciation, sunken eyes, decreased milk production, edema of hind quarters in bulls and udder edema in some cows, icterus and diarrhea in one calf, exhaustion and anemia. Before referring to the Veterinary Teaching Hospital, the cows were treated at a local veterinary clinic with penicillin (30000 IU/ kg, once a day for 3 days), cephalosporin (3 mg/kg once a day for 3 days) and oxytetracyclin (20 mg/ kg for 3 days) inter-muscular injections.

Clinical examination including determination of body temperature, heart rate and respiratory rate of all experimental animals were done. Blood samples with EDTA were taken from these animals (n=18) for determination of hemoglobin (Hb), red blood cell counts (RBC), hematocrit (Hct), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets (PLT) count, white blood cell counts (WBC) and differential leukocytic count (DLC) using Coulter Counter apparatus. Peripheral blood smears were prepared and stained with Giemsa stain then examined under a light microscope using oil emersion (x100). Positive results were defined if one infected erythrocyte was found in 200 observed RBC (Yuan et al., 2009). The data were expressed as mean ± standard deviation (SD).

Results

On clinical examination, ticks were not found on the animals except one, and swelling did not persist in lymph nodes. Infected animals showed signs of fever (40.5 ± 0.3Cº), tachycardia (90±12 beat/minute), tachypnea (45±3 breath/minute), loss of appetite, emaciation, hind limbs or udder edema and paleness of the mucous membranes. Some animals were recumbent. The hematological values of all infected cattle showed lower values of Hb, Hct, RBCs and platelets counts and higher values of total WBC, neutropenia, lymphopenia and monocytosis than the reference range o (Table 1).

The microscopic examinations of blood smears stained with Giemsa indicated that the epierythrocytic organisms in peripheral blood were morphologically consistent with *Mycoplasma wenyonii*. The organisms which affected nearly 90% of erythrocytes were small, basophilic, coccus and ring-shaped (Fig. 1). These organisms were observed both on the surface of erythrocytes and in the background. Most of *M. wenyonii* were approximately 1-2 µm in diameter with a number ranging from 1 to 50 organisms on the surface of the erythrocytes (Fig. 2). No organisms were detected penetrating erythrocytes. In addition, a few chain-shaped *Haemobartonella bovis* (0.1-0.5 µm) on the periphery of the erythrocytes were detected (Fig. 3). On treatment, five animals clinically recovered within three days following inter-muscular injection with Imidocarb dipropionate (4 mg/kg body weight) two times at 48 hours intervals. Eight days after treatment all animals were discharged as clinically normal and the blood smear examination was negative.

Table 1: Hematology values from cattle with *M. wenyonii* and *H. bovis*

Values	Result (Mean ± S.D.)	Reference range
RBC count ($x10^{12}$/L)	2.51 ± 0.63	5.0 – 10.0
Hb (gm/L)	55 ± 13	80 – 100.0
Hct (L/L)	0.20 ± 0.07	0.24 – 0.46
MCV (fl)	44.1 ± 4.1	37 – 51
MCH (pg)	14.4 ± 2.1	13 – 18
MCHC (gm/L)	327 ± 25	330 – 370
WBC count ($x10^9$/L)	13.78 ± 1.6	4.0 – 12.0
Neutrophils ($x10^9$/L)	0.4 ± 0.1	0.6 – 4.0
Lymphocytes ($x10^9$/L)	1.2 ± 0.2	2.5 – 7.5
Monocytes ($x10^9$/L)	1.34 ± 0.2	0.03 – 0.8
Platelets ($x10^9$/L)	100± 24	200 -730

* Significant difference in comparison with reference range ($P<0.05$) RBC= Red blood cell; Hb= Hemoglobin; Hct= Hematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration; WBC= white blood cell. Reference range from Meyer and Harvey, 2004

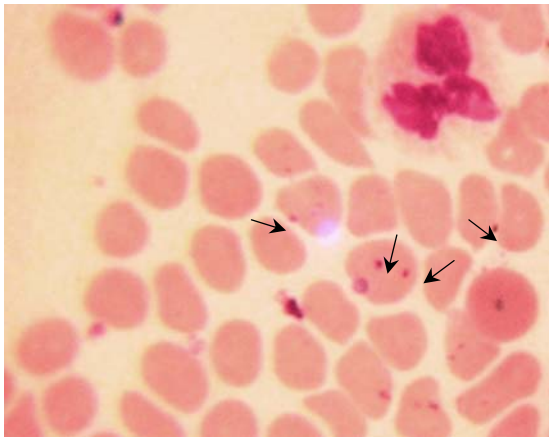

Fig. 1: Blood film of M. wenyonii on the surface of RBCs (100X)

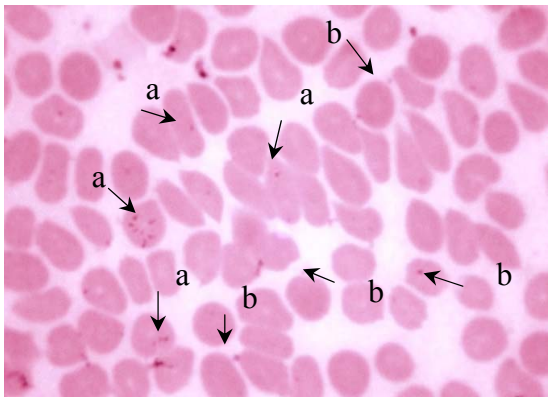

Fig. 2: Blood film from a cow infected with *M .Wenyonii*

A	B
infection organisms are visible on the surface of RBC (Leishman's stain)	Free in the background (arrows) (Giemsa stain x 100)

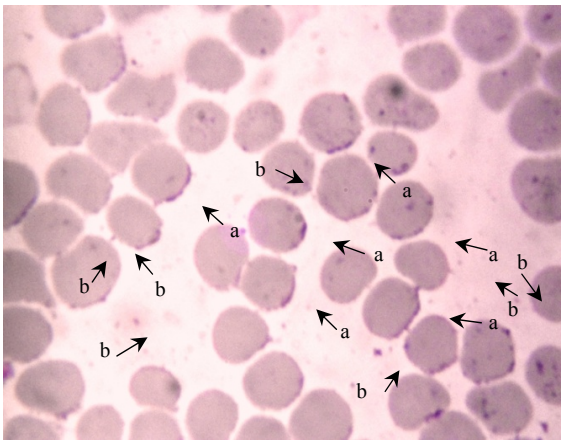

Fig 3: Blood film of *Haemobartonella bovis* observed as a chain on the surface of RBCs

Discussion

Eperythrozoonosis has not been reported previously in cattle or other animals in Iraq. It is expected that these concurrent infections are in animals imported from the neighboring countries such as Turkey. *E. wenyonii* and *H. bovis* in cattle were reported for the first time in Turkey by Şaki and Özer (2009). Differentiation of *Eperythrozoon* versus *Haemobartonella* is some arbitrary, but *Haemobartonella* does not usually occur as a ring form and it is not present free in the plasma, while *Epeyrethrozoon* does occur as a ring form and it can be found free in the plasma (Weiss and Moulder, 1984). To date, three morphologically and immunologically different hemoplasma species have been identified in cattle: *E. wenyonii* (Adler and Ellenbogen, 1934), *E. teganodes* (Hoyte, 1962), and *E. tuomii* (Uilenberg, 2009). Recently, cattle infected with a novel virulent hemoplasma species, named *Mycoplasma haemobos* were reported in Japan and northern Germany (Hoelzlea et al., 2010; Tagawa et al., 2010). Differentiation of the species of *Eperythrozoon* is difficult and is based on the species of animal in which the organisms is found as well as its morphological characteristics. The size and shape of the organisms in the cattle are similar to *Eperythrozoon wenyonii (M wenyonii)*.

Cattle that are commonly infected with *M. wenyonii* may show signs of depression, fever and anemia. This pathogen was also considered to be the potential cause of hind limb edema and udder swelling (Smith et al., 1990; McAuliffe et al., 2006). However, Tagawa et al. (2010) demonstrated that *M. wenyonii* infection did not produce obvious signs of anemia and icterus. The occurrence of these clinical signs is likely to be associated with the pathogen load. Some of the *M. wenyonii* infections in the present study also did not show obvious signs. However, most of the ill cows had received extensive antibiotic treatment (oxytetracycline) before the sample collection, which might have led to the underestimation of *M. wenyonii* prevalent in ill cows. It also hampered the clinical evaluation of the disease of *M. wenyonii* infection in the present study. This study showed that cows at the age of 2 to 4 years were most vulnerable to *M. wenyonii* infection. The reason for susceptibility of young animals to *M. wenyonii* infection was unclear. One calf of the animals in the present study appeared to be infected with eperythrozoonosis, and it can be suggested that the transmission of organism was through uterus.

In conclusion, the information about the occurrence of these two pathogens may provide basis for further research in cattle diseases in Iraqi environmental conditions.

Acknowledgement

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