



## The Exophthalmos of Eyes as An Unusual and Unregistered Sign of *Mycoplasma wenyonii* Infection in Newborn Calves in Basrah, Iraq (A case study)



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**E**XOPHTHALMOS with bulging and edema of both eyes was detected as an unusual and unregistered sign of *Mycoplasma wenyonii* infection in cross-bred newborn calves in Basrah, Iraq. Three male cross-bred calves 4- 10 days old, show different clinical signs, A blood sample of 2.5 mL was drawn from the jugular vein of each newborn calf in. The blood was mixed with EDTA to obtain the total erythrocyte count, level of Hemoglobin, packed cell volume, total leukocyte count using automatic fully digital cell counter. The stained blood smears were used for detection of the microorganism, Moreover, the microorganism was confirmed by the polymerase chain reaction technique. Exophthalmos in diseased newborn calves can affect both eyes (bilateral) or only one eye (unilateral). Furthermore, calves also showed increased abdominal respiration with panting, recumbency, inability to stand, pale mucus membranes, small hemorrhagic patches on the skin, and enlargement of superficial lymph nodes. Vital signs were also increased (body temperature, respiratory and heart rates). It has been concluded that the exophthalmos of eyes in infected animals with *Mycoplasma wenyonii* should be added as a new clinical sign through which the disease can be suspected and inferred.

**Keywords:** *Mycoplasma wenyonii*, Exophthalmos, Cross-breed calves, Basrah, Iraq.

### Introduction

*Mycoplasma wenyonii* (previously known as *Eperythrozoon wenyonii*) is a rickettsial organism belonging to the family Bartonellaceae [1]. It is also referred to as Hemomycoplas or Hemoplasma [2]. The organisms are described as prokaryotic, existing on the surface of the erythrocyte cells. Giemsa-stained smears are showed pleomorphic coccoid, rod, and/or ring-shaped structures present in isolation or chains on the red cell and exhibiting gram-negative like staining due to the absence of a cell wall, but there has been no evidence of any of the organisms being cultured outside their hosts [3]. The infections are worldwide spread which includes different parts of Africa, Southern Europe, Central Asia, and

elsewhere in the world in cattle and other species of domesticated animals [1,4]. It has also been indicated in most parts of Iraq [5].

In newborn calves, the disease has been registered mostly as an intrauterine type of infection as it has been strongly suggested that the infection appears to have taken place at the prenatal stage. Similarly, different calves born from different cattle breeds have exhibited *M. wenyonii* infection on days 1-17 postpartum [6].

Besides the various manifestations mentioned earlier, diseased calves are unable to suck their dam, have multiple petechial hemorrhages indicated on the sclera and conjunctival membranes, pale and/or icteric mucus

membranes. Furthermore, different hemorrhagic patches might be seen in different parts of the skin, enlargement of superficial lymph nodes, and hemoglobinuria, which might occur rarely [5].

Exophthalmos or bulging eye, refers to proptosis of eyeballs which might affect one or both eyes [7,8]. The main objective of this case study is to highlight exophthalmos as an unusual and non-registered clinical sign exhibited by diseased newborn calves with *M.wenyonii* infection.

### **Materials and Methods**

Three male cross-bred calves four to ten days old, were taken to the Consultant Veterinary Hospital, College of Veterinary Medicine, University of Basrah, Iraq. The calves showed different clinical signs, such as obvious bulging of both eyes, panting, and pale mucous membranes, which are the typical most prominent clinical signs.

A blood sample of 2.5 mL was drawn from the jugular vein of each newborn calf in a vein puncture procedure. The blood was mixed with EDTA to obtain the total erythrocyte count (RBC), level of hemoglobin (HB), packed cell volume (PCV), total leukocyte count (TLC) was obtained an automatic fully digital cell counter (Beckman, USA). The Giemsa stained blood smears were used for the detection of the microorganism [9].

Concerning the polymerase chain reaction (PCR) technique, the blood sample was kept in ethylenediaminetetraacetic acid (EDTA) tube. DNA was obtained from 200 $\mu$ l of blood with the commercial kit (Bioingentech Genomic DNA Purification Kit, BioInGentch, Chile) and following the instructions of the manufacturer' (*M.wenyonii* Detection Kit, BioInGentch, Chile) specifically to amplify a region (180-basepair) of 16S RNA gene (from *M. wenyonii*). The kit contained the ingredients which were sufficient to amplify genomic DNA, namely, *Mycoplasma wenyonii* Pre-mixture, *Mycoplasma wenyonii* PCR Positive control, PCR negative control, PCR internal control, DNase/Ranse free water, mineral oil solution, and brig<sup>TM</sup> molecular weight marker. The preparation of the *M. wenyonii* polymerase chain reaction mixture was done according to the instructions on the same detection kit. The amplification process included a single cycle of Initial Denaturation at 94 $^{\circ}$ C for 2 minutes, 30 cycles of denaturation at 94 $^{\circ}$ C, annealing at 57 $^{\circ}$ C,

and extension at 72 $^{\circ}$ C for 30 seconds, the final extension of one cycle at 72 $^{\circ}$ C for 5 minutes employing an automatic cycler. The output of the amplification was electrophoresed on 1.5% agarose gels. The staining of gels with Ethidium bromide was done, followed by examination with UV illumination. Bands were detected and visible expected (180- bp).

### **Results and Discussion**

Exophthalmos in diseased newborn calves can affect both eyes (bilateral) or only one eye (unilateral) (Fig. 1 and 2). Ophthalmic examination of both eyes for symmetry failed to indicate any kind of malformation or irregular vision, except for forwarding displacement of eyeballs. However, no evidence was detected to suggest that the animal had been physically traumatized. Nonetheless, sub-conjunctival ophthalmic edema of the eyelids and the area surrounding the orbits was noted with little mucopurulent discharges from the eyes. Moreover, there were no indications of any retraction of the eyelids. Calves also showed increased abdominal respiration with panting, recumbency, and inability to stand, pale mucus membranes, small hemorrhagic patches on the skin, and enlargement of superficial lymph nodes. On the other hand, vital signs were also increased (body temperature, respiratory and heart rates) (Table 1).

Results of hematological changes of diseased calves indicated anemia with high parasitemia of diseased animals (Table 2). The organism *M. wenyonii* appears in Giemsa-stained blood smears as small coccoid or rod structures with different shapes, and it could be arranged in a singular or a chains pattern on the erythrocyte cell membranes of infected RBC of diseased calves (Fig. 3). Moreover, infection was confirmed with a PCR test (Fig. 4).

Exophthalmos (Reduced globe retropulsion) is a protrusion and bulging of the globe which might result from the presence of a space-occupying lesion in the orbit (infection, inflammation, salivary gland enlargement, neoplasia, zygomatic vascular malformation, or myositis) [7,10].

It has been documented that, exophthalmos is a common clinical sign of some diseases, which may be accompanied sometimes by deviation of the globe, third eyelid prolapse, a widened palpebral fissure, conjunctival swelling/edema/chemosis, epiphora, and a decreased ability to blink and subsequent exposure keratopathy (also known



**Fig. 1. Bulging of both eyes**



**Fig. 2. The bulged eye is edematous, hemorrhagic, and protrudes outside the eyeball**

**TABLE 1. Clinical signs of diseased calves with *M. wenyonii* infection**

Clinical signs	Diseased calves n=3	%
Exomphalos with bulging and edema of both eyes (bilateral)	3	100
Increase abdominal respiration with panting	3	100
<i>Recumbency and unable to stand</i>	3	100
Pale mucus membranes	2	66.3
Small hemorrhagic patches on the skin	2	66.6
Enlargement of superficial lymph nodes	3	100
Increased vital signs (body temperature, respiratory, and heart rates)	3	100

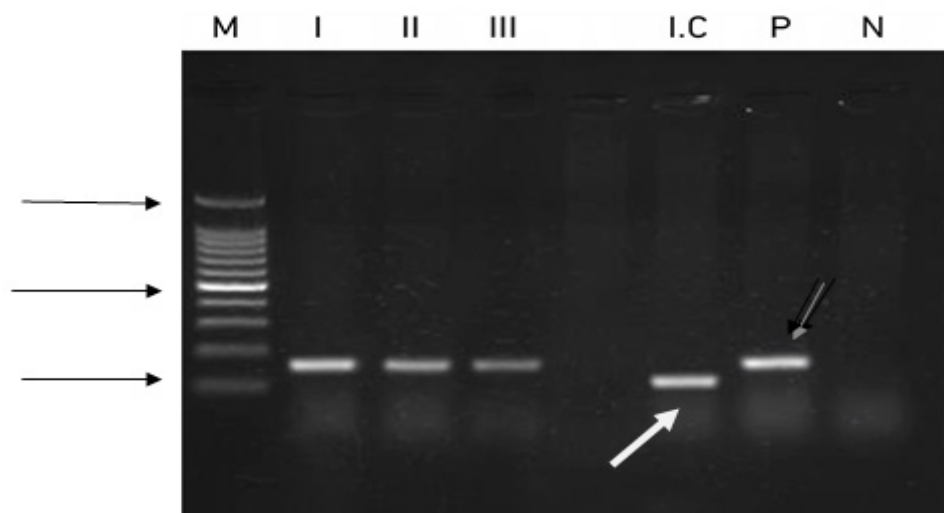
**TABLE 2. Hematological parameters of diseased calves**

Parameters	Diseased calves n=3	Reference range *
RBC x 10 <sup>6</sup>	4.85	5-10
Hb mg/dl	5.5	8-15
PCV %	22	24-46
TLC x 10 <sup>3</sup>	15.3	4-12
Parasitemia	< 200%	Zero

\*( Constable et al., 2017) [1]. Numbers are represent M ±SE of mean



**Fig. 3.** *Mycoplasma wenyonii* on Giemsa stain blood smear of diseased calves x100



**Fig. 4.** The Amplification of *M. wenyonii* genomic DNA by PCR

**M:** The Brig<sup>TM</sup> Molecular Weight Marker

**I,II and III :** positive samples of *Mycoplasma wenyonii* (180-bp)

**I.C.:** the Internal control(140-bp)

**P:** the control positive (180-bp)

**N:** the control negative



as exposure keratitis). Moreover, Retrobulbar tumors of dogs and cats had exophthalmos, with conjunctival hyperemia, fundic abnormalities [11]. On the other hand, exophthalmos is described as “the anterior displacement of a normal-sized globe within the orbit and most easily identified by viewing the head from the front and comparing the angle of eyelashes, the relative prominence of the globe, and the size of the palpebral fissure.” On the contrary, in other domestic species, there is a minimal additional benefit by observing from above the skull [12].

An ophthalmometer is defined as “an apparatus employed for measuring the adjacent globe prominence within the orbit, but it is seldom of clinical relation.” However, palpation of the globe concerning the orbital rim could produce the same outcomes as an ophthalmometer [13].

It was reported that noticeable exophthalmos can be normal in some dairy cattle breeds. Nevertheless, numerous instances of acquired exophthalmos have been observed in young cattle in respect of vascular malformations or a syndrome known as cavernous sinus syndrome and assessment should be done of this syndrome in any case of exophthalmos together with a comprehensive cranial nerve examination. In most cases, acquired exophthalmos in adult cattle is characteristically related to either orbital inflammations or orbit neoplasia. Usually, a standard history and clinical presentation can clarify the differences between these two wide classes. The most significant causal factors of inflammation in exophthalmos (cellulitis, foreign body, sinusitis, myositis, and so on) are either peracute or acute at the initial stage, with the patient feeling distinct pain as the affected eye is retropulsion into the orbit and when the jaw is opened [14].

It is important to note that, exophthalmos is not glaucoma or traumatic proptosis. In ruminants, the protruding of any eye from its orbit due to trauma has a serious prognosis and it might be related to other worrying craniofacial injuries and the possibility of neurologic signs [15].

Some scientific opinions support the following proposal. A proposed eye may need enucleation if ruptured or if there is extensive extraocular muscle avulsion. Nevertheless, and no matter the reason, any exposed eye must be immediately and regularly cleaned and moistened. In an emergency, the eye and periocular tissues can be treated by moistening with sterile saline, sterile eyewash, or

any non-peroxide contact lens solutions before the availability of medical attention [7].

### Conclusions

It has been concluded that unilateral or bilateral exophthalmos of eyes must be considered as an important clinical sign of *M. wenyonii* infection in diseased newborn calves.

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### Conflict of interest

There is no conflict of interest in this scientific article

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## جحوظ العين كعلامة غير عادية وغير مسجلة لعدوى الميكوبلازما وينيوني في عجول حديثي الولادة في البصرة ، العراق

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تم الكشف عن جحوظ مع انتفاخ ووذمة في كلتا العينين كعلامة غير عادية وغير مسجلة لعدوى الميكوبلازما وينيوني في العجول الهجين حديثي الولادة في البصرة ، العراق. اظهرت ثلاثة عجول هجين من الذكور بعمر ٤-١٠ أيام ، علامات سريرية مختلفة خاصة بالمرض ، تم سحب عينة دم بمقدار ٢,٥ ملتر من الوريد الوداجي لكل حيوان. ومزجها مع EDTA للحصول على إجمالي عدد كريات الدم الحمراء ، ومستوى الهيموجلوبين ، وحجم خلايا الدم المرصوفة ، وتم الحصول على إجمالي عدد كريات الدم البيضاء أيضاً باستخدام جهاز عداد خلايا الدم الرقمي . تم استخدام مسحات الدم المصبوغة للكشف عن الكائنات الحية الدقيقة المسببة للمرض ، كما تم تأكيد التشخيص النهائي للكائن الدقيق عن طريق اختبار تفاعل البلمرة المتسلسل. قد يؤثر جحوظ العينين في العجول حديثة الولادة المريضة على كلتا العينين (ثنائية) أو عين واحدة فقط (من جانب واحد). علاوة على ذلك ، أظهرت العجول أيضاً زيادة في سرعة التنفس البطني مع اللهاث ، والاستلقاء ، وعدم القدرة على الوقوف ، وشحوب الأغشية المخاطية ، وبقع نزفية صغيرة على الجلد ، وتضخم في الغدد الليمفاوية السطحي من ناحية أخرى ، تم أيضاً حدوث زيادة في المؤشرات الحيوية (درجة حرارة الجسم ، ومعدل التنفس ، ومعدل ضربات القلب)