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 mucous...
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 eyes, 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 of diseased animals (Table 2). The organism M. wenyonii appears in 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 200µl of blood with the commercial kit (Bioingentech Genomic DNA Purification Kit, BioInGentch, Chile) and following the instructions of the manufacturer’s (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™ 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°C for 2 minutes, 30 cycles of denaturation at 94°C, annealing at 57°C, and extension at 72°C for 30 seconds, the final extension of one cycle at 72°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
THE EXOPHTHALMOS OF EYES AS AN UNUSUAL AND UNREGISTERED SIGN OF …

**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|
| *Recumbency and unable to stand*                                               | 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|

*Egypt. J. Vet. Sci. Vol. 52, No.3 (2021)*
TABLE 2. Hematological parameters of diseased calves

| Parameters          | Diseased calves n=3 | Reference range * |
|---------------------|----------------------|-------------------|
| RBC x 10⁶           | 4.85                 | 5-10              |
| Hb mg/dl            | 5.5                  | 8-15              |
| PCV %               | 22                   | 24-46             |
| TLC x 10³           | 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 BrigTM 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

*Egypt. J. Vet. Sci. Vol. 52, No.3 (2021)*
THE EXOPHTHALMOS OF EYES AS AN UNUSUAL AND UNREGISTERED SIGN OF…

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.

Acknowledgments

The researchers offer their thanks and appreciation to the College of Veterinary Medicine, University of Basrah. Iraq.

Funding statement

The College of Veterinary Medicine / University of Basrah, Iraq is the financial support for this research

Conflict of interest

There is no conflict of interest in this scientific article

References

1. Constable, P.D., Hinchcliff, K.W, Done, S.H. and Grunberg, W. Veterinary Medicine. A textbook of the diseases of cattle, sheep, goats and horses. 11th ed, WB Saunders Co. (2017). ISBN-13: 978-0702052460.
2. Messick, J.B. Hemotrophic mycoplasmas (hemoplasmas): A review and new insights into pathogenic potential. Vet. Clin. Pathol., 33, 2-13 (2004). doi: 10.1111/j.1939-165x.2004.tb00342.x.
3. Adresi, Y and Saki, C.E. Clinical Eperythrozoon wenyonii (Adler and Ellenbogen, 1934) and Haemobartonella bovis (Donatin and Lestoquard,1934) Infection in A Cattle. F.Ü. Sağ. Bil. Vet. Derg., 23 (2), 117 – 118(2009).
4. Fard, R.M.N., Milad, V.M.S. and Mohammadkhan, F. Haemotropic mycoplasmas (haemoplasmas): a review. Int. J. Adv. Biol. Biom. Res., 2(5),1484-1503 (2014). http://www.ijabbr.com.
5. Al-Badrani, B.A. and Rhaymah, M.S.H. A clinical and diagnostic study of Mycoplasma wenyonii and Haemobartonella bovis infections in cattle of Mosul City, Iraq. Res. Opin. Anim. Vet. Sci., 2(1), 27-30 (2012). www.roavs.com
6. Girotto-Soares, A., Soares, J.F., Bogado, A.L.G., de Macedo, C.A.B, Sandeski, L.M.., Garcia, J.L. and Vidotto, O. ‘Candidatus Mycoplasma
haemobos’: Transplacental transmission in dairy cows (Bos taurus). *Vet. Microbiol.*, 15, (195),22-24 (2016). doi: 10.1016/j.vetmic.2016.08.020.

7. Gelatt, K. “Treatment of Orbital Diseases in Small Animals”. *Proceedings of the 27th World Congress of the World Small Animal Veterinary Association*. (2002). https://www.vin.com/apputil/content/defaultadv1.aspx?meta=&pId=11147

8. Sudan, V., Sharma, R.L., Borah, M.K., and Mishra, R. Acute bilateral proptosis in a cross bred calf naturally infected with Theileria annulata. *J. Parasit. Dis.*, 36(2), 215–219 (2012). doi: 10.1007/s12639-012-0111-6.

9. Soulsby, E.J.L. *Helminths, arthropods and protozoa of domesticated animals*, ELBS-7. London: Bailliere Tindal. (2005). PMC1697579

10. Mallick, K.P. Ocular signs of theileriosis in cross bred calves. *Agric. Pract.*, 14, 26–29 (1993). doi: 10.1007/s12639-013-0364-8.

11. Townsend, W.M., Renninger, M., Stiles, J., Townsend, J.R., and Krohne, S.G. Dexamethasone-induced exophthalmos in a group of Holstein calves. *Vet. Ophthalmol.*, 6(3),265-268 (2003). doi: 10.1046/j.1463-5224.2003.00287.x.

12. Mömke, S and Distl, O. Bilateral convergent strabismus with exophthalmus (BCSE) in cattle: an overview of clinical signs and genetic traits. *Vet. J.*, 173 (2), 272-277 (2007). doi: 10.1016/j.tvjl.2005.11.020.

13. Malatestinic, A. Bilateral exophthalmos in a Holstein cow with lymphosarcoma. *Can. Vet. J.*, 44(8):664-666 (2003).

14. Sharma, S.K and Tarunpreet, J.M. Acute Bilateral exophthalmos in crossbred calves affected with bovine tropical theileriosis. Indian *Vet. J.*, 94(6),46-48. (2017). https://www.researchgate.net/publication/318264936.

15. Manson, C.S., Buxton, D. and Gartsidej, F. Congenital ocular abnormalities in calves associated with maternal hypovitaminosis A. *Vet. Rec.*, 153, 213:214 (2003). doi: 10.1136/ vr.153.7.213.
جحوظ العين كعلامة غير عادية وغير مسجلة لعدوى الميكوبلازما وينيوني في عجول حديثي الولادة في البصرة، العراق

كمال الدين مهلهل السعد , علي جراد واسماء سالم طارق
فرع الطب الباطني والوقائي - جامعة البصرة – العراق.

تم الكشف عن جحوظ مع انتفاخ ووذمة في كلتا العينين كعلامة غير عادية وغير مسجلة لعدوى الميكوبلازما وينيوني في العجول الجين صناعي الولادة في البصرة ، العراق. أظهرت ثلاثة عجول مريضة من الذكور بعمر 2.5 ملايين يوم ذو الوريد الوداجي لكل حيوان، ومتزوجاً مع وحدهما الداخليين. وتم الحصول على إجمالي عدد كريات الدم الحمراء، ومستوى الهيموجلوبين، وحجم خلايا الدم المرصودة، وتم الحصول على إجمالي عدد كريات الدم البيضاء أيضاً باستخدام جهاز عداد خلايا الدم الرقمي. ثم استخدمت بطانيات الدم المصبوغة للكشف عن الكائنات الحية الدقيقة المسببة للمرض، كما تم تأكيد التشخيص النهائي للكائن الدقيق عن طريق اختبار فاصل الريجوستول. قد يتأثر جحوظ العين في العجول حديثة الولادة المريضة على كثافة العينين (ثنائية) أو عين واحدة فقط (من جانب واحد). علاوة على ذلك، أظهرت العجول أيضًا زيادة في سرعة التنفس البطني مع اللهاث، والاستلقاء، وعدم القدرة على الوقوف، وشحوب الأغشية المخاطية، ويفت نزيف صغيراً على الجلد، ويتضح في الغدد الليمفاوية السطحية من ناحية أخرى، تم أيضًا حدوث زيادة في المؤشرات الحيوية (درجة حرارة الجسم، ومعدل التنفس، ومعدل ضربات القلب).