

EPERYTHROZONOSIS (*Mycoplasma* sp.) IN MALAYSIAN PANGOLIN

JAMNAH O.¹, FAIZAL H.², CHANDRAWATHANI P.^{1*}, PREMAALATHA B.¹, ERWANAS A.I.¹, LILY ROZITA¹ AND RAMLAN M.¹

¹ Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak Darul Ridzuan, Malaysia.

² Faculty of Science and Biotechnology, University Selangor, Bestari Jaya, Selangor Darul Ehsan, Malaysia

* Corresponding author: chandra1959@gmail.com

ABSTRACT. The Malaysian Pangolin (*Manis javanica*) is an endangered species, but is widely hunted for its medicinal value in body parts. A total of sixteen pangolins were screened for blood protozoa and six pangolins were confirmed to be positive for eperythrozoonosis infection based on morphology from blood smears stained with 8% Giemsa. The causative organism, *Eperythrozoon* sp., with a size of 0.3 µm were observed under a compound microscope at 100× magnification on the surface of red blood cells as blue coloured dots. This is the first report of Eperythrozoon infection from pangolins in Malaysia. Further identification using polymerase chain reaction (PCR) is necessary to confirm *E. ovis* or *wenyonii*, which is uncultivable in artificial media.

Keywords: pangolin, eperythrozoonosis, *Mycoplasma* sp.

INTRODUCTION

Eperythrozoonosis is a zoonotic disease with worldwide distribution found in wild and domesticated animals. It is caused by microscopic blood parasite belonging to the genus *Eperythrozoon*

and first recognised in 1934. It usually infects goat or sheep (*Eperythrozoon ovis*), and pigs (*Eperythrozoon suis*). Several references now, refer to Eperythrozoon as *Mycoplasma* (Neimark *et al.*, 2001). This parasite infects the red blood cells of the host animal and infected cells are removed from the bloodstream by the spleen. With heavy infections, the host can become severely anaemic and this is the main effect of the disease. (Urquhart *et al.*, 1996). In sheep, once infected, may stay infected for life. This feature of the disease may affect the long term production of a flock. However, some sheep rapidly clear the parasite from their bloodstream but the infection can flare up again when they are stressed. Hence, this infection is associated with stress situations in animals such as poor feeds, transportation or overcrowding.

The role of *Mycoplasma wenyonii* (*M. wenyonii*) in disease is still controversial, infections caused by *M. wenyonii* have been reported to result in parasitaemia and anaemia, with infected cattle rarely dying but on occasions showing acute clinical signs (Smith *et al.*, 1990). In addition, dairy cows with swollen teats, oedema of the

distal portion of the hind limbs, transient fever, prefemoral lymphadenopathy, rough coat, dramatically decreased milk production and subsequent infertility and weight loss have been reported (Smith *et al.*, 1990). Recently, other as yet unclassified haemotrophic Mycoplasmas have been discovered in cattle in Switzerland with severe anaemia that were co-infected with *M. wenyonii* and *Anaplasma marginale* (Hofmann-Lehmann *et al.*, 2004).

However, since 2001, *Eperythrozoon* sp. are classified as *Mycoplasma* sp. (Neimark *et al.*, 2001) as there is an 80% similarity based on 16S rRNA gene sequences. However, it has been ascertained that *E. wenyonii* belongs to a group of Mycoplasmas that attach to and grow on the surface of red blood cells (Neimark *et al.*, 2001) and not able to be cultured artificially (Uilenberg *et al.*, 2006). Although the mode of transmission for this infection is unknown, there is evidence that flies, lice and mosquitoes may serve as mechanical vectors and oral transmission also seems likely (Prullage *et al.*, 1993; Smith *et al.*, 1990).

In general, Mycoplasmas are gram-negative bacteria, lack a cell wall and is only 0.2 μm to 0.3 μm in size. It is an aerobic and facultative anaerobic organism. Blood sucking insects (mosquitoes, flies) can be vectors for Mycoplasmas (Nicholas *et al.*, 2008). Mycoplasmas also infect the surface of red blood cells of the animals, and excessive infection can lead to illthrift, anaemia, jaundice, dark red urine and can be fatal. During an outbreak, which can

last for 14 to 28 days, it can be observed that 'Howell Jolly Bodies' are found in red blood cells. These are remnants of the nucleus in red blood cells which are still immature (Neimark *et al.*, 2001).

Pangolins, also referred to as a scaly anteater or trenggiling, is a mammal of the order Pholidota, and the genus *Manis*, has large keratin scales covering its skin, and is the only known mammal with this adaptation (Briggs, 2006). It is globally classified as an endangered species, commonly hunted worldwide and smuggled mostly to China for preparation of traditional medicine. Pangolins are listed as endangered from the International Union for Conservation of Nature (IUCN) Red List of Threatened species, due to high levels of hunting primarily for its meat and scales believed to cure cancer and asthma (Wassener, 2013). Every year, large numbers of pangolins are recovered from smugglers in Asia and Africa. In Malaysia too, live pangolins are sometimes recovered and sent to the Wildlife Department for health screening before being released into their natural habitat or rehabilitated in zoos and national parks. Under such stressful conditions, when caught, these pangolins may contract infections such as eperythrozoonosis. The Veterinary Research Institute, received blood samples in EDTA from sixteen pangolins under the care of the Wildlife Department. This paper describes the observations from blood smears of these pangolins.

MATERIAL AND METHOD

In June 2013, sixteen whole blood samples from pangolins were submitted to the Parasitology and Haematology Laboratory in Veterinary Research Institute (VRI), Ipoh, Perak. Thin blood smears were prepared, fixed with methanol and stained with 8% Giemsa in Phosphate Buffer Saline (7.2 pH) for 45 minutes. The stained smears were view with a compound microscope under 100× objective lens magnification (Urquhart *et al.*, 1996).

Eperythrozoon (*Mycoplasma*) sp. may be identified as blue dots on the surface of the red blood cells, approximately 0.2 µm to 0.3 µm in size. *E. wenyonii* and *E. ovis* are the only two species which cannot be cultured (Barrow & Feltham, 1993; Urquhart *et al.*, 1996). Other *Mycoplasma* species can be cultured in media and identified.

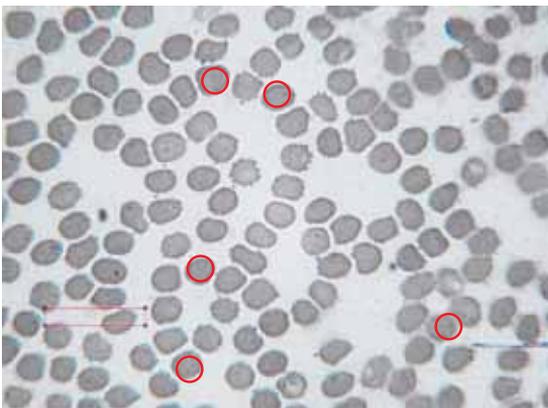


Figure 1. *Eperythrozoon* sp. (*Mycoplasma* sp.)

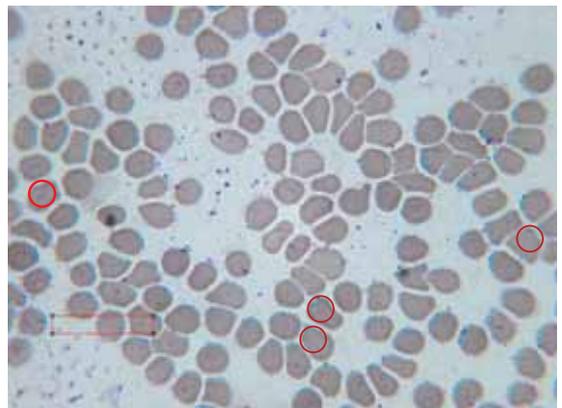


Figure 2. *Eperythrozoon* sp. (*Mycoplasma* sp.)

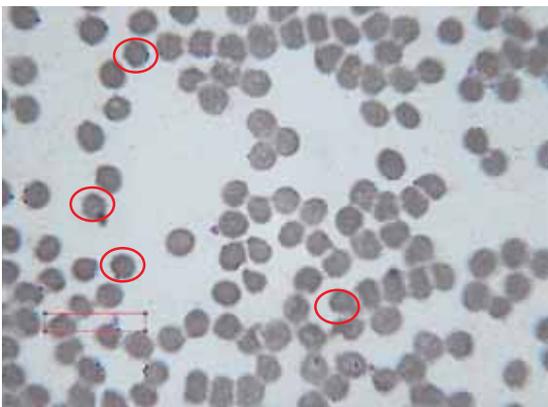


Figure 3. *Eperythrozoon* sp. (*Mycoplasma* sp.)

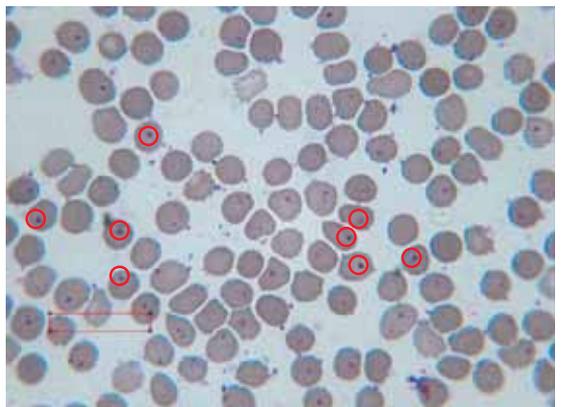


Figure 4. 'Howell Jolly Bodies' in red blood cell

RESULT

Examination of the blood smears indicated six pangolin samples showed positive for eperythrozoonosis (*Mycoplasma* sp.) as observed by the presence of tiny blue stained dots (approximate in 0.3 μ m in size) on the red blood cell surface.

Figures 1, 2, and 3 show the organism observed under 100 \times objective lens magnification. The 'Howell Jolly Bodies' are also observed in red blood cells as shown in Figure 4. This is an atypical observation in blood samples with eperythrozoonosis.

DISCUSSION

Eperythrozoonosis can infect mammals, thus it is found in pangolins. It is a stress related disease, whereby pangolins kept in crowded conditions with poor nutrition can elucidate this infection. In normal circumstances, this organism would be hibernating in the organs and flare up with the onset of stressful conditions. As this is the first report of eperythrozoonosis in pangolins, it is important that other wildlife, especially in captivity, be screened for protozoas and rickettsias.

Wildlife being transported or kept in captivity are at risk of harbouring severe infections and may cause mortality if untreated (Uilenberg, 2006). Drugs like tetracyclines can be used effectively for a range of eperythrozoon infections (*Mycoplasmas*) and in addition to good management, this disease can be averted.

One of the critical features of controlling this disease is the control and eradication of vectors such as insects and mosquitos. Although the pangolin has scales for protection, insects and ants are its main diet. The close proximity to these arthropods may allow the transmission of this infection. The wildlife livestock interface also brings higher risk to domestic mammals such as the ruminants, where eperythrozoon infections can cause severe morbidity and mortality in poorly managed farms in the jungle fringes.

In conclusion, steps to identify the species of eperythrozoon infecting wild mammals and its effects need to be studied. Although there is close resemblance to *Mycoplasma*, the eperythrozoon is still considered a parasite capable of causing much damage to livestock.

REFERENCES

1. Barrow, G. I., & Feltham, R. K. A. (1993). *Cowan And Steel's Manual for the identification of medical bacteria*.
2. Hofmann-Lehmann, R., Meli, M. L., Dreher, U. M., Gönçzi, E., Deplazes, P., Braun, U., Engels, M., *et al.* (2004). Concurrent Infections with Vector-Borne Pathogens Associated with Fatal Hemolytic Anemia in a Cattle Herd in Switzerland. *Clinical microbiology*, 42(8), 3775–3780.
3. Neimark, H., Johansson, K. E., Rikihisa, Y., & Tully, J. G. (2001). Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of "Candidatus *Mycoplasma haemofelis*", "Candidatus *Mycoplasma haemomuris*", "Candidatus *Mycoplasma haemosuis*" and "Candidatus *Mycopl*". *International journal of systematic and evolutionary microbiology*, 51(3), 891–899.
4. Nicholas, R., Ayling, R., & McAuliffe, L. (2008). *Mycoplasma Diseases of Ruminants* (p. 233).

5. Prullage, J. B., Williams, R. E., & Gaafar, S. M. (1993). On the transmissibility of Eperythrozoon suis by Stomoxys calcitrans and Aedes aegypti. *Veterinary parasitology*, 50(1-2), 125–135.
6. Smith, J. A., Thrall, M. A., Smith, J. L., Salman, M. D., Ching, S. V., & Collins, J. K. (1990). Eperythrozoon wenyonii infection in dairy cattle. *Journal of the American Veterinary Medical Association*, 196(8), 1244–1250.
7. Uilenberg, G., Thiaucourt, F., & Jongejan, F. (2006). Mycoplasma and Eperythrozoon (Mycoplasmataceae). Comments on a recent paper. *International journal of systematic and evolutionary microbiology*, 56(1), 13–14. doi:10.1099/ijs.0.63998-0
8. Urquhart, G. M., Armour, J., Dungan, J. L., Dunn, A. M., & Jennings, F. W. (1996). *Veterinary Parasitology* (p. 299).
9. Briggs, Mike, Briggs, Peggy (2006). The Encyclopedia of World Wildlife. Paragon Books. p. 63.
10. Bettina Wassener (March 12, 2013). "No Species Is Safe From Burgeoning Wildlife Trade". The New York Times. Retrieved March 13, 2013.