

Letter to the editor: *Theileria* in loggerhead sea turtles *Caretta caretta*

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The article by Di Santi et al. (2012) titled “Hemoparasitization by *Theileria* in the loggerheads *Caretta caretta* of the Mediterranean Sea” describes various stages of what the authors interpret as *Theileria* organism within erythrocytes and leukocytes of loggerhead sea turtles based on morphologic features of the suspected organism. The identification of an organism that has not been previously documented in reptiles requires its ultrastructural and molecular characterization. For example, Paporini et al. (2012) describe novel *Babesia* and *Theileria* genotypes in erythrocytes of marsupials having 0.8–2 µm in diameter intra-erythrocytic inclusions with clearly visible signet ring or dot-like forms containing dark purple nuclear material consistent with a piroplasm. Molecular analysis was used for genotype confirmation and phylogenetic analysis. For reasons provided below, we do not believe that *Theileria*, or any infectious organism, is present in the images presented in Di Santi et al. (2012).

Figure 1c–f by Di Santi et al. (2012) describes schizonts in lymphocytes. However, we believe these to be granules within granulocytes; Fig. 1g is consistent with features of a heterophil with drying or fixation artifact rather than a *Theileria* schizont. Likewise, inclusions in cells in Fig. 1h–j do not have morphology consistent with schizonts. Figure 1j may be a lysed melanomacrophage, a non-specific finding in blood films of healthy reptiles (Strik et al. 2007).

Figures 2 and 3 by Di Santi et al. (2012) have images of erythrocytes that are reported to contain intraerythrocytic rounded, bacilliform, or crescent-shaped parasites. Inclusions were variably described as dividing merozoites, tiny bacilliform parasites, curved trophozoites, and gametocytes. These colorless, refractile, and variably sized “inclusions” are not consistent with *Theileria* organisms, but rather appear to be drying or fixation artifacts, which can be observed in erythrocytes and leukocytes of any species, especially in thicker areas of a blood film (Fig. 1 in this correspondence below).

Figure 4 a,b by Di Santi et al. are reported to show heterophils phagocytizing parasitized erythrocytes. The morphologic description of erythrophagia in loggerhead turtles has been recently addressed (Stacy and Harvey 2015) in response to another publication by this laboratory (Ruggiero et al. 2013). As stated previously, we disagree that erythrophagia is present in the images in which it is described. The erythrocytes are next to the heterophils but the cell membrane of heterophils does not encircle erythrocytes. The amorphous orange discoloration of the background is consistent with an artifact that may originate from drying, staining (residual stain), or smearing (cytoplasmic contents leaked from damaged heterophils).

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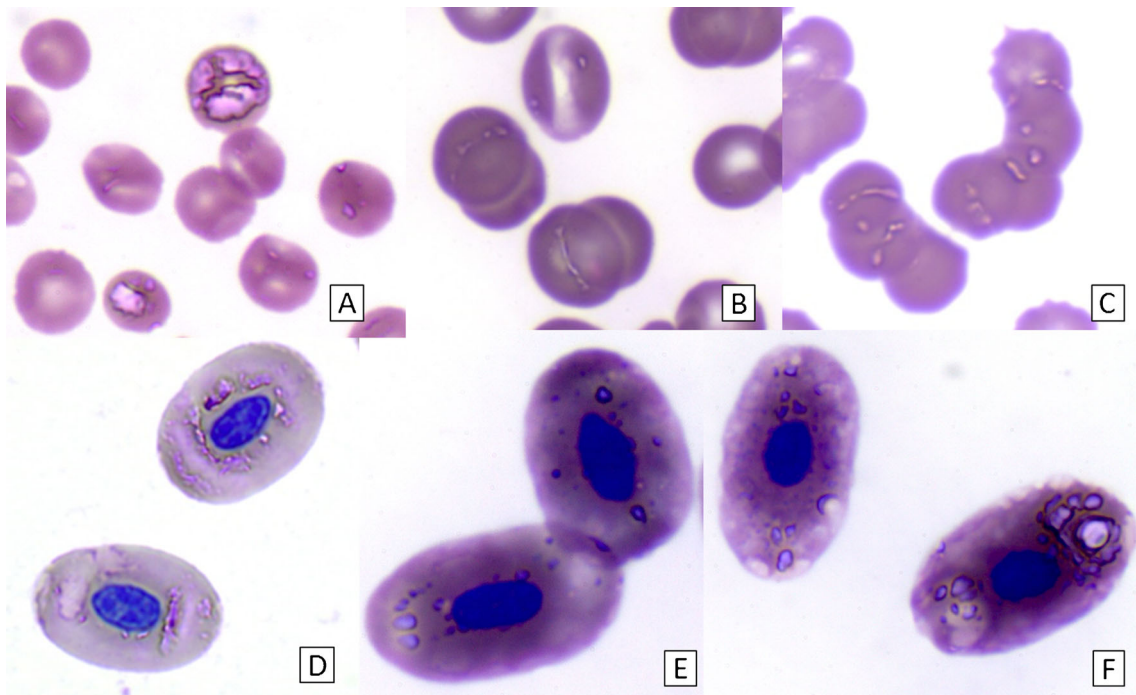


Fig. 1 Colorless, refractile, and variably sized drying or fixation artifacts in erythrocytes of a **a** cow, **b** sea lion, **c** manatee, **d** gopher tortoise, **e** green turtle, **f** Kemp's ridley sea turtle. Wright-Giemsa. $\times 100$

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