J. Parasitol., 99(3), 2013, pp. 546–547

Lice Outbreak in Buffaloes: Evidence of *Anaplasma marginale* Transmission by Sucking Lice *Haematopinus tuberculatus*

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ABSTRACT: Lice infestations are commonly seen in buffaloes, causing damage directly to the animal, i.e., itching, skin lesions, and anemia. In addition, these insects can also be vectors for infectious diseases. The present study describes an outbreak of lice in buffaloes as well as evidence for *Haematopinus tuberculatus* acting as a vector of anaplasmosis. Lice and blood were collected from 4 young buffaloes (2- to 4-mo-old) and a molecular analysis for the presence of *Anaplasma marginale* was conducted. DNA of *A. marginale* was detected in the blood of all 4 animals. Twelve lice were collected and separated in 4 groups, with 3 insects each, to comprise a pool of samples. After DNA extraction and molecular analysis, a positive PCR for *A. marginale* was found in all pooled samples. These results identify sucking lice as potential vectors of anaplasmosis. However, additional studies are necessary to fully evaluate the vector potential of *H. tuberculatus* for *A. maginale* transmission.

The growing interest of farmers to increase herd size suggests that buffaloes (*Bubalus bubalis*) may become a source for producing proteins of animal origin, due mainly to buffaloes' great adaptability to different regions of the world. The Brazilian herd is currently estimated at 1.3 million animals. This population has increased by >1,900% in the last 40 yr, which is strong evidence of their adaptive capacity and of being a huge resource for the country (Barbosa, 2005). Some specific particularities of this species, such as their preference for humid environments may, however, encourage frequent parasitic infestations. These habitats are compatible with the life cycles of different parasites as well as with the maintenance and propagation of disease-transmitting vectors (Cockrill, 1981).

Buffaloes may be hosts for many endoparasites such as nematodes, cestodes, and trematodes as well as numerous ectoparasites such as the flies, lice, and mites which are responsible for transmitting infectious diseases. Included among these diseases are babesiosis and anaplasmosis (Gomes et al., 2008). Parasitic diseases of buffaloes play an important role in tropical countries where climatic conditions are favorable for parasite dispersal. However, there is generally a muted interest for the study of parasitic diseases in buffaloes because they do not cause great economic losses in this host species. More often, buffaloes are considered reservoirs for other animals. For example, scientific information regarding *Anaplasma marginale* infections in buffaloes is very incomplete (Gomes et al., 2008). Recently though, researchers have reported the presence of both *Anaplasma* spp. and *Rickettsia* spp. DNA in sucking lice collected from ruminants and pigs (Hornok et al., 2010).

Lice are species specific and the primary ectoparasite affects buffaloes (Láu, 1999). Although it is known that buffaloes are affected by lice infestations in Brazil, there are few studies on minimizing their prevalence. Louse infestation on buffaloes is known in many countries and is typically caused by a single species, i.e., *Haematopinus tuberculatus*; the species is of considerable size (3.5 mm), visible to the naked eye and, therefore, is easily diagnosed (Jorge and Francisco, 2011). The present study was aimed at describing an outbreak of lice in buffaloes as well as to investigate the possibility of sucking lice acting as a vector for anaplasmosis transmission. A louse outbreak on buffaloes was detected during the winter of 2011 in Taquara, a small town in the south of Brazil. The herd was comprised of 140 animals of all ages. Many of the female buffaloes were domesticated and used for milk production. In lactating females, as well as in the young animals, a large number of lice were detected accompanied by clinical signs such as itching, alopecia, scaling skin, and pale mucous membranes.

Twelve lice identified as *H. tuberculatus* (8 males and 4 females) were collected from 4 young buffaloes aged between 2 (n = 1) and 4 mo (n = 3). Blood was also collected from these animals and stored in tubes with EDTA 10%. In the laboratory, ethanol was added to blood (v/v) and lice samples. These samples were stored at room temperature until analyses were performed.

DNA extraction from whole blood samples was executed as described by Brito et al. (2006). For the extraction of DNA from blood samples, a GFX TM Genomic Blood DNA Purification kit (Amershan Pharmacia Biotech Inc., Little Chalfont, U.K.) was used following the manufacturer's recommendations. DNA samples were subjected to a specific PCR for *A. marginale* as described by Brito et al. (2006).

Twelve lice were divided in 4 groups, containing 3 lice each, to form a pooled sample. The insects were washed with distilled water to remove ethanol. DNA extraction and molecular investigation was performed according to the technique described by Hornok et al. (2010), adapted to search for *Anaplasma* sp. and *Rickettsia* sp. in lice. DNA samples were subjected to PCR specific for *A. marginale* (Master Mix™, Promega©, Madison, Wisconsin) according to Brito et al. (2006).

DNA of *A. marginale* was detected in the whole blood of 4 young buffaloes and in all pooled samples. According to the owner's verbal communication, lice infestation occurs frequently during autumn and winter but not in summer. In recent years this farm did not report mortality or cases of anaplasmosis. This farmer used cypermethrin to control the louse infestation. There is no report of any animal entering or leaving the property in the prior year.

In the present study, buffaloes infested by *H. tuberculatus* exhibited clinical signs with ruffled fur, skin peeling, anemia, and pale mucous membranes, especially on young animals. A literature review reported cases of severe anemia and mortality in young buffaloes infested by *Linognathus vituli* (Otter et al., 2003). Another epizootiological study revealed the prevalence of lice infestation on buffaloes in the Marathwada region of Maharashtra state of India (32.2% for *H. tuberculatus*), with occurrence mainly in the coldest seasons of the year (Sonule et al., 2011), findings similar to the present study. Despite growth of the buffalo population in Brazil, epizootiological studies on louse prevalence and their economic impact have yet to be performed.

Our results suggest the possibility that *H. tuberculatus* may act as a vector for *A. marginale* between buffaloes. The literature reports various forms of transmission of *A. marginale* by tick-borne (*Rhipicephalus* (*Boophilus*) *microplus*) and hematophagous insects (species of *Tabanus*, *Stomoxys*, *Chrysops, Siphona*, and *Psorophora*) in addition to transplacental and iatrogenic transmission (Kessler, 2001). Similar to our study, researchers have found DNA of *Anaplasma platys* in lice collected from dogs (Brown et al., 2005) as well as *Anaplasma* and *Rickettsia* species

DOI: 10.1645/GE-3260.1

collected from ruminants and pigs (Hornok et al., 2010). Experimental studies with lice infected by *Anaplasma* sp. could confirm this transmission route as perhaps the most important source for buffalo, as lice are the main ectoparasites of these animals.

Based on these results, it is concluded that lice infestation on buffalo deserves attention, especially because of problems directly caused for the animals. Moreover, the present study suggests that lice may be a potential vector for anaplasmosis to buffaloes.

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