Haematopinus Infestations and Mycoplasma Infections of Water Buffalo (*Bubalus bubalis*) Herds in National Parks of Hungary

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Abstract: The biology, epidemiology and pathology of sucking louse infestation and *Mycoplasma* infection of water buffalo (*Bubalus bubalis*) herds in Hungarian national parks were studied between 19 December 2011 and 4 May 2012. A total of 333 water buffaloes were examined in buffalo stocks of the Balaton Uplands, Fertő-Hanság and Kiskunság National Parks. The objective was to determine the prevalence and rate of sucking louse infestation and mycoplasma infection among water buffaloes. Always an area of identical size (2 cm^2) was examined on the right or left side of the middle part of the animals' neck. A total of 3106 eggs, 10 nymphs and 105 adults of the sucking louse *Haematopinus tuberculatus* were identified with the help of a Conrad USB microscopic camera and a Wild-Leitz-Leica M420 photomacroscope. The data were evaluated using the Quantitative Parasitology software QP 3.0. The prevalence of mycoplasmas was determined in 20 randomly selected buffaloes of two national parks with the help of sterile nasal and vaginal transport swabs (Sarstedt). All of the 10 nasal swabs collected from buffaloes in the Balaton Uplands National Park contained *Mycoplasma bovirhinis* and three swab samples yielded *M. bovis* as well. *Mycoplasma bovirhinis* was cultured from 8 out of 10 swabs taken from the vagina, and three vaginal samples also yielded *M. bovis*. Similar results were obtained by testing samples collected from buffaloes in the Kiskunság National Park (Mórahalom). All ten nasal swab samples yielded *M. bovirhinis*. From two samples a mixture of *M. bovirhinis* and *M. bovis* was cultured. Nine out of the 10 vaginal swabs yielded *M. bovirhinis* while two showed a combined infection by *M. bovis* and *M. bovirhinis*.

Keywords: *Haematopinus*, sucking louse, *Mycoplasma*, infection, water buffalo, Hungary.

INTRODUCTION

In Hungary, the water buffalo (*Bubalus bubalis*) is kept for breeding and area reconstruction purposes, under extensive rearing conditions. The objective of the work reported here was to survey the sucking louse fauna of Hungarian water buffalo stocks, given that before this survey only very old data [1,2] had been available on its distribution and faunal characteristics, and for example, *Haematopinus tuberculatus* infection of buffaloes had not been described since 1945.

Five louse species are known to be able to infect cattle: three species of the genus *Haematopinus,* along with the species *Linognathus vituli* and *Solenopotes capillatus*. *Haematopinus tuberculatus* is a typical parasite of the domesticated Asian buffalo, which is known to infest cattle as well [3].

Throughout the world, louse infestation of ruminants is an important problem that impairs the growth and performance parameters of both beef and dairy stocks. Louse infestation has been reported to cause blood loss [4], weight reduction of as much as 25–30 kg, diminished milk production and development of stress [3, 5-8]. The blood-sucking lice mentioned above may also be carriers of different pathogens [9].

Host animals infested with blood-sucking lice tend to keep scratching and biting their skin, thus causing surface injuries to themselves. By rubbing their body against different objects, cattle infested with lice may damage fences or trees. Skin that has been irritated by lice has a rough surface with complete loss of hair in some areas, which gives the animals an unthrifty appearance and reduces both the slaughter value and the usability of skin and hide for industrial processing [10].

In a survey conducted in Pakistan [6], the prevalence of lice was significantly ($P<0.05$) higher in cattle than in buffaloes: 144 out of 600 randomly selected cattle (24%) and only 113 out of 600 randomly selected buffaloes (18%), kept under conditions identical to those of the cattle, proved to be infected. The prevalence of louse infestation in cattle has been reported by researchers from different countries [11-13] have reported varying prevalence rates of louse infestation in cattle in association with differences in the ecological, geographic and weather conditions. Animals kept in closed management systems are not exposed to direct sunlight which favours the survival of lice. When cattle are kept in open barns, houses with outdoor runs or in free range management system,

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their skin surface is directly exposed to sunlight and consequently becomes drier, which reduces the survival chances of lice and decreases the intensity of their propagation [6].

Factors determining the severity of infestation with blood-sucking lice include the animals' age and sex and also the season. Numerous studies have been conducted to investigate correlations between the distribution of lice and the age of host animals within herds. Islam [14] reported higher incidence (65.8%) of *H. tuberculatus* infestation among one-year-old buffaloes than in the two-year-old groups. On the other hand, according to Chowdhury [15], the incidence of *H. tuberculatus* was higher in buffaloes belonging to the two- to four-year-old group (60.0%). Rawat *et al.* [16] studied the prevalence of *H. tuberculatus* infestation among buffaloes in Dehradun, India, and found that 60.57% of the 373 buffaloes were infested, with the adult buffaloes showing more severe infestation than the younger animals. In contrast, Mamun *et al.* [17] established that the host animal's age had a significant effect ($P<0.01$) -on the severity of infestation with ectoparasites. According to their findings, buffalo calves were more susceptible (73.7%) than young (70.7%) or adult buffaloes (58.52%), thus indicating that susceptibility to ectoparasites infestation decreases with increasing age. They found blood-sucking lice on a significantly higher percentage ($P<0.01$) of female buffaloes (85.7%) than of male buffaloes (56.7%). *Haematopinus tuberculatus* was the most common louse species in both sexes. There is no definite explanation as to why buffalo cows are more commonly infested; this phenomenon is probably related to the hormonal background of the females. This was demonstrated previously, when Lloyd [18] reported that higher prolactin and progesterone levels may make animals more susceptible to different infections. In addition to these, the production stress, pregnancy and lactation also tend to 'debilitate' female animals.

Occurrences of bovine pediculosis do not show seasonal variation in countries with a warm climate. However, in the temperate zone and in colder regions the most severe infestations occur in late winter and early spring, when the weather is cold and damp and the animals have the thickest coat of hair. The coat of hair serves as a habitat and shelter for lice, and provides optimum conditions for their propagation. During the year, the highest increase in the louse population occurs when cattle or buffaloes are kept indoors for the winter. In late spring, the number of lice suddenly decreases. It than remains at a low average level during the summer months when the hair coat becomes thinner, which provides a less favourable habitat for lice, because the high temperature of the skin surface and direct exposure to sunlight reduces the intensity of their development [10, 19]. Other authors have also observed seasonal occurrences of pediculosis, reporting that the population of sucking lice starts to grow in late winter, reaches its peak in the spring and its nadir in the summer and autumn months [20]. In India, the highest 'louse index' was found in January and the lowest in June (Rawat *et al*., 1992) [16]. According to the results obtained by Hussain *et al.* [6] (2006), the louse population reaches its highest level in February, and the environmental conditions continue to be favorable for survival and propagation of lice in March and April.

Skin temperature has also been correlated with the severity of louse infestation. Islam [14] found large numbers of lice belonging to the species *H. tuberculatus* and *H. bispinosa* during the winter season. Chowdhury [15] observed a high level of *H. tuberculatus* infestation both in summer and in winter. From November 2007 to October 2008, a total of 236 buffaloes were examined in the Kurigram district of Bangladesh and-, nearly 62% of them were found to be infested by one or multiple ectoparasite species. The highest level of infestation was observed in the winter and it,- then decreased in the summer, reaching its lowest level in the rainy seasons [17]. *Haematopinus tuberculatus* is known to be a vector for the species *Trypanosoma evansi* and *Anaplasma marginale*.

Mycoplasma bovis was first isolated from the milk of a mastitic cow in 1961 [21]. After the first report about bovine mastitis due to mycoplasma infection in Europe [22], a series of mastitis cases due to *M. bovis* (earlier known as *M. agalactiae* var. *bovis*) were reported in 1962 in the USA [21] and the pathogen was subsequently described as *M. bovimastitidis* [23], until finally it was elevated to species level as *M. bovis* [24]. Since that time, *M. bovis* infection has frequently been detected in the United States and also in most European countries [25], Canada [26], Latin America [27] and Africa [28]. This infection has been associated not only with mastitis but also with respiratory disease [29, 30], arthritis [31], meningitis [32], otitis [33, 34] and infertility in cattle [35], and it has been recovered from the eyes of bullocks [36] and the reproductive organs of cows [26].

In a survey conducted by the World Organization for Animal Health (Office International des Epizooties,

(-OIE)) in over 48 countries, *M. bovis* was a major component of the calf pneumonia complex, with isolation rates of 23 to 35% [37]. In another study, serological tests for *M. bovis* gave positive results in 76% of beef cattle and in an even higher percentage of veal calves [38]. In buffaloes, mastitis associated with *M. bovis* was described in Egypt [9, 28, 39, 40]; however, buffaloes were found to be less susceptible than cattle [41]. In Kansas (USA), during a high-mortality outbreak caused by *M. bovis* in North American bison (*Bison bison*) a total of 53 out of 194 animals died. There were abscesses in the lung and liver, fibrinosuppurative pleuritis, polyarthritis, and disseminated microabscesses in various organs [42]. A similar disease was reported from North Dakota (USA), where necrotic pharyngitis associated with *M. bovis* infection occurred in American bison [43].

The study presented in this paper was undertaken to obtain data on the biology, epidemiology and pathology of sucking louse infestation and *Mycoplasma* infection of water buffalo herds in national parks of Hungary.

MATERIALS AND METHODS

A total of 333 buffaloes were included in our study, which was conducted between December 19, 2011 and May 4, 2012. The buffaloes were selected from the stocks of three national parks, the Balaton Uplands (Zalavár), Fertő-Hanság (Hany) and Kiskunság (Kígyós, Mórahalom) National Parks.

To our knowledge, in three of the buffalo stocks in the three national parks, insecticidal treatment to prevent or control louse infestation is performed twice a year. In one herd, no ectoparasiticide treatment is used at all. In the herds included in this study, hair samples were taken only from animals infested with blood-sucking lice in which the hair was covered with louse eggs that were easily visible with the naked eye. These hair samples were consistently collected from a 2 cm^2 area on the side of the middle part of the neck.

During the laboratory tests, the numbers of the different developmental stages, i.e. louse eggs (Figure **1**), lice and nymphs, were determined using a Conrad USB microscope.

The sex and species of adult parasite specimens were determined with the aid of a Wild-Leitz-Leica M420 photomacroscope, in accordance with the description of Ferris [44], primarily on the basis of the paratergal plates and the so-called dermal pattern. Since herd-level parasitic infections can be most precisely assessed using quantitative parasitological values, the Quantitative Parasitology software (QP 3.0) developed by Rózsa *et al*. [45] and Reiczigel *et al*. [46] was used during our studies.

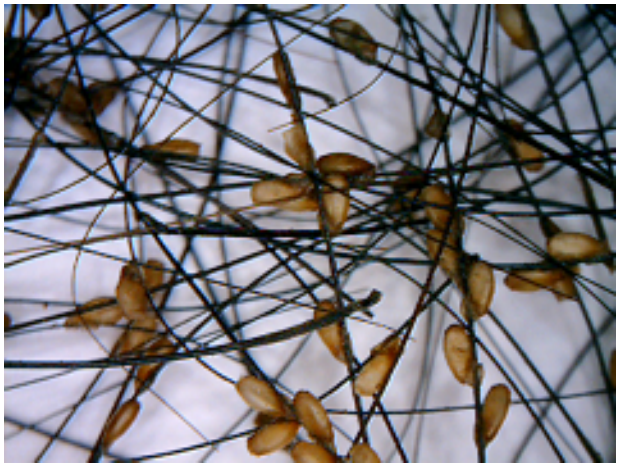

Figure 1: Eggs of *Haematopinus tuberculatus* glued to hairshaft of a buffalo (x16).

For mycoplasma detection, swabs from the nasal cavity and vagina were collected from 20 randomly selected adult female buffaloes in two national parks. The animals were kept free on pastures of the national parks of Balaton Uplands and Mórahalom. Nasal swab samples were cultured by means of inoculation into broth media, then plated on PPLO agar media [47] and incubated at 37 °C for 7 days. Genus determination for the isolates was performed through biochemical characterization of the purified strains. Film and spot formation was tested as described by Fabricant and Freundt [48] and Freundt *et al*. [49]. The isolates were identified by means of growth inhibition, capture ELISA [50] and polymerase chain reaction [51-53].

RESULTS

The evaluations reported here demonstrated that the samples collected from the 333 buffaloes contained only a single blood-sucking louse species, *H. tuberculatus* (Figure **2**).

According to our observations, the female host animals and, among them, the cows showed the most severe louse infestation. Larvae of the parasite accounted for only 0.3% and adult lice represented 3.2% of all the developmental stages recovered, while 96.4% of the stages found were louse eggs. The hair samples from the bulls yielded five adult lice and 83 louse eggs, while those from the cows yielded 78 adult lice, eight larvae and 2348 louse eggs. The hair

samples from the buffalo heifers yielded 12 adult lice, two larvae and 641 louse eggs, while from those of young males seven adult lice and 16 louse eggs were recovered. The hair samples collected from animals without an ear-tag number yielded three adult lice and 18 louse eggs. These buffaloes did not have an identification within SIRIC (the System of Individual Registration and Identification of Cattle). Most of the infested buffalo cows were in the age category of 3 to 11 years.

Figure 2: *Haematopinus tuberculatus* female (x11.7).

During the study, we collected a total of 3106 *H. tuberculatus* eggs, 105 adult stages of *H. tuberculatus* and 10 *H. tuberculatus* nymphs. The quantitative parasitological data on louse infestation in the herds studied are presented in Table **1**.

The buffalo herd at Hany (Fertő-Hanság National Park) showed the most severe infestation with louse eggs, with an egg prevalence of 52.5%. Given that the confidence interval for the entire population examined was best characterized by the confidence interval of prevalence, this value was 0.2102–0.3304 for the entire study population, while for the most severely infested herd kept at Hany, it varied within wider ranges, i.e. between 0.3792 and 0.6651. Both the median intensity (15.0) and its confidence interval (5–23) were the highest in the herd kept at Hany and, exceeded the corresponding values of the entire study population (12; 8–22). The discrepancy index (D) showed a linear correlation with the distribution according to Crofton (1971), i.e. with the fact that only a low number of parasites are usually found on many hosts. In our samples, the lowest D value was found in the most severely infested herd kept at Hany (D = 0.768). Even the D value of the total sample (D = 0.896) was higher than that, thus proving that the distribution of louse eggs was less aggregated in the most severely infested herd. All these statements proved to be true for louse infestation as well [54].

The mycoplasma infection of buffalo herds living in Hungary was studied in two areas, in the Balaton Uplands National Park and at Mórahalom. In both herds, samples were collected from randomly selected female animals. All of the 10 nasal swabs collected from buffaloes in the Balaton Uplands National Park contained *Mycoplasma bovirhinis* and three swab samples yielded *M. bovis* as well. *Mycoplasma bovirhinis* was cultured from 8 out of 10 swabs taken

Table 1: Quantitative Parasitological Data on *Haematopinus tuberculatus* Infestation in the Buffalo Herds Studied

	Eggs	Nymphs	Lice
Total number of buffaloes examined	333	333	333
Number of infested buffaloes	98	8	46
Prevalence (%)	29.4	2.4	13.8
Confidence limits for the prevalence (P = 0.99)	0.2333–0.3628	0.0077–0.055	0.0945–0.1939
Mean intensity	98.0	8.00	46.0
Confidence limits for the mean abundance	(P = 0.95) 24.13–33.50	(P = 0.99) 0.05–0.38	(P = 0.99) 4.14–8.70
Median intensity	98.0	8.0	46.0
Confidence limits for median intensity	(P = 0.99) 98–98	(P = 0.99) 8-8	(P = 0.95) 46-46
Mean abundance of sample	28.84	0.19	6.35
Minimum intensity	1	1	1
Maximum intensity	384	2	8

from the vagina, and three vaginal samples yielded *M. bovis* as well. Similar results were obtained by testing samples collected from buffaloes in the Kiskunság National Park (Mórahalom). All ten nasal swab samples yielded *M. bovirhinis*. From two samples a mixture of *M. bovirhinis* and *M. bovis* (Figure **3**) was cultured. Nine out of the 10 vaginal swabs yielded *M. bovirhinis* while two showed combined infection by *M. bovis* and *M. bovirhinis*.

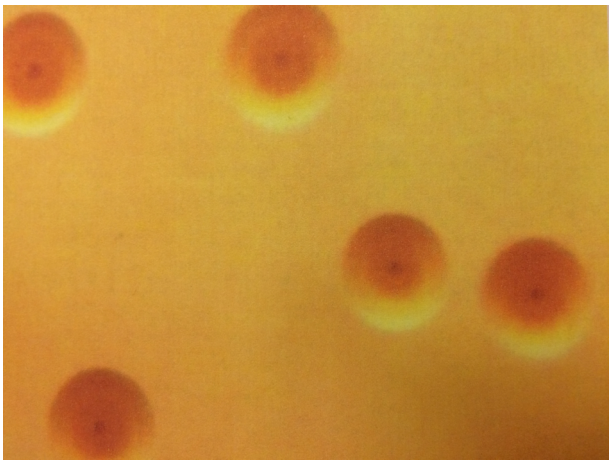

Figure 3: *Mycoplasma bovis* isolated from a buffalo 121x116 mm (96x98DPI).

DISCUSSION

According to Rózsa (oral communication), the species *H. tuberculatus* had not been described in Hungary since 1945, and thus the demonstration of its occurrence on buffaloes can be considered to be a new scientific fact.

Islam [14] reported that the incidence of pediculosis among buffaloes was most common in the one-year-old age category, which is at variance with our findings, since we could not demonstrate louse infestation in buffalo calves. Chowdhury [15] found *H. tuberculatus* in 60% of two to four-year-old buffaloes, which is also inconsistent with the findings of the present study, in which the majority of the louse-infested buffaloes belonged to the age category between 3 and 11 years. Rawat *et al*. [16] reported a 60.57% louse infestation rate among 373 buffaloes examined, with pediculosis more severely affecting the adult animals. This finding is consistent with the results from our analyses. Mamun *et al*. [17] observed that susceptibility to infection decreased with the advancement of age; however, this was not supported by the findings of the present study.

In their above-cited study, Mamun *et al*. [17] found a much higher incidence of *H. tuberculatus* infestation in female than in male buffaloes. This statement is consistent with our own findings, given that in the buffalo herd of Kígyós, the ratio of females to young males was 14:8 among the infested animals. Since young males do not have a cyclical sexual hormone production, conception and milk production did not occur in their case. As a result, we can agree with the statement of Lloyd [18], i.e. that higher prolactin and progesterone levels may make animals individually more susceptible to different infections (and thus also to louse infestation), and that 'production stress', pregnancy and lactation may compromise the resistance of female animals.

M. bovirhinis is not considered to be a pathogenic species. However, *M. bovis* is regarded as an important pathogen and it has been reported to cause significant mortality in young bovine calves in Hungary due to severe pneumonia and arthritis [31]. *M. bovis* has been implicated in reproductive disorders (such as infertility and abortion) as well in severe mastitis in cows [35].

CONCLUSIONS

This is the first published paper on mycoplasma infection among buffaloes in Hungary. It provides important information about the presence of *M. bovis* in buffaloes. Parasitic invasion might play a role as a vector in the intensive spreading of mycoplasma infection among buffaloes. The results obtained in this study draw attention to the importance of preventing the spread of mycoplasma infection and implementing control programs against parasitoses of animals.

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