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Technical note

Estimation of the frequency of lice collected from water buffalo (*Bubalus bubalis*) at production units in the state of Veracruz, Mexico

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Abstract:

This research aimed to determine the occurrence of lice in Buffalo Production Units (BPU) in Veracruz, Mexico. Fifty-seven (57) buffaloes of different sexes and ages were inspected in two BPUs located in the municipalities of Cotaxtla and Medellín de Bravo (BPU1 and BPU2). The lice were collected from different regions of the body and preserved in vials with 70 % ethanol for transfer to the laboratory. Taxonomic identification was performed with

stereoscopic and scanning electron microscopy. The frequency, abundance, and intensity of lice in each BPU, anatomical region, and developmental stage evaluated were calculated. The overall frequency of lice infestation was 78.9 ($CI_{95\%}$: 65.7 - 88.2). The frequency for BPU1 and BPU2 was 60 % and 100 %, respectively. Six hundred ten lice were collected from the two BPU and only the louse species *Haematopinus tuberculatus* was found. The abundance was 610 lice/57 buffaloes in total (10.7) and the intensity was 610 lice/45 infested buffaloes (13.6). The costal region was the place with the highest presence of lice. The nit/egg stage was the most abundant, followed by adult lice and nymphs (78.4 %, 16.1 %, and 5.1 %, respectively). The high frequency of lice in buffaloes at BPU in Veracruz can compromise animal health and meat and milk production in buffalo production systems.

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In Mexico, buffalo production is an alternative for meat and milk production due to their high rusticity, docility, and ability to adapt to various ecosystems. However, buffalo can also contract viral, bacterial, fungal, and parasitic diseases[1,2,3]. Ectoparasites are of medical-veterinary importance because they can be vectors of diseases. In buffaloes, *Haematopinus tuberculatus*, or the sucking louse, is the most common and is present in Europe, Asia, South America, Africa, and Australia[4]. *H. tuberculatus* causes lesions that produce anorexia, cachexia, and reduced production. While the infestation can be asymptomatic, it causes negative effects on milk production and weight gain in growing animals[4]. Besides, its presence has been associated with the transmission of *Anaplasma marginale*, *Trypanosoma vivax*, and *Brucella abortus*[5,6]. In Mexico, there are reports of the presence of *A. marginale* in lice collected from buffalo[7]. Additionally, other lice species, such as *H. quadripertusus*, *H. eurysternus*, and *Linognathus vituli*, have been reported in buffalo from Iraq and Pakistan, respectively[8]. Therefore, this work aims to determine the occurrence of lice in water buffalo in buffalo production units in the state of Veracruz.

The study was conducted from January to June 2023 in Buffalo Production Units (BPU) selected with the non-statistical convenience method in the municipalities of Medellín de Bravo (BPU1, N: 27) and Cotaxtla (BPU2, N: 30) in the state of Veracruz. In both units, the animals were of the Murrah breed, of different sexes and ages.

Firstly, the BPU that had animals infested with lice were identified. All animals from each BPU positive for lice were included in the study. The animals were individually inspected

from head to tail for lice for two or three minutes, without distinction of sex and age; the presence or absence of ectoparasites and the affected body region were recorded by using specific tables. A positive animal was considered to be one that had at least one louse at any stage of development. The collected specimens were deposited in vials containing 70 % ethanol for conservation and transferred to the Parasitology Laboratory of the Diagnostic Unit of the Torreón del Molino Ranch of the Faculty of Veterinary Medicine and Zootechnics of the Veracruzana University.

In the laboratory, each development stage (adults, nymphs, and eggs) was clarified with 10 % KOH for 3 to 5 min, following the methodology described by Shakya[9]; subsequently, they were mounted on slides for examination under the stereo microscope. The species of louse was determined through the taxonomic keys developed by Guzmán-Torres and Cano-Santana[10]. In addition, a scanning electron microscopy (SEM) was performed in the Advanced Molecular Studies Laboratory of the Institute of Ecology of Xalapa, Veracruz, in order to describe the main morphological characteristics of the lice. As a first step, a washing was carried out using an ultrasonic cleaner (Cole-Parmer 8848), followed by chemical fixation/dehydration with ethanol and xylene and drying using a critical point dryer (Quorum model K850). The specimens were then covered with gold using a metal ionizer (Quorum model Q150R S) for analysis in the field emission SEM (FEI Quanta 250 FEG).

To analyze the information, descriptive statistics were obtained by means of online statistical software (VassarStats: Website for Statistical Computation), which was used to estimate the frequency and 95 % confidence intervals. Frequency was obtained from the number of infested buffaloes/number of buffaloes examined × 100. Mean abundance refers to the total number of lice collected/total number of buffaloes tested. Mean intensity refers to the total number of lice collected/number of buffaloes infested.

In total, all 57 buffaloes from the two BPU were included in the study. Table 1 shows the frequency of animals positive for some stage of lice development. The overall frequency of lice infestation was 78.9 % (45/57). In the BPU1 located in Cotaxtla, there was a frequency of 60.0 % (18/30) and the BPU2 located in Medellín de Bravo presented 100 % (27/27) of infested buffaloes. The mean abundance was 610 lice collected from 57 buffaloes (10.7 lice/buffalo) and the mean intensity was 610 lice collected from 45 infested buffaloes (13.6 lice/buffalo).

Table 1: Frequency of lice found in the BPU in Cotaxtla and Medellín de Bravo

BPU	Municipality	n	Infested	Frequency (%)	*CI95%
BPU1	Cotaxtla	30	18	60.0	40.7 - 76.8
BPU2	Medellín de Bravo	27	27	100.0	84.5 - 100
Total		57	45	78.9	65.7 - 88.2

CI= 95 % confidence interval.

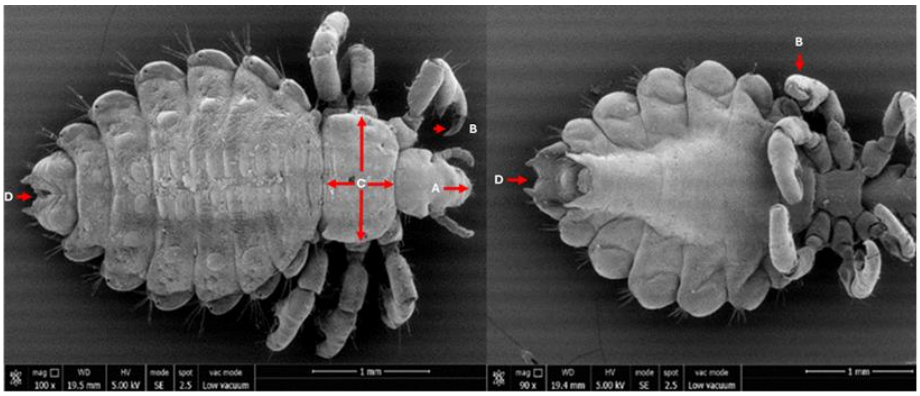
In total, 610 lice were collected, of which 481 were egg/nits and 129 lice (31 nymphs and 98 adult lice). The only species identified was *Haematopinus tuberculatus* (Table 2).

Table 2: Frequency of infestation by nits, nymphs, and adult lice of *H. tuberculatus* collected in the different body areas of *Bubalus bubalis*

Body region	Infested animals (n)	Body areas (n)	Lice (n)	Nits (n)	(%)	Nymphs (n)	(%)	Adults (n)	(%)
Auricular	45	2	5	5	0.8	0	0	0	0
Cervical or neck	45	36	165	128	21.0	9	1.5	28	4.6
Scapular	45	19	73	53	8.7	6	1.0	14	2.2
Costal	45	45	350	295	48.4	12	2.0	43	7.0
Ventral	45	9	11	0	0	3	0.5	8	1.3
Coccygeal	45	5	6	0	0	1	0.1	5	0.8
Total	45		610	481	78.9	31	5.1	98	16.0

Figure 1 shows a female *H. tuberculatus*. This species of louse was characterized by being an apterous insect with a dorsoventrally flattened body. The head was elongated and arrow-shaped and the thoracic area was much wider than it was long (Figure 1A). Likewise, the thoracic area appeared to be fused (Figure 1C)[5,11]. Another characteristic of this louse is that it has the "nail-finger" complex in the tarsal segment, forming a tarsal claw that allows it to attach to the host's hair (Figure 1B). It also had a sclerotized abdominal segment, forming lateral lobes whose function is to provide rigidity to the abdomen. The genitalia had finger-like projections or gonopods that serve to guide, manipulate, and stick the eggs to the host's hairs (Figure 1D)[12].

Figure 1: Scanning electron microscopy, *H. tuberculatus* dorsal (left) and ventral (right) views



A) Arrow-shaped head, B) hook-shaped tibial-tarsal claw, C) thoracic area with fused appearance, D) vulva in the shape of fingers.

In countries where buffalo is a common productive species, lice prevalence of 11.6 % (Brazil)[13] to 100 % (Iraq and India)[8] has been informed. In Mexico, there are recent reports of prevalences of 19 to 100 % of *H. tuberculatus* in adult buffalo and calves in the states of Veracruz and Tabasco, respectively[7,14]. Likewise, only the level of infestation has been reported in buffaloes, with low infestation (1-10 lice) in adult animals and medium infestation (11-25 lice) in calves[14]. On the other hand, in cattle, an average intensity of 8 to 36 lice and an average abundance of 36 to 56 lice of the species *Linognathus vituli* and *H. Eurysternus* have been informed. In this study, similar results were found with respect to intensity; nevertheless, the abundance found was lower than what has been previously described in cattle (10 lice/buffalo)[15].

The frequency of infestation of the animal's body areas was also obtained, and it was found that the costal region was the most frequent, 100 % (45/45), followed by the cervical or neck region with 80 % (36/45) frequency, the scapular region with 42.2 % (19/45) and, to a lesser extent, the ventral, coccygeal, and auricular regions with 20 % (9/45), 11.1 % (5/45), and 4.4 % (2/45), with significant differences among the different regions ($P<0.05$).

Chaudhuri and Kumar[16] indicate that the location of *H. tuberculatus* depends on the stage of development since adult lice are most often found on the back, neck, and hind legs, but not on the head and tail. Nonetheless, other researchers[14] reported the presence of *H. tuberculatus* in the head region in a BPU of Tabasco.

In relation to the frequency of each stage of development of *H. tuberculatus* present by region, Table 2 shows that there were high frequencies of eggs/nits and adult stages in the costal, cervical or neck, and scapular regions.

The results of this research are different from those informed in another study[17], where they mention that the most infested region is the tail (77.7 %) and less frequently, the face (22.2 %); likewise, Ojeda-Robertos *et al*[14] mention that the head, body, and forelimbs were the regions of predilection of the adult phases. The results of this study coincide with those reported by Figueiredo[18], who found the neck area to be the region with the highest frequency.

On the other hand, it is mentioned[18] that adult lice are usually not found where they oviposit their eggs, which coincides with what was found in this work, where nits/eggs were found in the ear and neck regions, whereas the adult stage was found in the ventral and coccygeal regions. It has been reported that the nit/egg phase represents the highest percentage of the development stages present in the animals[11], a result similar to that described in this study, where nits/eggs represented 78.8 % of the stages found in the buffaloes evaluated.

This study demonstrated the presence of animals with *H. tuberculatus* in BPU in the municipalities of Medellín and Cotaxtla, Veracruz. Likewise, there was a higher frequency of lice in the costal and cervical regions.

The information generated in this study highlights the need to control this ectoparasite because it has been associated with the transmission of *Anaplasma marginale* and *Brucella* spp[7,19]. In addition, it is necessary to determine what factors are associated with its presence in order to implement control programs with directed designs.

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Conflict of interest

The authors state that they have no conflict of interest.

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