Detection of deltamethrin, cypermethrin and flumethrin efficacy against buffalo lice—Haematopinus tuberculatus

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Detection of deltamethrin, cypermethrin and flumethrin efficacy against buffalo lice—*Haematopinus tuberculatus*

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Abstract

The cattle and buffalo farm practices have been adopted differently by farmers in India but the infestation of ectoparasites including louse has been advocated in high population of animals across the country. The aim of this study was to identify the louse morphologically and determine the in vitro efficacy of the insecticides deltamethrin, cypermethrin and flumethrin against the buffalo louse, *Haematopinus tuberculatus*. The present research work was conducted using lice collected from organized buffalo dairy farms of Mhow block, Indore district of Madhya Pradesh, India. The adult's lice were collected from heavily infested regions of the body and tail of buffaloes. Some of the collected adult's lice were preserved for morphological identification in 70% alcohol. Briefly, in vitro treated surface bioassay utilizing a cloth rectangle that allows lice to move freely has been used. The concentrations were prepared as 30, 60, 90 and 120 ppm for deltamethrin and flumethrin, whereas for cypermethrin, 100, 200, 300 and 400 ppm concentrations were prepared in distilled water. The 600 µl of each concentration was spread evenly over a cloth rectangle held in the bottom of a Petri plate. Ten adult lice were used for each concentration in triplicate ($n=30$) and the same is maintained for control. The vitality of the louse was assessed at various intervals: 30, 60, 120, 180 and 240 min. The lousicidal efficacy was determined by using in vitro bioassays with deltamethrin, cypermethrin and flumethrin. It is observed that as the concentration of insecticides increases with exposure time, mortality of lice is also increased. The current study reveals that cypermethrin and flumethrin were effective in their recommended doses but in the case of deltamethrin, the lice showed a low level of resistance. Furthermore, this type of study on buffalo louse has not conducted in Mhow region of Madhya Pradesh where heavy infestation of lice occurs on buffalo.

Keywords Cypermethrin · Deltamethrin · Flumethrin · Buffalo · Lice · *Haematopinus tuberculatus*

Introduction

Buffaloes (*Bubalus bubalis*) are important contributor of livestock economies of several countries, including India (Zicarelli 2004). The *Haematopinus tuberculatus* is a sucking louse, that belongs to Phylum Arthropoda, Class Insecta, Order Phthiraptera, Suborder Anoplura, Family Haematopinidae, and is a specific louse of buffaloes. It is an important permanent ectoparasite which attaches the buffaloes (Bastianetto et al. 2002). Various reports have been published on buffalo lice infestation from different continents such as Asia, Africa, Australia, Europe and South America (Meleney and Kim 1974a, b). From Europe alone, numerous reports have been available from Albania, Macedonia, France, England and Italy (Veneziano et al. 2003). Other animals such as cattle, camel and American bison are also susceptible to lice infestation (Chaudhuri and Kumar 1961).

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Lice are wingless insects living as permanent ectoparasites of different mammals. Their mouth parts are mainly adapted for sucking the blood and tissue fluid of their host. The eyes are absent, the head has forward prolongations behind the antennae and the thorax is broadly developed and the parasite having marked paratergal plates on the body and a single row of spines on each abdominal segment (Soulsby 1965). The lice mainly affect their host by making them restless and causing irritation to the animals. Their infestation mainly occurs in winter season as animals try to combat low temperature by coming close to each other and thus succumb to lice infestation. The animals become restless, do not feed properly, damage their hide and hair and occasionally milk production is also reduced (Soulsby 1965). Sucking lice causes various damaging effects through blood loss, anaemia, abortion and in extreme cases death of animals also happened. Since reports sometimes show the presence of two or more species in a herd at the same time, the specific species is less significant than the total number of lice on the animal (Lancaster and Meisch 1986). There may also be a connection between lice and the fungus *Trichophyton verrucosum*, which causes ringworm (Kamyszek and Tratwal 1977).

In the UK, ectoparasites cause 70 to 90% of the damage to hides (costing the bovine leather industry £20 million a year), with lice responsible for 40 to 60% of the damage (British Leather Confederation, personal communication). The economic influence of lice on cattle and buffalo production is inconsiderable, owing to the fact that their impact on leather is not a direct concern for the producer. According to a report, *Bovicola ovis* causes a serious problem for wool sheep breeds, with annual costs and losses to wool growers reported to be over AU$160 million in Australia (McKenzie and Whitten 1984).

Specific treatment regimens for control of lice infestation in animals are atypical throughout the world including India. However, commercial product available for management of other ectoparasites like ticks, mites and flies is used effectively on buffalo lice populations (Levot 2000). The lice infestation in buffalo needs to be managed by drugs, particularly when their health condition is affected adversely (Veneziano et al. 2004). On buffaloes, numbers of products marketed for control of cattle lice have been tested in field trials against *H. tuberculatus*, in particular macrocyclic lactones such as ivermectin (Lau and Singh 1985), avermectin, doramectin (Bastianetto et al. 2002) and eprinomectin (Veneziano et al. 2004) and deltamethrin (Singh et al. 2015). However, indiscriminate application of these chemicals at insufficient doses results in resistance in these arthropods (FAO 2004). The synthetic pyrethroids such as deltamethrin and cypermethrin are commercially available in India from long time and in the present situation, they are the predominantly used insecticides for management of ectoparasites in the country (Sharma et al. 2012). Currently, a number of synthetic pyrethroid resistance reports available from India, which have been experimentally proved in Indian isolates of cattle ticks *Rhipicephalus microplus* (Sharma et al. 2012; Kumar et al. 2016; Fular et al. 2018; Sagar et al. 2020; Upadhaya et al. 2020) and *Hyalomma anatolicum* (Shyma et al. 2012). Although a number of dairy farmers and small animal owners have confirmed the failure of these chemical compounds to treat lice in the field, there is currently no data on lice resistance to these chemicals in the region.

This is why there is little knowledge in the literature about the use and efficacy of most insecticides and herbicides on buffalo lice. Considering the need to preserve the efficacy of these expensive insecticides, multinationals' aversion to funding insecticide science, and the costs of developing and selling a new class of chemicals for arthropod control, it is becoming essential to develop resistance data for implementation of future arthropod control measures. Because there is no data on the in vitro efficacy of the different groups of insecticide on buffalo louse, the aim of this study was to determine in vitro efficacy of the deltamethrin, cypermethrin and flumethrin resistance status of *H. tuberculatus* collected from Mhow, Madhya Pradesh, India.

Materials and method

Collection of lice

The study was performed on lice collected from animals (buffalo) of organized dairy farm of Mhow, Madhya Pradesh. The buffaloes had heavy infestation of lice on body, hair and tail region. Lice were collected from 33 buffaloes by natural picking and with the help of a comb. Around 500 lice were collected for experiment purpose. All lice collected from buffaloes were pooled and used in the present study. Few lice were also collected in 70% alcohol for their morphological identification as described by Soulsby (1965).

Identification of lice

Lice specimens were studied by using the following routine procedure, lice were preserved in 70% alcohol followed by treating them with 10% KOH (heating the louse by adding 2 ml of 10% KOH for 3–5 min), and then dehydration in ascending grades of alcohol (30, 50, 70, 90 and 100%). The clearing was done with xylene followed by mounting with Phenol balsam; afterwards, the mounted slides were examined under light microscope. The morpho-metric data of louse were also recorded and compared with available literature. The keys suggested by Chaudhuri and Kumar (1961) and Meleney and Kim (1974a, b) were used to determine the species.

Bioassay

Laboratory *in vitro* treated surface bioassay

The methodology used after a minor modification, briefly, in vitro treated surface bioassay utilizing a cloth rectangle that allows lice to move freely has been used which was initially developed in Australia (Levot and Hughes 1990). Three 60 × 60 mm cloth squares were prepared for each insecticide concentration and labelled. From the centre of each cloth, approx 800–1000 µl of each concentration was pipetted onto each cloth square and allowed to dry at room temperature. Cloths impregnated with distilled water were used for control group. Impregnated cloths were then inserted into glass tubes with the help of forceps. Ten live lice were placed into each glass tube and the tubes were sealed with the muslin cloth and incubated in dark at 34 °C for 4 h. In the present study, we used SP insecticide and maintained a relative humidity of 70 to 80%, using a 10% solution of KOH. Lice were examined at different time intervals and dead or live lice numbers were recorded. Dead lice were motionless and exhibited symptoms of dehydration.

Insecticides used

A commercial preparation of deltamethrin (1.25%), cypermethrin (100 g per litre) and flumethrin (1%) was used to prepare different concentrations used in experiments. For deltamethrin and flumethrin, 30, 60, 90, 120 ppm concentrations were used whereas for cypermethrin, 100, 200, 300, 400 ppm concentrations were used. All the concentration was were prepared in distilled water.

Data analyses

The data were analyzed by probit regression (Bany et al. 1995) and the LC50 and LC95 calculated (Fig. 1).

Results

Lice were collected from 33 buffaloes of an organized dairy farm in Mhow block of Indore district. All the buffaloes were heavily infested with lice and nits throughout the body of animals including the tail. The lice were identified as *H. tuberculatus*. The morphometric data of lice collected in the present study were similar to morphological

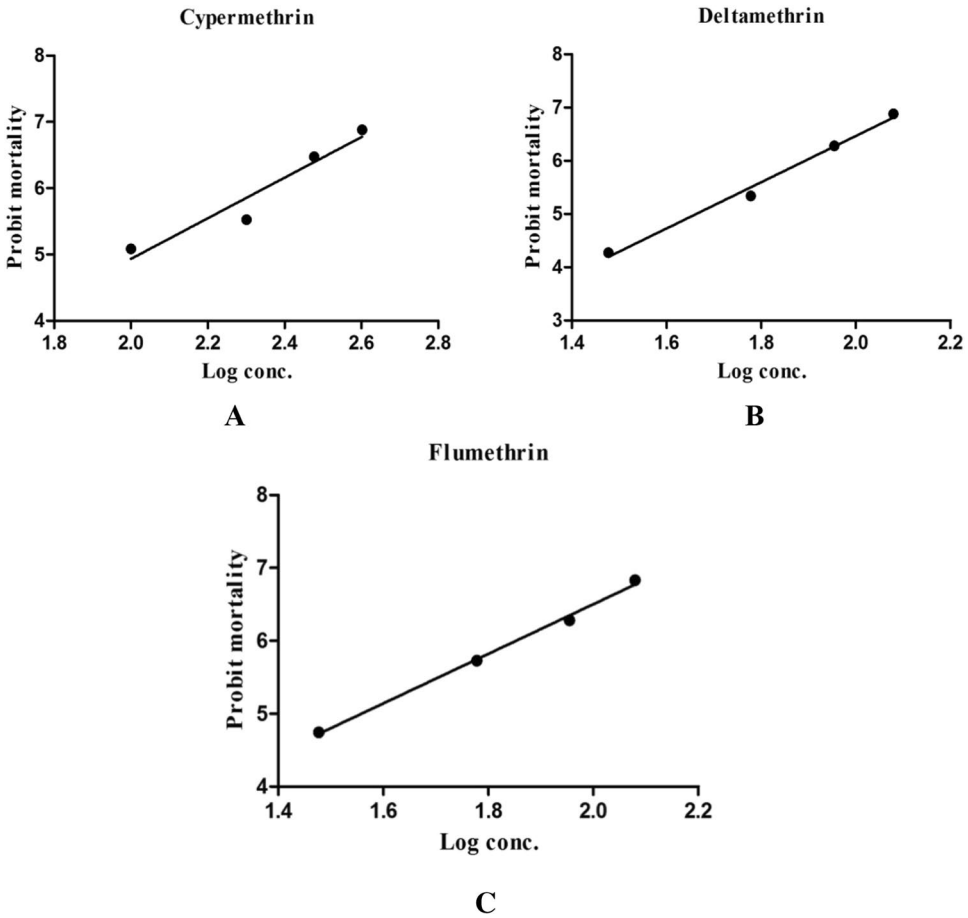

Fig. 1 Dose mortality curve of *H. tuberculatus* against deltamethrin (**A**), cypermethrin (**B**) and flumethrin (**C**)

characters reported for *H. Tuberculatus*, the average length and width of females were 4.7 mm and 2.9 mm, respectively, and males are smaller than the females, 3.5 mm in length and 2.1 mm in width (Fig. 2A and B). The thorasic part of lice was smaller and much wider than longer (Fig. 2A and B). Data on the effects of various concentrations of deltamethrin, cypermethrin and flumethrin on buffalo louse (*H. Tuberculatus*) are presented in Tables 1, 2

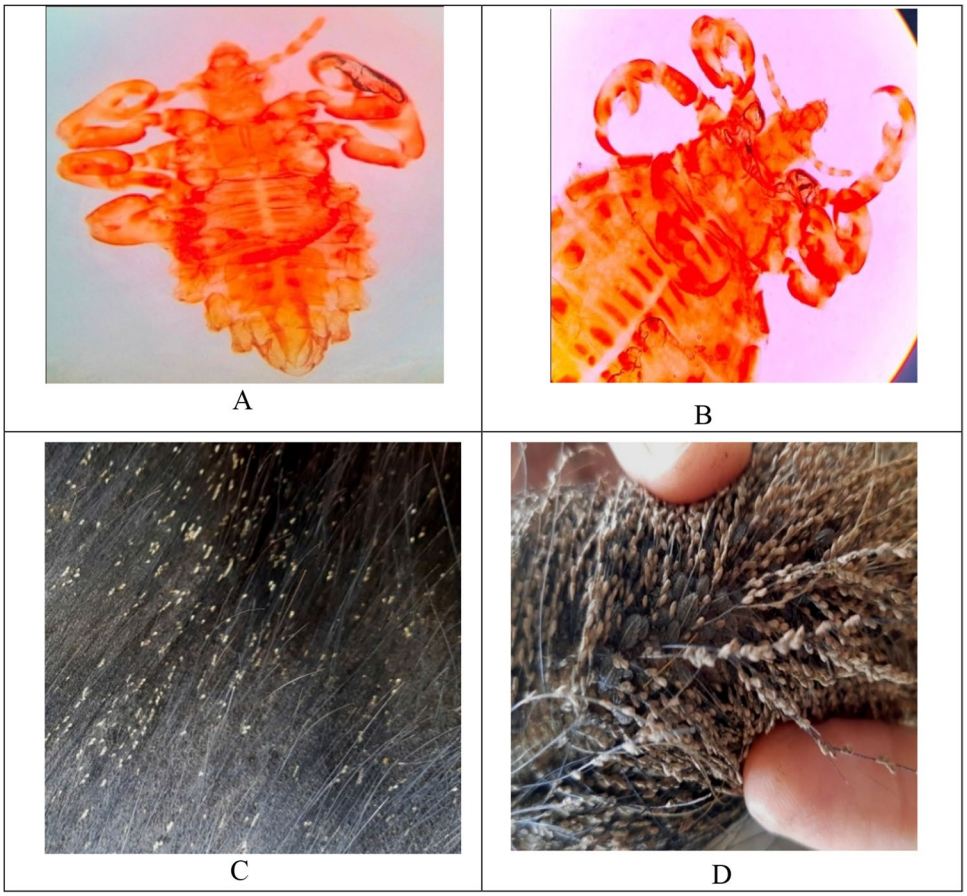

Fig. 2 **A** and **B** Microphotograph of *H. tuberculatus*. **C** and **D** Nits infestation on buffaloes and animals infestation on the tail

Table 1 Effect of different concentrations of deltamethrin on *H. tuberculatus*

Concentration (ppm)	Number of lice died						Mortality (%)
	N	30 min	1 h	2 h	3 h	4 h	
30	30	0	4	2	3	0	23.33
60	30	0	6	5	7	1	63.33
90	30	2	8	7	8	2	90.00
120	30	5	10	11	2	1	96.67
Control	30	0	0	0	2	3	16.66

Table 2 Effect of different concentrations of cypermethrin on *H. tuberculatus*

Concentration (ppm)	Number of lice died						Mortality (%)
	N	30 min	1 h	2 h	3 h	4 h	
100	30	0	1	5	7	3	53.33
200	30	0	3	10	6	2	70.00
300	30	0	3	17	8	0	93.33
400	30	0	5	20	3	1	96.67
Control	30	0	0	0	1	2	10.00

Table 3 Effect of different concentrations of flumethrin on *H. tuberculatus*

Concentration (ppm)	Number of lice died						Mortality (%)
	N	30 min	1 h	2 h	3 h	4 h	
30	30	3	1	2	5	1	40.00
60	30	5	7	8	1	3	76.66
90	30	8	5	11	2	1	90.00
120	30	17	2	6	4	0	96.67
Control	30	0	0	1	1	2	13.33

and 3. The results of the in vitro study revealed that maximum louse responded in terms of mortality within 1–3 h of exposure. After 4 h of exposure, almost no concentration-dependent change in mortality was seen following treatment with insecticides (deltamethrin, cypermethrin and flumethrin). The lice were incubated for 4 h in various chemical concentrations. The experimental lice were incubated at 34 °C in dark and maintained a relative humidity of 80% to minimize the stress produced by environmental conditions.

With increasing concentrations of deltamethrin, cypermethrin, and flumethrin, lice mortality increased and maximum mortality recorded as 96.67% at 120 ppm (Table 1), 400 ppm (Table 2) and 120 ppm (Table 3) in the case of deltamethrin, cypermethrin and flumethrin. In the present study, it was observed that lice exposed to the concentrations in which deltamethrin is commonly used (25–30 ppm) died at a rate of less than 25%. At 100 ppm concentration of cypermethrin achieved 53.33% mortality, while flumethrin achieved 40% mortality. Even the much higher concentration used in experiment of 120 (deltamethrin), 400 (cypermethrin) and 120 (flumethrin) ppm failed to produce 100% mortality, thus indicating development of resistance against deltamethrin, cypermethrin and flumethrin. About 10–16.67% mortality of control group also takes place and this may be due to suffocation or some other reason unknown to us.

The regression graph of probit mortality of lice plotted against log values of progressively increasing concentrations of deltamethrin, cypermethrin and deltamethrin is shown in Fig. 2. The slope of mortality was 4.347 ± 0.3022 (deltamethrin), 3.063 ± 0.6043 (cypermethrin) and 3.402 ± 0.1478 whereas the value of co-efficient of correlation (R2) was in the range of 0.93–0.99. From the regression equation, the LC_{50} and LC_{95} values of deltamethrin, cypermethrin and flumethrin were calculated as 45.99 and 109.65; 105.00 and 360.28; and 36.18 and 109.79, respectively. The RF_{50} were observed as 3.67, 1.05 and 1.20 for deltamethrin, cypermethrin and flumethrin, respectively. However, without a reference population of pyrethroid-naive *H. tuberculatus*, it is impossible to prove definitively that the lice evaluated have established resistance. Due to the shortage of untreated livestock, reference populations could not be collected for this analysis.

Discussion

In the present scenario, the valuable data available on the in vitro efficacy of drugs against the buffalo louse are lacking; the results of this study are comparable only to data reported by Khater et al. (2009) and Singh et al. (2015). The authors showed, in a similar type of experiment, where all treated lice were killed within a minute after treatment with some essential oils (Khater et al. 2009). Same authors also reported the effect of in vitro treatment of *H. tuberculatus* with different concentrations of d-phenothrin determined the 100% mortality within 20 min. In another study, Singh et al. (2015) reported the development of resistance in buffalo louse against the deltamethrin. According to WAAVP guidelines (Holdsworth et al. 2006), lousicidal efficacy of insecticides determined by that drug should provide a reduction in the parasitic population of at least 95% to demonstrate its efficacy in louse control.

The incubation time is very important and an optimum 16-h exposure is recommended, despite highest mortality within 2–4 h (Levot 2000). In the present study, lice were incubated for 4 h in darkness at 34 °C and 70 to 80% RH to reduce the stress caused by environmental conditions as stressed lice may be affected by lower concentrations and give false susceptibility readings (Levot and Hughes 1990).

Few reports were available on SP pour-on compound, where treatment failure reported in *B. ovis* and first published in Australia experimentally (Boray et al. 1988). Farmers' repeated and improper applications trigger the majority of complaints, but resistance has been involved in a growing number of cases (Levot 1995). Species of lice with decreased susceptibility to SPs have now been reported in most states of Australia (Johnson et al. 1988). Levot (1995) confirm resistance development in lice against cypermethrin, deltamethrin, cyhalothrin and alpha-cypermethrin.

Like in the present study, by using in vitro treated surface technique many workers reported the resistance development in lice and observed wide variation in LC_{50} and LC_{95} values of deltamethrin, cypermethrin and flumethrin (Levot and Hughes 1990; James et al. 1993; Keys et al. 1993). This indicated that ineffective lice control was caused by factors other than pyrethroid resistance. In the present study, a low level of RF observed in the case of

cypermethrin and flumethrin whereas in the case of deltamethrin, somewhat high RF observed which was an indication of absence of resistance against cypermethrin (1.05) and flumethrin (1.20) in studied population of lice. But in the case of deltamethrin (3.67), a low level of resistance was observed. Similarly, Singh et al. (2015) reported the deltamethrin resistance *H. Tubeculatus* population from Ludhiana, India. SP resistance was also reported from New Zealand by James et al. (1993) where authors recorded moderate to high resistance against cypermethrin by using treated surface bioassay with RF ranging from 1.0 to 12.4. Workers such as Johnson et al. (1988) and Johnson et al. (1989) reported that efficacy of long wool SP treatment was reduced dramatically when the resistant lice infested the animals and often recorded little or no reduction in lice population was observed. After SP resistance in lice, Levot (2000) recommended the use of diazinon spray on in Australia to control *B. ovis*.

However, there is relatively very small comparable reports published data on the efficacy of deltamethrin, cypermethrin and flumethrin against buffalo lice. Pyrethroid resistance has previously been identified in Australian field populations of the sheep body louse, *B. ovis* (Johnson et al. 1992; Levot 1995; Jazayeri 2004). Four populations of *B. ovis* from the UK demonstrated potential tolerance to deltamethrin in related studies (Bates 2001). Singh et al. (2015) reported development of deltamethrin resistance in lice collected from Ludhiana, Punjab, India. Reports on resistance in different species (*Haematopinus eurysternus*, *H. quadripertusus*, *H. tuberculatus*) of lice have been reported against BHC and DDT (FAO 1991).

The development of resistance against deltamethrin, cypermethrin and flumethrin in lice from Mhow, Madhya Pradesh, may be attributed to the fact that farmers began treating animals with commercially available insecticides on a regular basis rather than maintaining an optimal dose regime for ectoparasite control, especially in ticks and mites. Insecticides were often applied by spray (deltamethrin and cypermethrin), and others such as flumethrin applied by pour-on in Madhya Pradesh (Sagar et al. 2020). The widespread and indiscriminate use of synthetic pyrethroids, especially deltamethrin and cypermethrin, resulted in the development of lice resistance. Furthermore, the higher reproductive rate of lice with heritable resistance factors, as well as the consequent increase in the proportion of the population of lice carrying these genes, results in the establishment of resistance in the population (FAO 2004). Furthermore, for a variety of reasons, lice populations can develop resistance quickly: lice are very host-specific obligate parasites and there is no or relatively little immigration from different populations. As a result, under the selection pressure exerted by insecticides, any resistant genotypes easily replace the susceptible (Ellse et al. 2012).

In lice, pyrethroid resistance can evolve in one of two ways: through single point mutations in the gene coding for the drug target protein, or through multiple point mutations in the gene coding for the drug target protein (Lee et al. 2000) or by upregulation of metabolic, monooxygenase enzymes (Scott 1999). As a result, future research aimed at determining the mechanism of resistance would be extremely beneficial in the development and implementation of effective control strategies for ectoparasites, especially lice in dairy animals, which affect dairy animals both directly and indirectly. This is the first study on resistance development in lice against deltamethrin, cypermethrin and flumethrin from Mhow, Madhya Pradesh, India.

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Author contribution MK and KG conceived and designed research. SJ, MS, AF and BB conducted experiments. GR contributed new reagents or analytical tools. VA, GPJ, GR and SK analyzed data. MK and SK wrote the manuscript. All authors read and approved the manuscript. This is approved by all authors of the manuscript.

Data availability Raw data were generated at the Department of Parasitology, College of Veterinary Science & Animal Husbandry, Mhow (NDVSU), Indore, Madhya Pradesh. Derived data supporting the findings of this study are available from the corresponding authors on request.

Declarations

Ethical Statement It is certified that the research manuscript is entitled "Detection of deltamethrin, cypermethrin and flumethrin efficacy against buffalo lice—*Haematopinus tuberculatus*". The research work was not funded by any department. It was conducted by using a facility in the Department of Parasitology, College of Veterinary Science & Animal Husbandry, Mhow (NDVSU), Indore, Madhya Pradesh and certified by the animal ethical clearance committee of the University.

Consent for publication Yes.

Conflict of interest The authors declare no competing interests.

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