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Muhammad Sohail Sajid, Asif Iqbal, Hafiz Muhammad Rizwan, Saima Naz, Mahvish Maqbool, Muhammad Abdullah Malik, Muhammad Jawad-ul-Hassan

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Epidemiology and therapeutic efficacy of asynthetic pyrethroid against *Haematopinus tuberculatus* (Phthiraptera: Haematopinidae) in *Bubalus bubalis*

Sajid M. S.^{*1,2}, Iqbal A.³, Rizwan H. M.^{**4}, Naz S.^{5,6}, Maqbool M.¹,
Malik M. A.¹, Jawad-ul-Hassan M.¹

¹Department of Parasitology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad (UAF), Pakistan

²Department of Epidemiology and Public Health, UAF, Pakistan

³Department of Parasitology, Riphah College of Veterinary Sciences, Lahore

⁴Section of Parasitology, Department of Pathobiology, KBCMA College of Veterinary and Animal Sciences, Narowal
Sub campus UVAS, Lahore

⁵Department of Veterinary Science, Faculty of Agrobiological Sciences, Food and Natural Resources, Czech University of Life
Sciences, Prague, Czech Republic

⁶Department of Zoology, University of Sindh, Jamshoro-76080, Pakistan

ABSTRACT: Louse (Insecta: Phthiraptera) are common ectoparasites of buffalos (*Bubalis bubalis*) causing huge economic loss. An epidemiological survey was conducted in selected towns (administrative division) of Faisalabad district to determine the prevalence of louse infestation, the associated risk factors and the efficacy of cypermethrin (CYP) against louse infestation in buffalos. To determine the efficacy of CYP, 40 louse-infested buffalos were divided into two groups i.e. drug-treated group and control group (each group contains 20 animals). Cypermethrin drug was used as pour-on at day 0 and louse counts were conducted on days 0, 7, 21, 35, 49, and 63 from predilection sites of each animal. Only one species of sucking louse *Haematopinus tuberculatus* was found in buffalo population of Faisalabad district. The overall prevalence of louse infestation was 37.76%. The variable like age and sex showed significant ($P < 0.05$) variation of louse infestation while breed showed insignificant variation. Among extrinsic factors, seasons, floor pattern, and feeding system showed significant association ($P < 0.05$) with louse infestation, while animal keeping as free or tethered and towns showed an insignificant association. On day 7, 100% effective results were obtained while 99.6% at day 21 and 100% from day 35 till day 63. No clinical adverse reaction was observed during this trial and cypermethrin was found efficacious. Based on these results it can be concluded that cypermethrin is an effective drug against louse infestation.

Keywords: Buffalo, Cypermethrin, Efficacy, *Haematopinus tuberculatus*, Prevalence.

Corresponding Authors:
Muhammad Sohail Sajid
E-mail address: drsohailuaf@hotmail.com

Hafiz Muhammad Rizwan
E-mail address: hm.rizwan@uvas.edu.pk

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INTRODUCTION

Buffalos play an important role in livestock economy, and among these *Bubalis (B.) bubalis* is the most important animal in Pakistan, Iraq, India, Turkey, China, Brazil, England, and Italy (McFarland and Coles, 2002; Zicarelli, 2004; Hussain et al., 2006; Batista et al., 2018). In Pakistan, there are five breeds of buffalos i.e. Azi Kheli, Nili, Ravi, Nili-Ravi, and Kundhi. Among these, Kundhi (from Sindh province) and Nili-Ravi (from riverine Punjab) are the major breeds of buffalo (Khan et al., 2007). Ectoparasite infestation is considered as a basic hindrance to the productive potential of *B. bubalis* so the distribution and control of these ectoparasites are considered a crucial step to prevent the economic losses (Veneziano et al., 2007).

Prevalence of louse infections was reported worldwide including in Pakistan (Hussain et al., 2006; Veneziano et al. 2007; Kakar and Kakarsulemankhel, 2009; Tasawar et al., 2009; Regasa et al., 2015). Louse infestation is considered as the most crucial ectoparasitic infestation among buffalos. Among louse species, *Haematopinus tuberculatus*, also known as buffalo sucking louse is one of the most significant phthirapteran louse (Bastianetto et al., 2002). *Haematopinus tuberculatus* is found more prevalent in Europe, Asia, South America, Africa, and Australia, and also a source of transmission of pathogenic agents (Veneziano et al., 2003; da Silva et al., 2013; Bosco et al., 2018).

Cypermethrin (CYP) is a synthetic pyrethroid insecticide that alters the sodium ion channel and ultimately effecting the central and peripheral nervous system of the insect. Cypermethrin has been found effective for a variety of insects concerned with public and animal health including ticks, blowflies, louse, etc. (Hussain et al., 2005). Cypermethrin against chewing louse infestations in horse and donkey population has also been observed successful (Veneziano et al., 2012; Sands et al., 2016).

In case of cattle, there is dosage regimen for antiparasitic compounds while in case of buffalos there is no specific drug regimen due to which many antiparasitic compounds remain ineffective (Veneziano et al., 2004). There is a lack of information regarding the prevalence of louse infestation, associated risk factors, uses, and efficacy of insecticide compounds in buffalos including synthetic pyrethroids. Therefore, the current study was conducted to determine the frequency distribution and efficacy of cypermethrin as

pour-on against louse infestation in buffalos in district Faisalabad, Punjab, Pakistan.

MATERIALS AND METHODS

Selection of Site and Epidemiological Survey

The study was conducted in district Faisalabad (31.4504° N, 73.1350° E), Punjab, Pakistan. Three towns named Faisalabad Town, Madina Town, and Jinnah Town were selected for the screening of buffalo population for louse infestation. It was a year based epidemiological study to determine the prevalence and association of various intrinsic factors like sex, age, and breed, and extrinsic factors like floor pattern, animal keeping methods, feeding system, seasons, and towns with louse infestation. Louse specimens were collected from randomly selected buffalos and preserved in 70% ethanol. Samples were transported to the Department of Parasitology, University of Agriculture Faisalabad for identification under the stereomicroscope. Species identification was done by using the key designed by Adhikary and Ghosh (1994).

Experimental Animals

After identification of louse species, the experiment was executed on 40 louse-infested female buffalos from which louse specimens were collected. The mean age of experimental animals was 4 (± 2.0) years and it was also recorded that they were not previously treated for louse infestation. Animals were kept indoor pens to avoid rainfall influence and ear tagging was done to recognize them from day 0 to day 63. Animals were fed by total mixed ration including wheat straw, vitamin, mineral supplements, corn fodder, and vegetable fat along with *ad libitum* water supply. The experiment followed the ethics and guidelines approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan to use animals for research purposes and guidelines provided by World Association for the Advancement of Veterinary Parasitology (WAAVP) to determine ectoparasiticidal efficacy (Holdsworth et al., 2006). Animals were divided into two groups based on their louse abundance. Animals with high louse abundance were kept in the treatment group and others were kept in untreated groups.

Parasitological Trial

At day 0, all the animals were kept in the treatment group that received pour-on cypermethrin treatment as per the manufacturer's recommendation i.e 10mL/animal (10 mg/kg). The topical application method

was applied, using a syringe from midline to withers and tail. While in the case of control group, no treatment was used. Each group was transferred to separate indoor pens at a distance of ten meters to avoid any physical contact. Louse collection was started from day 0 at different sites, determined based on louse distribution. Predilection sites were determined based on louse distribution on buffalos (Veneziano et al., 2003; Emmanuel et al., 2017; Bosco et al., 2018). Observed predilection sites include a neck, dewlap, cheek, ear on both sides, foreleg, tail-head, withers, back and hind leg, and perineum (10cm² area of each). When no louse were available from the above-mentioned prediction sites, then a whole-body examination of that animal was performed.

Experimental animals were examined on day 1 to examine any adverse reaction of the drug and followed by daily examination for week 1, and then once every 14 days till the end of the experiment. Skin examination was also used to examine any erythema, edema, and eschar formation along with loss of hair.

Statistical Analyses

The association of louse infestation with different risk factors was statistically analyzed using the chi-square test. Louse counting was performed at day 0 followed by day 7, 21, 35, 49, and 63 from all the prediction sites for each animal in a group. Cumulative

count for each buffalo during each inspection was calculated through arithmetic mean (AM). Efficacy of cypermethrin was checked at days 7 followed by day 21, 35, 49, and 63 in terms of percentage (%) reduction in louse prevalence with the help of Abbott's formula. Mann-Whitney test was used to analyze the difference in louse counts and comparison of treatment group with the control group. P values of <0.05 were considered to check significant differences. SPSS 16.0 software was used to examine all analyses at 95% confidence interval.

RESULTS

The overall prevalence of louse infestation in the buffalo population of Faisalabad district was 37.76%. Only one species of sucking louse, *Haematopinus tuberculosis*, was found in buffalo population of Faisalabad district. The variable like age and sex showed significant (P<0.05) variation of louse infestation while breed showed insignificant variation. Among extrinsic factors, floor pattern (uncemented soiled, semi cemented, and cemented) and feeding system (grazing, stall-feeding) have significant (P<0.05) association with louse infestation while animal keeping (free, tethered) showed an insignificant association. Among seasons, summer showed 65.66% with a significantly (P<0.05) higher prevalence of louse infestation followed by autumn (30.34%), spring (29.70%),

Table 1. Association of different parameters with louse infestation in buffalos of Faisalabad district, Punjab, Pakistan

Parameters	Groups	Animals examined	Infested animals	P-value ($\alpha=0.05$)	Chi-Square value
Sex	Male	104	17	0.000	27.83
	Female	280	128		
Age	Young	158	79	0.000	17.112
	Adult	226	66		
Breed	Nili Ravi	283	103	0.356	0.853
	Kundi	101	42		
	Uncemented	173	78		
Floor pattern	Semi cemented	146	52	0.005	10.565
	Cemented	65	15		
Animal keeping	Free	218	86	0.434	0.612
	Tethered	166	59		
Feeding system	Grazing	264	88	0.001	11.176
	Stall-feeding	120	57		
Season	Spring	101	30	0.000	45.079
	Summer	99	65		
	Winter	95	23		
Towns	Autumn	89	27	0.169	3.544
	Faisalabad Town	129	45		
	Madina Town	134	46		
	Jinnah Town	121	54		

Table 2: Total louse counts, arithmetic means (AM), and efficacy (percentage louse reductions) for the buffalos of the control (untreated) group compared to buffalos of the cypermethrin (treated) group at each time study points

Day	Control group		Cypermethrin treated group		Efficacy (%)
	Intensity of louse infestation (mean \pm SE)	Arithmetic mean	Intensity of louse infestation (mean \pm SE)	Arithmetic mean	
0	701 \pm 12	70.1	819 \pm 23	81.9	-
7	629 \pm 86	62.9	0 \pm 00	0	100*
21	685 \pm 21	68.5	1 \pm 00	0.1	99.6*
35	559 \pm 18	55.9	0 \pm 00	0	100*
49	1170 \pm 21	117.0	0 \pm 00	0	100*
63	1260 \pm 30	126.0	0 \pm 00	0	100*

*Significant ($P < 0.05$).

and winter (24.21%).

Selected towns of district Faisalabad showed an insignificant ($P > 0.05$) variation of louse infestation, however, Jinnah Town showed relatively higher prevalence (44.63%). The higher frequency of louse distribution was found in head region i.e. 47% followed by withers (32%), neck (17%), cheeks (8%), forelegs (7.5%), ears (4.4%), and tail with minimum frequency (0.9%). Association of different parameters with louse infestation is given in Table 1 while prevalence of louse infestation in buffalo population of Faisalabad district is given in Figure 1.

Intensity (total number \pm SE) of louse for CYP-group on day 0 was 819 \pm 23 (AM 81.9) while for the control group it was 701 \pm 12 (AM 70.1). The intensity

of louse for control group at day 7, 21, 35, 49, and 63 was 629 \pm 86 (AM 62.9), 685 \pm 21 (AM 68.5), 559 \pm 18 (AM 55.9), 1,170 \pm 21 (AM 117.6), and 1,260 \pm 3 (AM 126.0) respectively. In case of efficacy trails, the efficacy was observed 100% throughout the experiment except at day 21 when it was 99.6% as one buffalo remain infested with *H. tuberculatus* first instar nymph (Table 2). Significantly ($P < 0.05$) lower louse intensity was determined in CYP-group as compared to the control group at all post-treatment days. No adverse effect was recorded on skin during the experiment.

DISCUSSION

A wide range of domestic livestock population is being infested with louse including cattle, buffalo, sheep, goat, and pigs throughout the world. Louse in-

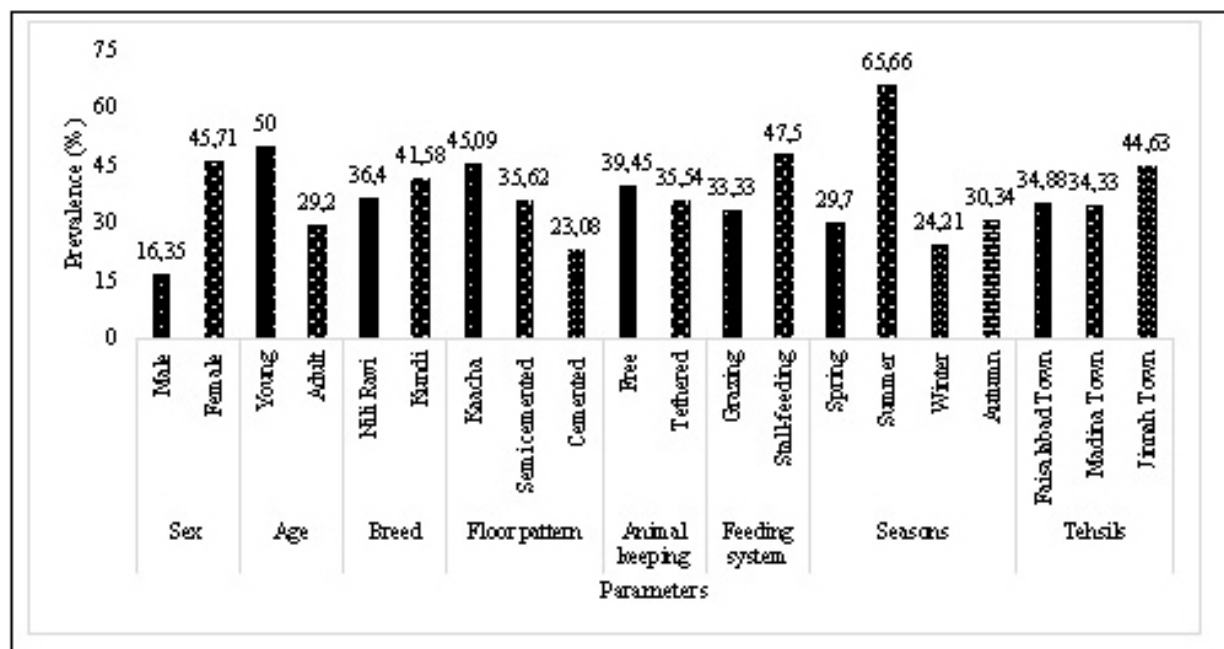


Figure 1. Prevalence (%) of louse infestation in the buffalos observed, with different variables in the selected population of district Faisalabad, Punjab, Pakistan

festation causes dermatological problems i.e pediculosis characterized by itching, biting of hair or fleece, irritation. Negative impact on animal health caused by louse infestation is anxiety, loss of blood, and decrease in reproductive efficacy of female buffalos (Ahmed et al., 2009). However, the survival of louse depends upon the hosts (Madeira et al., 2000; Hornok et al., 2010).

Generally, a lower prevalence of louse infestation in buffalos has been determined prior to the present study (Azam et al., 2002; Hussain et al., 2006; Kakar and Kakarsulemankhel, 2008; 2009; Cringoli et al., 2009; Al-Mayah and Hatem, 2018; Batista et al., 2018). However, Al-Lahaibi and Al-Tae (2019) recorded higher prevalence, whereas Shamim et al. (2015) and Khatoun et al. (2018) have affirmed zero prevalence of louse infestation in buffalo in Pakistan.

At present, a single species of louse in buffalo population was reported, similar to Batista et al. (2018) and Al-Lahaibi and Al-Tae (2019) while Kakar and Kakarsulemankhel (2009) and Al-Mayah and Hatem, (2018) found two species (*H. quadrpertusis* and *H. eurysternus*). Hussain et al. (2006) reported three species of louse (*Linognathus vituli*, *H. eurysternus*, and *H. tuberculatus*). This variation might be due to the change in the climate of study area, geographic, ecological, and weather conditions (Hussain et al., 2005).

In contrast to our study, Batista et al. (2018) reported a higher prevalence in males as compared to females. The higher prevalence in females might be due to the rearing of animals mainly for dairy and feeding purposes. The other main reason for higher infestation in females may be due to continuous hormonal variation and stress during lactation and reproduction (Das et al., 2016). In present study, adult animals showed a higher frequency of louse infestation which is in line with the result of Hussain et al. (2006), while Batista et al. (2018) found a higher prevalence in younger animals. The higher frequency in adults might be due to longer exposure with the environment and congested farming system of the study area. However, in younger animals, the higher distribution may be related to grazing habits, grazing areas, movement of animals, and exposure of a more substantial proportion of the body with contamination (Batista et al., 2018). The rate of louse infestation in different breeds of buffalo was found insignificant which is in line with the result of Hussain et al. (2006). The infestation in all kinds of breeds might be due to the practice of keeping all breeds together under the same management conditions.

The uncemented soiled floor pattern revealed a higher distribution of louse, the same has been reported by Hussain et al. (2006) than semi cemented and cemented floor. This might be due to less exposure to sunlight of animals because the uncemented soiled floor mostly related to the covered or close type of houses in Pakistan. Animals settled for stall-feeding are more prone to infection than grazing animals in the current study, while Batista et al. (2018) considered a various type of grazing with the highest prevalence in alternating grazing (57.1%) followed by continuous grazing (26.1%), and rotational grazing (25.0%).

Animal farms have been adopted for farming systems by replacing grazing with a constant supply of concentrate (cereal grains and their by-products) and forages due to which a reduction in helminth infection is reported (Fekete and Kellems, 2007). However, the intensive farming system favors ectoparasites especially louse like *H. tuberculatus* because of high density of animals (Tasawar et al., 2009; Mennerat et al., 2010; Batista et al., 2018).

Regarding the highest prevalence of louse infestation in summer, a similar result was observed to that of Al-Mayah and Hatem (2018), while Hussain et al. (2006) reported a higher prevalence in spring. The variation of louse infestation in different seasons is due to change in temperature, humidity, environmental hygienic conditions, precipitation, the intensity of light and release of skin moisture like secretion of sebum and sweat by animal's skin prevail the higher infestation of louse (Milnes et al., 2003; Hussain et al., 2005; Arijo et al., 2018; Al-Mayah and Hatem, 2018; Batista et al., 2018). A louse is extremely helpless to environmental impact, the development of all stages of louse is remarkably temperature reliant. It has been documented that louse requires a somewhat tightened temperature range of about 30 to 40°C for development (Lehane, 2005). However, it was also reported that louse can survive better at 25°C. Concerning area, an insignificant association of louse infestation in buffalo was observed by Hussain et al. (2006) as reported in the present study. This might be due to a similar kind of environmental condition in study areas.

In buffalo's treatment, options against ectoparasites are meager because of limited availability of the licensed products. Insecticides used in buffalos for ectoparasites are mainly those which are firstly applied in cattle. Therefore, without any designed clinical trials, topical insecticides are being used by veterinary practitioners in buffalos (Bastianetto and Leite, 2005).

Limited data is available on the safety and efficacy of parasiticides compounds used in buffalos.

Little data is present regarding the use of pyrethroids against *H. tuberculatus* on buffalos. Formulation of cypermethrin 10mL/animal (10 mg/kg) used in this trial was found 100% effective from day 7 to day 63 of post-treatment and 99.6% at day 21. Similar results were described by Khater et al. (2009) for pour-on of D-phenothrin (0.6ml/Kg body weight) in naturally infected buffalos against *H. tuberculatus* and found 100% efficacy recorded at day 9 post-treatment. Comparative efficacy against louse infestation in buffalo determined by Hussain et al. (2006) showed 100% efficacy of ivermectin and 94.7% of cypermethrin at day 28. Veneziano et al. (2013) declared that Alphacypermethrin is 100% effective at day 7, 99.8% at day 14, and 100% from day 21 to 56.

Different reports are published regarding the efficacy of macrocyclic lactones for *Haematopinus* spp. Ivermectin showed 85-100% efficacy by using the subcutaneous route (Lau and Singh, 1985). Hussain et al. (2006) described 100% efficacy when single dose of Ivermectin was used in bovines against *H. tuberculatus* while 80-83% efficacy in bovines are reported by Ahmed et al. (2009). Avermectin bases i.e doramectin, abamectin, and ivermectin are found effective in buffalos naturally infested with louse at day 2 post-treatment (Bastianetto et al., 2002). Complete efficacy of eprinomectin was recorded in water

buffalos against *H. tuberculatus* infestation at day 56 post-treatment by using the pour-on method with a dose rate of 500µg/Kg b.w. (Veneziano et al., 2004). Some essential oils (peppermint, onion, camphor, and chamomile) were also found effective for control of louse and other ectoparasite in animals (Khater et al., 2009; Khater et al., 2014; Faraone et al., 2015; Greive et al., 2018).

The current study describes that intrinsic factors like age and sex directly were associated with the disease. However, among extrinsic factors, seasons, floor pattern, and feeding systems play a significant role in the prevalence of louse infestation ($P<0.05$). The topical application of cypermethrin using pour-on formulations, according to the manufacturer's recommendations, was found highly effective in buffalos for the treatment of sucking louse (*H. tuberculatus*) in district Faisalabad, Pakistan. However, studies are needed regarding the safe use of antiparasitic against endoparasites and ectoparasites in buffalos. This pour-on application test of pyrethroid is important for farmers to control louse infestations in their herds. This study is considered as the first study in Pakistan, focusing on association of risk factors and the evaluation of field efficacy of cypermethrin in buffalos against *H. tuberculatus* infestations.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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