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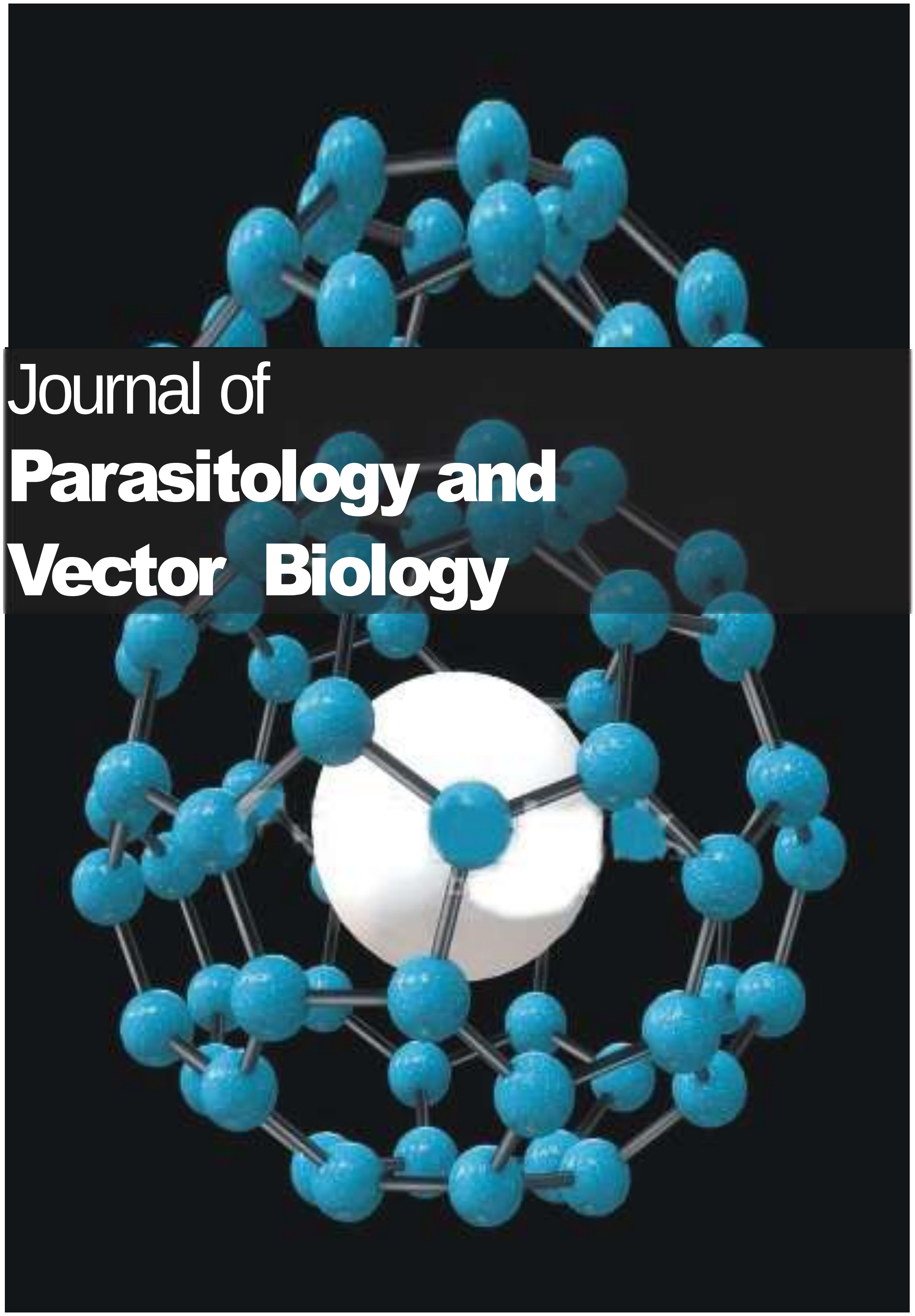

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Journal of Parasitology and Vector Biology

Full Length Research Paper

Cytotaxonomic study of the larvae of blackfly (Diptera: Simuliidae) from River-Ose, Ondo State, Nigeria

Ajayi, O. J.* and Olusi, T. A.

Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria.

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A study of the cytotaxonomy of the larvae of blackfly was undertaken at three sites, Imeri A, Imeri B, and Ose-oba, along River Ose in Ondo State, Nigeria. Based on the morphology of the larvae and the resultant pupae, three species of blackflies were identified. These included *Simulium damnosum* complex, *Simulium alcocki*, and one yet to be identified (YI). Investigations on the polytene chromosomes from the salivary glands of each of these species revealed that the *S. damnosum* complex comprises of three cytospecies identified as *Simulium squamosum*, *S. damnosum* s.s, and *Simulium sirbanum*. The *S. alcocki* was also found to be a complex of sibling species but the YI remained as a single species. There was no significant difference between the distribution of *S. damnosum* complex and *S. alcocki.* However, these two species were different from the YI in occurrence. Results of the present study which conform with earlier reports suggest that the study area (River-Ose) has been and is still a good breeding site for *S. damnosum* and *S. alcocki* species of black flies.

Key words: *Simulium damnosum*, *Simulium alcocki*, 'yet to be identified species' (YI), sibling species.

INTRODUCTION

Based on their impact on the health and economic well-being of humans, black flies are generally regarded as the second most medically important group of insects (Adler et al., 2004). They are responsible for the transmission of onchocerciasis, a debilitating disease caused by the filarial nematode *Onchocerca volvulus* Leukart (Nematoda: Filarioidea). Although, males usually feed on nectar, females obtain nourishment by feeding on the blood of humans and animals. There are several areas of the world where black flies are the most dreaded noxious arthropods due to their biting nuisance. In many rural settlements of the world they have a wide distribution particularly in Mexico, Yemen, Brazil, Venezuela, Ecuador, Colombia and Africa (Malau and James, 2009). The distribution of the flies in Africa covers the sub-tropical belt from Senegal in the West to Somalia in the East. It occurs in savanna, rainforest, plateau, and in the highland areas. The insects breed mainly in fast flowing, well-oxygenated streams and rivers. Their distribution and breeding is not even across Nigeria and their biting activities can be highly seasonal (Adeleke et al., 2011). The disease transmitted by them is prevalent in 35 countries of the world of which 28 are in Africa and Nigeria accounts for one quarter of the global infection

*Corresponding author. E-mail: joshfaj@yahoo.com.

rate (CDI Study Group, 2010).

Species complex occurs widely within the blackfly. A species complex (complex of sibling or cryptic species) is a group of closely related species (Crosskey and Lane, 1993). Although the species may satisfy the criterion of being reproductively isolated from each other, they are not readily or reliably distinguishable on morphological basis. This makes the use of cytological, genetic and/or ecological attributes for distinguishing between them an absolute necessity. The cytology of blackfly larvae has so far relied on studying their polytene chromosomes (Crosskey and Lane, 1993; Post et al., 2007). The micromorphology of polytene chromosomes has provided many key features for dipteran species characterization and phylogenetic investigations. The polytene chromosomes of simuliid populations have been analyzed for reasons such as the identification of species and cytotypes with vectorial capacity and the resistance or susceptibility to insecticides for the purpose of improvement of control programmes (Charalambous et al., 1995).

It has been established that not all the species of blackfly are vectors. The painful bites and disturbing effects of other species of *Simulium* in many riverine areas are intolerable nuisance. These bites could sometimes lead to blood losses and the sites could serve as portal of entry for viruses, bacteria, protozoans and nematodes which the flies may be carrying on their bodies or which are existing in the environment (Ubachukwu, 2004; Usip et al., 2006). These have consequential effects of low productivity, sickness and abandonment of infested areas.

This present study was undertaken to identify the larvae of different species of blackflies present in the study sites of River-Ose, Osun State, Nigeria, in order to reveal the different cytoforms present in the river by studying their polytene chromosomes.

METHODOLOGY

Study area

A cytotaxonomic study of the larvae of blackflies was undertaken at three breeding sites, Imeri A, Imeri B, and Ose-Oba which are located along River Ose in Ose Local Government Area of Ondo State. The segment of the river studied lie between longitude 05°66' and 05°93' east of the Greenwich Meridian, latitude 07°30' and 07°31' north of the equator. The three breeding sites (Imeri A, Imeri B, and Ose-Oba) are 224, 217, and 192 m above the sea level, respectively. The river is characterized by the presence of rocks, trees, vegetation, and sections where rocky substrata and submerged logs obstruct the flow of water, creating rapids. These rapids make the river a suitable environment for the development and survival of the aquatic larval stages of blackflies.

Sample collection

The three sites were visited biweekly for larval collection between the period of January and May 2015 during which 343 specimens of larvae were obtained. The immature stages of blackflies (larvae and pupae) were collected from submerged stones, trailing vegetations, twigs, and leaves, where the water flows rapidly, by using a pair of forceps. The larvae collected were fixed in freshly prepared cold Carnoy's fixative which contained 1:3 acetic acid and ethanol mixture, and brought to the laboratory.

Sorting of specimens

The larvae collected were sorted into different species under the objective lens of a dissecting microscope. The parameters for sorting included mainly nature of the post-genal cleft, head pigmentation patterns, pattern of gill spot, shape and length of larvae with other identification criterium such as the presence of scales on the abdominal region as described by Crosskey and Lane (1993).

Cytological studies

The larvae fixed inside Carnoy's solution were removed and blotted. The polytene chromosomes preparations were made following the method described in Sorungbe (2014) which was a modification of that of Olorode (1974). Full karyotyping and cytospecies identification were based on the criteria described by Vajime and Dunbar (1975), Boakye (1993) and Post et al. (2007).

RESULTS

Distribution of the black fly larvae

The cytotaxonomical investigation on the blackfly in the present study which was based on larvae examination revealed three species of the flies in Ose River. These included *Simulium damnosum* Theobald complex, *Simulium alcocki* group, as well as one 'yet to be identified' (YI). In all the surveyed sites of the river, members of the *S. alcocki* group were abundantly present, while *S. damnosum* were present in abundance only at Imeri sites A and B, but few at Ose-Oba site. However, a few members of the 'YI' species were present at the two sites in Imeri, but none was found at Ose-Oba site (Table 1).

Morphological identification of specimens

Matured *S. damnosum* larvae (Figure 1a) have barrel shaped structure and are usually 5 to 6.5 mm in length. The larval head capsule is strongly pigmented and spotted. The hypostomium is with 9 apical teeth. The larvae appear to be 'unshaved' as a result of the cuticular covering of small upstanding black spines and scales and they also have very prominent paired dorsal abdominal protuberances (tubercles). The setae of the larval cuticle occur even on the thoracic prolegs. The Pupae (Figure 1b) gill filaments look somewhat like hands of bananas (branching stoutly and tubular). The cocoon is shoe-shaped, closely woven, and appear blackish in colour.

The length of a matured larva of *S. alcocki* (Figure 2a)

Table 1. Overview of the abundance of various species of blackfly at the three breeding sites.

Location	No. of specimen collected	No. of male analyzed	No. of female analyzed
S. squamosum			
Imeri A	16	6	7
Imeri B	19	6	9
Ose-Oba	10	4	3
S. damnosum			
Imeri A	34	14	18
Imeri B	36	13	16
Ose-Oba	15	4	7
S. sirbanum			
Imeri A	24	10	11
Imeri B	19	9	8
Ose-Oba	12	5	5
S. alcocki			
Imeri A	50	19	25
Imeri B	53	21	23
Ose-Oba	42	18	17
YI			
Imeri A	8	3	3
Imeri B	5	2	3
Ose-Oba	-	-	-
Total	343	134	142

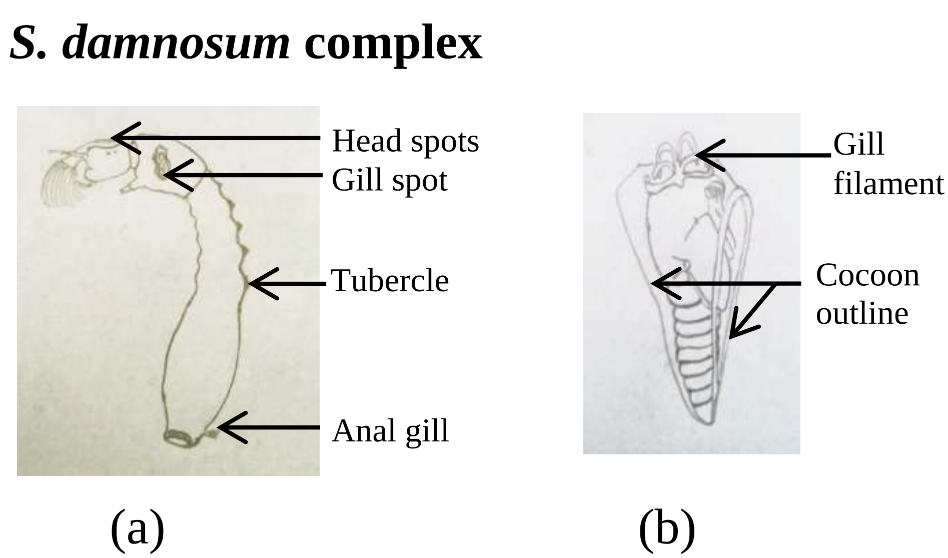

Figure 1. Lateral view of matured: (a) Larvae and (b) Pupa of *S. damnosum* (x20).

ranges between 6 and 8 mm. It has an elongated mid-section which enlarges gradually towards the posterior end. The head capsule has strong brown pigmentation. It is pale-brown but in a few of its members, the colour is

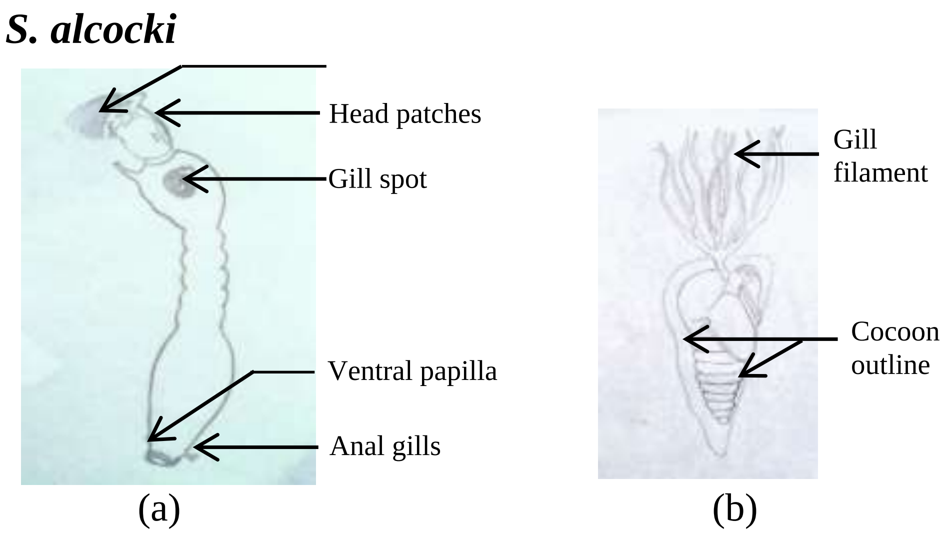

Figure 2. Lateral view of matured: (a) Larva and (b) pupa of *S. alcocki* (x20).

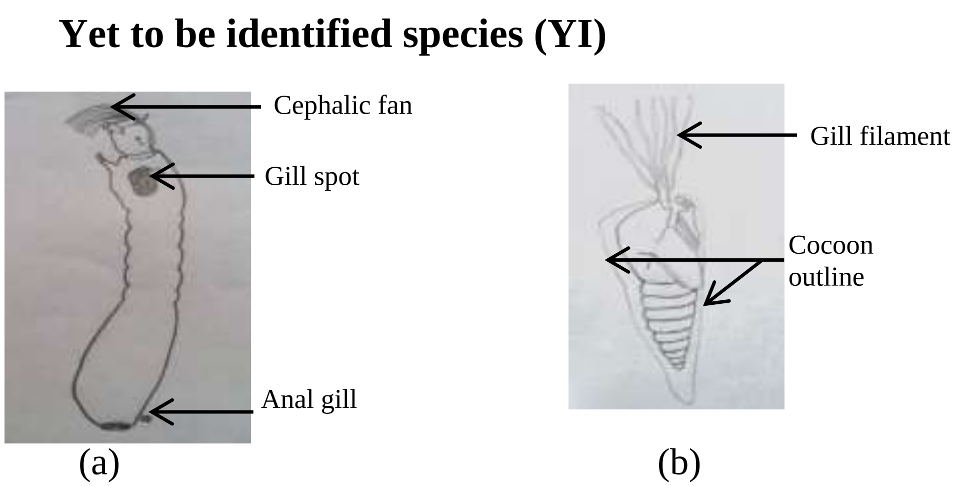

Figure 3. Lateral view of matured (a) Larva and (b) Pupa of YI species (x20).

deep-brown. The dorsal part of the abdomen is covered with setae. Each abdominal segment possesses two pairs of spots. An 'X'-like anal sclerite lies between the anus and the posterior circlet. The gill coils extensively to form a gill spot with a clear region at the center. The pupa (Figure 2b) has gills with three main branches and 9 to 12 filaments. Pupal gill arrangement is variable within samples of this species obtained in the present study. The cocoon is entirely slipper-shaped and closely woven.

Matured larvae of the yet to be identified 'YI' species (Figure 3a) were stout and with average lengths of between 4.5 and 5.5 mm. The head capsule has a clear zone with few spots but without pigmentation. Larval abdomen lacks conspicuous setae, but is covered with light brown scales. The gill is coiled extensively to form a very dark gill spot with shape somewhat similar to that of *S. alcocki* except that it has no cleared center.

The pupa (Figure 3b) of the 'YI' species has gills with 7 to 9 filaments and each of the filaments branched out right from the origin. The pupal cocoon is slipper-shaped and has darkish brown colouration.

Cytotaxonomy of the specimens

Cytological study of each of the morphologically identified species revealed three cytospecies from the *S. damnosum* complex (Figure 4). These include, *S. squamnosum* belonging to *S. squamnosum* subcomplex, *S. damnosum* s.s and *S. sirbanum* belonging to *S.*

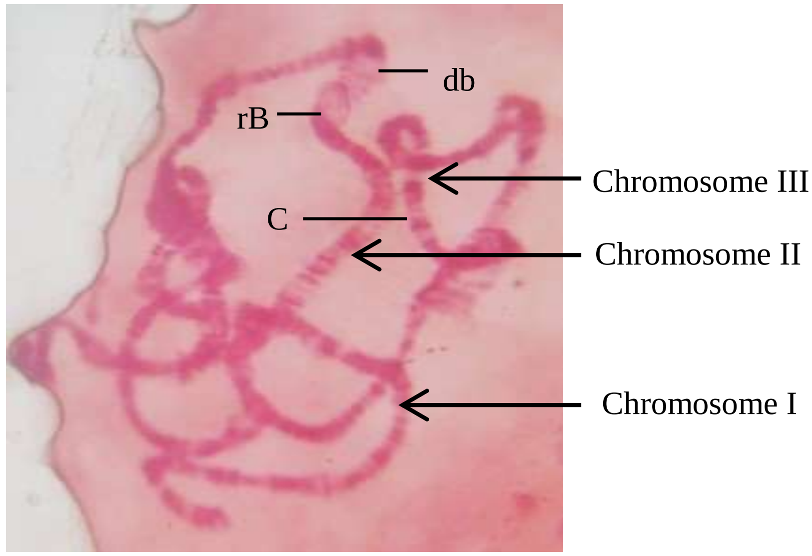

Figure 4. *S. damnosum* chromosome (x1000). db: Double bubble, rB: Ring of Balbiani, C: Centromere.

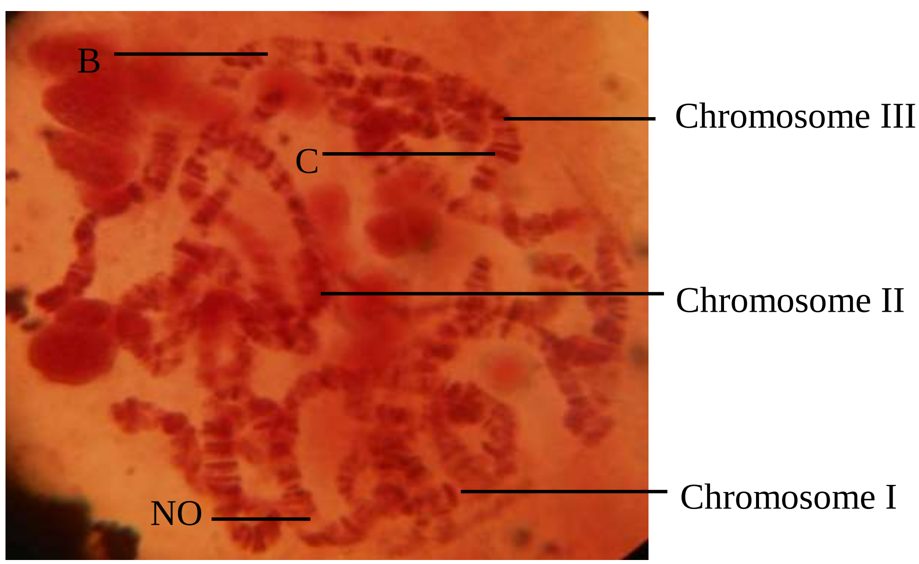

Figure 5. *S. alcocki* chromosome (x1000). B-Blister, NO-Nuclear Organizer, C- Centromere.

damnosum subcomplex. *S. squamosum* was recorded from all the breeding sites in the study area. Intraspecific chromosomal variation was observed in the *S. squamosum* encountered. Most of them showed complete synapsis in chromosome I, while some of the remaining few have short asynaptic centromeric region of chromosome I. All the specimens had homozygous inversions at both arms of chromosome I. Some specimens found at Imeri A and B showed floating inversions on IL and IIL, which was not present on any of the specimens obtained at Ose-Oba.

S. damnosum s.s were observed to be abundant in the two breading sites at Imeri (Imeri A and Imeri B). The females had homozygous inversion (C/C) on the long arm of chromosome II (IIL). Most of the specimens were homozygous, apart from some males that showed heterozygous inversion (C/C.8) on IIL (long arm of chromosome II). Amongst the *S. sirbanum* examined, inversions IL-3.1 on long arm of chromosome I was sex-linked, two inversions IS-1.3 and IIL-C.8 that were on the short arm of chromosome I and long arm of chromosome II, respectively were fixed within the specimen.

Majority of the *S. alcocki* specimens (Figure 5) had one or two inversions at either the short or long arms of

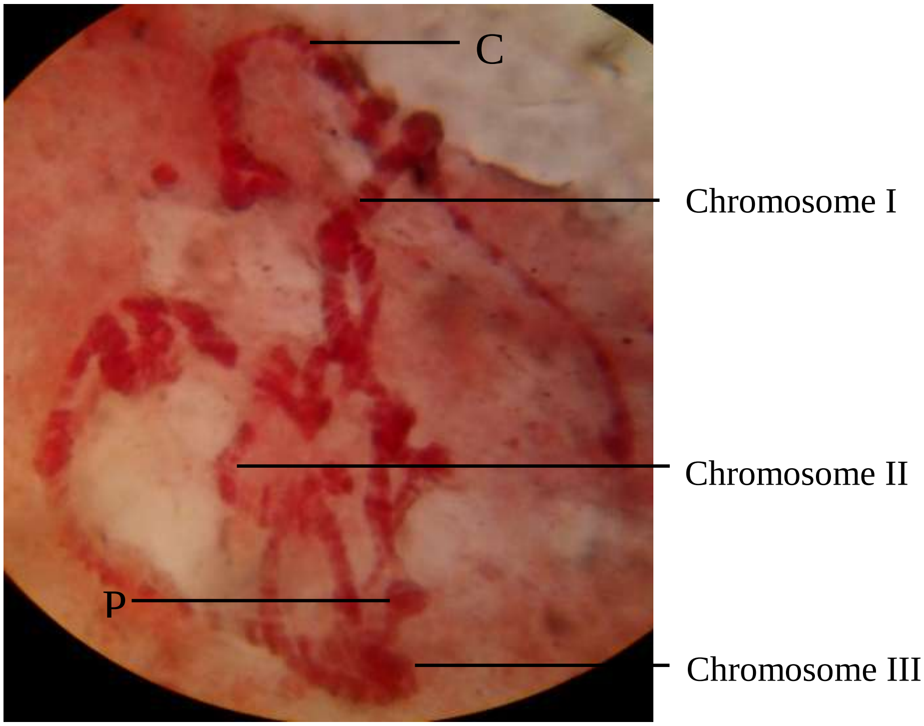

Figure 6. YI secies chromosome (x1000). C- Centromere, P-puff.

chromosome II (IIS or IIL). These inversions appeared to be fixed across specimens from each of the breeding sites, while specimens of the yet to be identified (YI) species (Figure 6) showed homogeneous and constant banding patterns. Asynaptic regions were also not frequent in this population. Arbitrary alphabets were adopted to denote inversions in *S. alcocki* and the yet to be identified species for better understanding since there was no documented standard chromosome map for comparison.

DISCUSSION

Based on morphological studies carried out on the larvae found in segments of Ose-river, findings revealed sympatric composition of three different species, although there was significant variation in the occurrence of these species along the river. This observation agrees with the earlier report of Opoku (2006) who observed that ecology plays a key role in the distribution of black flies. The most wide spread species in the three sites investigated were *S. alcocki* and *S. damnosun* which was consistent with the earlier findings of Tangjura et al. (2015) in Nasarawa State where higher occurrences of *S. damnosum* and *S. alcocki* were recorded from some rivers. The third species 'YI' was only recorded in two of the sites investigated. This species, as described in this present study, has similar morphology with the one reported earlier by Sorungbe (2014), which he denoted as unspecified species (US2) from Osun State.

The inversions on chromosomes I, II, and III of *S. damnosum* complex revealed three different cytospecies. *S. damnosum* s.s is the most wide spread member of the complex and the one having the highest occurrence in all the sites investigated. There was diversity in this sibling species across the breeding sites. Similar findings had also been reported by Post et al. (2007) and Olusi et al. (2012). This probably suggests that the flies must have invaded Ose River from different parts of the country since blackflies are reputed to undertake regular wind assisted migration covering distances of over 400 km, coupled with the fact that River OSE appears to be a good breeding site.

S. sirbanum was also found to be present in all the breeding sites and in sympatry with *S. damnosun* s.s. Their presence in this study was consistent with the work of Olusi et al. (2012); and Crosskey (1981) in a review of Nigerian cytospecies of the *S. damnosum* complex. Other workers, Wilson et al. (1994), and Onyenwe et al. (2007) also confirmed the presence of *S. sirbanum* in Nigeria. Diversity existed within the *S. sirbanum* identified in this study which was also in conformity with the findings of Vajime (1989) who synonymized *S. sirbanum* and *S. sudanense* under the name *S. sirbanum* and described them as 'sub-siblings'. However Mafuyai (1992) did not recognize the different cytotypes within *S. sirbanum*.

S. squamosum was found abundantly at Imeri A and

Imeri B sites. Its occurrence at Ose-Oba was found to be very low. Its presence was consistent with the list made available by Vajime and Gregory (1990) where eight cytospecies of *S. damnosum* complex which included *S. squamosum* were documented. Wilson et al. (1994) confirmed the presence of *S. squamosum* as reported by earlier findings.

S. alcocki were well distributed in the three different sites of the present study. However, the specimens obtained at Ose-Oba revealed more of similar inversions, and were different in parts to the inversions obtained from the other two sites of Imeri A and Imeri B. This suggests that this species comprises of more than one sibling species since there were differences in their cytology as well as breeding sites. Although the result obtained in the present study alone is not enough to justify this suggestion as there was no antecedent records to back it up, Sorungbe (2014) and Tangjura et al. (2015) had reported it's occurrence in Osun and Nassarawa States, respectively.

The specimens of 'YI' species obtained were very few. They were found mainly at imeri A and Imeri B, with no occurring in Ose-Oba. The members of this group showed little variation in distribution of inversions which suggests that they were monotypic species. This particular species was similar to the US2 species described by Sorungbe (2014) from Osun State.

Meanwhile, members of *S. damnosum* complex are solely responsible for transmission of onchocerciasis in West Africa (Boakye, 1999). Although some species like *S. alcocki* had hardly ever been reported as vectors, they can however constitute biting nuisances which can be almost unbearable. Their females, like other black flies, swarm about the head of humans in large numbers, intermittently landing and crawling on any exposed skin or darting into the eyes, ears, mouth and nostrils. All these could have implications on socio-economic well-being of the human population and consequentially culminate in overall low productivity of residents of *Simulium* infested areas. It has also been reported that the sites of bites of these flies can serve as portal of entry for bacterial infection.

The awareness of the species richness of these flies is a necessary imperative in the quest for an effective and successful control measures against them and the diseases they transmit.

Conclusion

This present study which involved identification of larvae based on morphology, as well as some pupae revealed three different species; *S. damnosum* complex, *S. alcocki,* and a yet to be identified species 'YI'. However, investigations carried out on the banding pattern of their polytene chromosomes showed that the *S. damnosum* was a complex of sibling species from which three siblings, *S. damnosum* s.s., *S. sirbanum*, and *S. squamosum* were reported, and they were widely distributed in the entire breeding sites studied along Ose-river. *S. alcocki*; as well as the yet to be identified (YI) species showed various patterns of bands with certain cytotypes having similar bands that were quite different from one another. This suggests that these species comprises of cytospecies.

RECOMMENDATION

Despite the effectiveness of Onchocerca Control Programs (OCP) and other programs directed at eliminating Onchocerciasis, blackflies still breed at such a rate that bring about great economic loss and threat to the success of any forms of control measure. Therefore, more work is indeed needed to be carried out in the aspect of the biology of the fly. Such study might probably provide better insights in carrying out more effective control measures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Prevalence of the major ectoparasites of poultry in extensive and intensive farms in Jimma, southwestern Ethiopia

Wario Mata, Wako Galgalo and Kula Jilo*

School of Veterinary Medicine, Jimma University, Jimma, Ethiopia.

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Ectoparasites pose a serious health threat and constitute major impediments in poultry production in many countries of the world including Ethiopia. However, they are paid less attention as endoparasites and infectious diseases; the huge economic burden of the parasites need a comprehensive study encompassing both intensive and free range poultry in order to generate accurate information about the disease. The current study was designed to identify species composition, estimate prevalence and assess associated risk factor of ectoparasites of poultry in extensive and intensive farms in and around Jimma town. A cross sectional study was conducted from January to June 2017 and a total of 384 chickens from purposively selected two intensive farms (n=222) and randomly selected free range systems (n=162) were sampled by systematic random sampling technique. Ectoparasites were collected from different parts of the body including skin scrapings from the shank and base of the wing. Breed, ages, sexes and management system were recorded. This study showed overall prevalence of 65.6% and lice, fleas and mites were predominant ectoparasite in the current area with prevalence rates of 28, 26.6 and 10.9% respectively. Logistic regression analysis showed that ectoparasite infestation was significantly higher in local than exotic chickens (OR=12; CI=7.320-19.673; P<0.001). Regarding ages, adults were found to be 6.29 more likely susceptible to ectoparasites than young chickens (OR=6.29; CI=3.745-10.587; P<0.001). Similarly, statistically significant variation was encountered between sexes as females were more infested than male chicken in the current study (OR=1.48; CI=1.277-2.242; P=0.040). Additionally, chickens kept under extensive management were significantly prone to ectoparasites than that kept under intensive management system (OR=8.12; CI=5.012-13.164; P<0.001). Generally, the study revealed that ectoparasites are highly prevalent in extensive farming system than in intensive farming system and in exotic than local chicken. Therefore, control of ectoparasites and creation of awareness in the community on the overall effect of ectoparasites on productivity of poultry is highly recommended.

Key words: Prevalence, chicken, poultry, ectoparasites, intensive, extensive farm.

INTRODUCTION

Ethiopia is endowed with a very large and diverse livestock resources that is composed of approximately 56.71 million cattle, 29.33 million sheep, 29.11 million goats, 2.03 million horses, 7.43 million donkeys, 0.4

*Corresponding author. E-mail: kula.jilo1@gmail.com.

million mules, 1.16 million camels, 56.87 million poultry and 5.88 million bee hives (CSA, 2016). Ethiopia's economy is predominantly agricultural where the livestock sub-sector plays a substantial role by providing meat, milk, hide, power, and traction for agricultural purpose and fertilizer for increasing the productivity of smallholding (Minjauw and Mcleod, 2003). Despite the large animal population with a high potential for production, its utilization is far lower than could be expected due to cattle production in Ethiopia is constrained by the compound effects of animal diseases, poor management and low genetic performance (Jilo et al., 2016, 2017a, b; Dabasa et al., 2017a, b). Poultry has been accepted as one of the most important sources of animal protein for humans in Ethiopia and elsewhere. In most parts of Ethiopia, consumers have high preferences for poultry products particularly during festivals. It is also suggested that poultry products provide proteins of high biological value (Kondombo, 2005).

The proportional contribution of poultry to the total animal protein production of the world by the year 2020 is believed to increase to 40%, the major increase being in the developing world (Delgado et al., 1999). The Ethiopian Livestock Master Plan stipulates that poultry will make up 30% of national protein demand by 2030 from current 5%, demanding meat production to grow by 235% and egg production by 828% (AACCSA, 2016). In Ethiopia, indigenous chickens can be found in almost all households in rural areas and about 99% of chickens are maintained under a traditional system with little or no inputs for housing, feeding or health care and are characterized by low output levels (Tadelle, 2003). Studies revealed that parasitic diseases particularly, ectoparasites are the major impediment to animal health worldwide by the direct and indirect losses they cause (Swai et al., 2007; Jilo et al., 2016; Dabasa et al., 2017a, b). They can affect bird health directly by causing irritation, discomfort, competing for feed, tissue damage, blood loss, toxicosis, allergies and dermatitis which in turn alleviate quality and quantities of meat and egg production. Also, they act as mechanical or biological vectors transmitting number of pathogens (Mekuria and Gezahegn, 2010; Tamiru et al., 2014; Ikpeze et al., 2017). Some of the ectoparasites of poultry like ticks, lice and mites play an important role in the transmission of certain pathogens which cause heavy economic losses to poultry industry in addition to direct effect of causing morbidity by sucking blood and causing irritation to the birds which adversely affects economic production of poultry (Arends, 2003; Sofunmade, 2003; Maina, 2005).

In developing countries, concomitant factors such as suboptimal management, lack of supplementary feed, low genetic potential, high morbidity and mortality rate due to various diseases may be attributed to low production and productivity (Zarith et al., 2017). External parasites are common in tropical countries because of the favorable climatic conditions for their development and the poor standards of husbandry practices (Mungube et al., 2006; Jilo et al., 2016, 2017a, b; Dabasa et al., 2017a, b). In Ethiopia, chicken infestation with external parasites poses a challenge to free-range chickens' productivity and associated benefits since there is inappropriate housing and lack of appreciable pest control efforts (Amede et al., 2011).

The incidence of mortality and morbidity due to different ectoparasitic diseases in chicken demands serious efforts to curtail the diseases. However, despite their devastating effects, ectoparasites receive less attention than endoparasites and infectious diseases in almost all the production systems. Even though, it has been attempted by few researchers (Belihu et al., 2010; Mekuria and Gezahegn, 2010; Amede et al., 2011; Tolossa and Tafesse; 2013; Dabasa et al., 2017a, b) there is no enough information concerning the species composition, distribution, burden, and economic impact of ectoparasite in different parts of Ethiopia (Dabasa et al., 2017b). Particularly, there is limited information in the prevalence and species composition of poultry ectoparasites in the current study area. This contributes to a problem in poultry disease control, planning, monitoring and evaluation strategy of the country for rural poultry programs (Arends, 2003). Given the huge economic burden of ectoparasites in poultry, a comprehensive study encompassing both intensive and free range poultry rearing is of paramount importance to generate accurate information and thereby, design effective disease control and prevention strategies accordingly. Therefore, the objectives of this study were to identify species composition, assess prevalence and associated risk factor of ectoparasite of poultry in extensive and intensive farms in and around Jimma town.

MATERIALS AND METHODS

Study area

The study was conducted from January to June 2017 to determine the prevalence and associated risk factors of poultry ectoparasites in and around Jimma town, south western Ethiopia. Jimma town is located in Oromia region, south west of Ethiopia, at a distance of about 352 km from Addis Ababa. Geographically, Jimma is located at 7°13' and 8°56' N latitude and 35°52' and 37°37E longitude. The climatic condition of the area is 'Woynadega' with altitude ranging between 1720 and 2110 m above sea level and receives annual rainfall which ranges between 1200 and 2000 mm. There are two rain seasons, short rainy season (November to April) and long rainy season (July to October). The annual mean temperature ranges from about 12.1 to 28°C; Jimma zone has a livestock population of about 570,241 poultry, 2,200,106 cattle, 824,208 sheep, 411,180 goats, 92,093 horses, 71,880 donkeys, 20,011 mules and 570,241 beehives (CSA, 2016).

Study population

The population of interest was poultry, and 384 chickens were randomly selected from different production systems in and around

Table 1. Prevalence of ectoparasites types and their respective predilection sites in extensive and intensive farms in Jimma, southwestern Ethiopia.

Ectoparasite	No. of infested chicken	Prevalence (%)	Predilection sites
Lice	108	28	All body parts
Flea	102	26.6	Head, comb, neck and wattle
Mite	42	10.9	Subcutaneous tissues of tigh and base of wings
Total	**252**	**65.6**	

Jimma town and all age, sex and breed were considered. Kitto Furdisa campus and Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) poultry farms were purposely selected for their intensive farming system, and extensive farms of small holder poultry rearing in around Jimma town was also randomly sampled at JUCAVM open air clinic. Information like biodata and management system of poultry were obtained from the owners visiting JUCAVM clinic to treat their flock while age of the chickens were determined based on the size of crown, length of spur and flexibility of the xiphoid cartilage together with observing color of the shank and growth of the spur and categorized as young grower (less than 12 weeks of age) and adult (greater than 12 weeks of age) (Tamiru et al., 2014).

Study design and sample size determination

A cross sectional study was carried out from March to May 2017 by sampling ectoparasites from 384 poultry to identify species composition, determine prevalence and other risk factor of poultry ectoparasites in the study area. Sample size was determined based on the formula provided by Thrusfield (2005).

$$n= (1.96)^2 P_{xep} (1-P_{xep}) /d^2$$

Where, n = required sample size, P_{exp} = expected prevalence and d = desired absolute precision.

Sample size was determined using 95% level of confidence; 50% expected prevalence since there was no previous work in this study area and 0.05% desired absolute precision was taken. Accordingly, a total 384 chickens were sampled for this study.

Study methodology

During sample collection, bird’s legs were tied with the help of an assistant and feathers manually deflected to observe the presence of parasite. After restraining, samples were taken randomly from head, vent, neck, leg, back, wattle, comb, and wing by using naked eye and hand lenses. A systematic approach was employed to detect and collect ectoparasites and thus, head was examined first and followed by the neck, body sides, back, ventral part of the abdomen; wings, vent area and legs. Lice and fleas were collected from hosts by parting the hairs or feathers, gently brushing the base of the feathers with a fine soft brush so as to prevent the chickens from injuries and some of them was collected by hand picking and non-toothed thumb forceps whereas, mites were collected by scraping the skin surface with scalpel blade and shank scraps were collected on clean Petri-dish. Each chicken examined were assigned a serial number on the sampling bottle for easy identification. Likewise, bio data of each chicken like sex, breed, age, and predilection sites and managements systems were recorded on format prepared for this purpose.

Representative of ectoparasite found in body of the chickens were put in universal bottle (film holders, vial) containing 70% alcohol and predilection sites of the body and hypothesized risk factor were also simultaneously labeled with water proof pencil. At JUCAVM Veterinary Parasitology Laboratory, lice and fleas were transferred from universal bottle to the clean Petri-dish, mounted under stereomicroscope and identified whereas, wet film was prepared from the scrap and 10% potassium hydroxide was added to digest debris and then examined under light microscope. Identifications of all ectoparasites were performed according to their morphological characteristics using, entomological keys as described in standard books such as Soulsby (1982), William (2010), Urquhart (1996), and Wall and Shearer (2001).

Data managements and statistical analysis

All collected data were entered into Micro-Soft Excel sheet 2010 and analyzed by SPSS version 20. Descriptive statistics was used to determine the frequency and percentage of both dependent and independent variables. The prevalence was calculated as a percent of infected animals from the total number of animals examined. Pearson’s chi-square(X^2) and logistic regression were applied to assess association of different variables. For statistical analysis, a confidence level of 95% and P-values less than 5% was judged as significant.

RESULTS

Overall prevalence of ectoparasites

In the current study, a total of 384 chickens of local and exotic breed kept under different management systems were examined and 252 (65.6%) of them were found infested with one or more species of ectoparasites. Accordingly, three major groups of poultry ectoparasites identified were lice, fleas and mites with prevalence rates of 28, 26.6 and 10.9% respectively (Table 1). Lice were encountered from all body parts examined and relatively highly infesting among ectoparasites of poultry in this area followed by fleas and mites respectively. However, fleas encountered were restricted to head, comb, neck and wattle while mites were found on subcutaneous tissues of thigh and base of wings (Table 2).

Regarding species of ectoparasites of poultry in the present study, seven species were identified. *Echidnophaga gallinacean* was the most prevalent ectoparasite with prevalence of 26.6% while Menacanthus *stramineus* (1.5%) was the least. *Lipeurus caponis* (14%) was the second most prevalent species infesting poultry followed by *Menopon gallinae* (7.8%),

Table 2. Prevalence of ectoparasites species and their respective predilection sites in extensive and intensive farms in Jimma, southwestern Ethiopia

Species of ectoparasite	Number of infected chickens	Prevalence (%)	Predilection sites
Dermanyssus gallinae	30	7.8	Thigh, base of wing
Cnemidocoptes mutans,	12	3.1	Thigh, base of wing
Lipeurus caponis	54	14	All body parts
Menopon gallinae	30	7.8	All body parts
Menacanthus stramineus	6	1.5	All body parts
Cuclotogaster heterographus	18	4.7	All body parts
Echidnophaga gallinacean	102	26.6	Head, comb, neck and wattle
Total	**252**	**65.6**	

Table 3. Distribution of lice infestation of poultry among different risk factors in extensive and intensive farms in Jimma, southwestern Ethiopia.

Variable		Number examined	Number positive	Prevalence (%)	X^2(P-Value)
Breed	Local	180	66	36.7	32.8(0.000)
	Exotic	204	42	20.6	
Age	Young	174	12	6.9	45.7(0.000)
	Adult	210	96	45.7	
Sex	Female	240	78	32.5	30.2(0.000)
	Male	144	30	20.8	
Management	Extensive	162	66	40.7	43.1(0.000)
	Intensive	222	44	19.8	

X^2 = Pearson Chi-square.

Cuclotogaster *heterographus* (4.7%) and *Cnemidocoptes mutans* (3.1%) in that order (Table 3).

Lice infestation

In the current study, 108 (28%) chickens were found positive for lice infestation and four species were identified. *L. caponis, M. gallinae*, *C. heterographus* and *M. stramineus* were identified species with prevalence rates of 14, 7.8, 4.7 and 1.5% respectively. The infestation of lice was higher in local breed (36.7%) than exotic breed (20.6%) and this variation was statistically significantly (X^2=32.8; P<0.001). In the age wise prevalence, adult chickens (45.7%) were more infested than young (6.9%) with a statistically significant variation (X^2=45.7; P<0.001). There were significant differences between the two sexes and management system as females (32.5%) were more infested than males (20.8%) (X^2=30.2; P<0.001). The extensively managed chickens (40.7%) were more infested than intensively managed chickens (19.8%) (X^2=43.1; P<0.001) (Table 3).

Fleas infestation

In the current study, 102 (26.6%) chickens were found infested with fleas that were collected from head, comb, neck and wattles of sampled chickens. *E. gallinacean* (stick tight flea) was the only species of flea identified from the present study area and its distribution among different animal related risk factors and management system employed were found varying. Accordingly, the prevalence of flea infestation was higher in local breed (43.3%) than exotic breed (11.7%). This variation was statistically significantly (X^2=48.85; P<0.001). Similarly, there was statistically significant difference between age groups as adult birds (25.8%) were found more prone to flea infestation than growers (6.9%) (X^2=15.59; P<0.001). Regarding the management system, significant variation was also revealed and infestation of fleas was higher in extensively managed (37%) than intensively managed (18.9%) chickens (X^2=15.76; P<0.001). Regarding sex, prevalence of fleas infestation was higher in male (27.8%) than in female chickens (25.8%). However, this variation was not statistically significant (X^2=0.17;

Table 4. Distribution of flea infestation of poultry among different risk factors in extensive and intensive farms in Jimma, southwestern Ethiopia.

Variable		Number examined	Number positive	Prevalence (%)	X^2(P-Value)
Breed	Local	180	78	43.3	48.85(0.000)
	Exotic	204	24	11.7	
Age	Young	174	12	6.9	15.59(0.000)
	Adult	210	90	42.9	
Sex	Female	240	62	25.8	0.17(0.721)
	Male	144	40	27.8	
Management	Extensive	162	60	37	15.76(0.000)
	Intensive	222	42	18.9	

X^2 = Pearson chi-square.

Table 5. Distribution of mite infestation of poultry among different risk factors in extensive and intensive farms in Jimma, southwestern Ethiopia.

Variable		Number examined	Number positive	Prevalence (%)	X^2(P-Value)
Breed	Local	180	42	23.3	53.45(0.000)
	Exotic	204	0	0	
Age	Young	174	0	0	17.05(0.000)
	Adult	210	42	20	
Sex	Female	240	12	5	26.45(0.000)
	Male	144	30	20.8	
Management	Extensive	162	42	25.9	64.62(0.000)
	Intensive	222	0	0	

X^2 = Pearson chi-square.

P=0.721) (Table 4).

Mite infestation

This study revealed an overall prevalence of 10.9% of mite infestation on the body surface subcutaneous tissue of the chicken. *C. mutans* (14%) and *D. gallinae* (7.8%) were two species of mites identified from the current study area. The occurrence of mite infestation was found to vary among different categories of studied chickens and their respective management system. To this effect, statistically significant difference in mite infestation was observed between breeds, age groups, sexes of chicken and management system. The local breed (23.3%) was found highly susceptible to mite infestation than exotic breed (0%) (X^2=53.45; p<0.001). Similarly, chickens kept under extensive management system (25.9%) were highly prone to mite infestation than that kept under intensive management (0%) (X^2=64.62; p<0.001). Regarding age groups of examined chickens, the mite infestation was encountered only in adult birds (20%) (X^2=17.05; P=0.000). Concerning sex, male chicken was more infested than female ones with prevalence rates of 20.8 and 5% respectively and this variation was statistically significant (X^2=26.45; p<0.001) (Table 5).

Risk factors

In this study, variables like breed (local and exotic), ages, sexes and management system were considered as risk factors for ectoparasite infestation of poultry in and around Jimma town. Likewise, the total of 384 chickens was examined and the overall prevalence 65.6% was recorded showing significant variations among all hypothesized risk factors for infestation of chickens with ectoparasites at current study area.

Table 6. Association of chicken ectoparasite infestation with assessed risk factors.

Risk factors		Number examined	Number positive	Prevalence (%)	OR	95% CI	P-Value
Breed	Local	180	150	83.3	12	7.320-19.673	0.000*
	Exotic	204	60	29.4	Ref*	0.172-0.338	
Age	Young	174	96	55.2	Ref*	0.540-0.718	0.000*
	Adult	210	186	88.6	6.29	3.745-10.587	
Sex	Female	240	140	58.3	1.48	1.277-2.242	0.040*
	Male	144	70	48.6	Ref*	0.735-1.011	
Mgt	Ext	162	132	81.5	8.12	5.012-13.164	0.000*
	Int	222	78	35	Ref*	0.076-0.200	

OR = Odd ratio; CI = Confidence of Interval; Mgt = Management; Ext = Extensive; Int = Intensive;Ref* = Reference;* = Significant.

Generally, local breed of chicken was found more prone to ectoparasites than exotic breed with statistically significant variation (OR=12; CI=7.320-19.673; p<0.001). Regarding age of examined chickens, statistically significant variation was observed and adults were found more susceptible for ectoparasites than young chickens (OR=6.29; CI=3.745-10.587; P=0.000). Similarly, statistically significant variation was encountered between sexes of chickens as females were more infested than male chicken in the current study (OR=1.48; CI=1.277-2.242; P=0.040). In the same way, chickens kept under extensive management were significantly prone to ectoparasites than that kept under intensive management system (OR=8.12; CI=5.012-13.164; P=0.000) (Table 6).

DISCUSSION

Poultry provides a valuable protein to the diets of people worldwide and is an important source of egg production and many types and species of ectoparasites such as flies, lice, mite, and ticks are known to infest chicken (Ensminger, 1992). Ectoparasites damage feathers, irritate and cause skin lesions, resulting in reduced performance of old chickens and direct harm to young chicks (Arends, 2003). Controlling ectoparasites in poultry flocks results in healthier and more economically productive birds for the pleasure and benefit of rural families (Moyer et al., 2002). In the present study, lice, fleas and mite were common ectoparasites infesting chickens with the overall prevalence of 65.6% out of 384 examined of which chickens 252 were found harboring at least one species of external parasites.

The observed overall prevalence of 65.6% of ectoaparasite infestation in the current study conceded with results of 67.95 and 70.73% from ambo district (Tamiru et al., 2014) and Meerut, (Kansal and Singh, 2014) respectively. However, the lower prevalence of 41 and 2.6% was reported by Nnadi and George (2010) and Tolossa and Tafesse (2013) from Nigeria and Fayoumi Farm, Ethiopia Ethiopia, respectively. On other hand, higher prevalence of 86.67% from Bangladesh (shanta et al., 2006), 91.5% from Central Ethiopia (Belihu et al., 2010), and 100% from Nigeria (Bala et al., 2011*)* were reported. The difference between the current and previous findings may be due to difference in breed, season of study, management, agro ecological, and implemented methods of disease control and prevention practiced in the study area, which exposes the chickens to poor hygiene on the farm and chicken houses thus, enabling them to contract a wide range of harmful ectoparasites.

The current study revealed that lice infestation was the most common among the chickens examined in small holder poultry rearers in and around Jimma Town and intensive poultry farms of Jimma University. The overall prevalence of lice infestation obtained in the present study (28%) was lower than reports of 35.1, 72.72, 81.33, 84.3, 88 and 90% from East Ethiopia (Amede et al., 2011), Fayoumi farm Ethiopia (Belihu et al., 2010), Nigeria (Malann et al., 2016), Nigeria (Sadiq et al., 2003), Wolayta (Mekuria and Gezahegn, 2010), and Kenya (Sabuni et al., 2010), respectively.

These differences in prevalence may be attributed to differences management system, breed of chickens examined, in geographical areas, sample size and period of study. Different geographical areas and period of study have different climatic conditions (temperature and humidity) which may alter the population dynamics of the parasites (Tamiru et al., 2014). In addition, it might be associated with the poor hygienic practice in rural regions, which creates a favorable environment for parasites and the free-range system, which provides a more sustainable environment for the parasites.

During the present study, four species of lice were identified namely, *L. caponis*, *M. gallinae*, *C. heterographus* and *M. stramineus* with prevalence rates of 14, 7.8, 4.7 and 1.5%, respectively. Among the identified lice species, *L. caponis* (14%) was the most frequently occurring species while *M. stramineus* (1.5%) was the least prevalent. The prevalence of *L. caponis*(14%) is higher than those of Bala et al. (2011), Sadiq et al. (2003), and Biu et al. (2007), who reported 5, 3.7 and 6.27%, respectively.

M. gallinae was the second most prevalent lice species in the present study area with prevalence of 7.8% that closely conceded with the finding of Bala et al. (2011) who reported 8.1% in Nigeria. However, the current finding was lower than 14.3, 40.12 and 97.7% reported by Amede et al. (2011), Sabuni et al. (2010) and Sadiq et al. (2003), respectively.

C. heterographus (4.7%) was third in prevalence among lice species encountered during the current study. This result was lower than the findings of Amede et al. (2011), Belihu et al. (2011) and Mekuria and Gezahegn (2010) who reported prevalence of 7.4, 25 and 40%, respectively. *M. stramineus* (1.5%) was the least prevalent lice species in the present study while it was the most prevalent in Bangladesh (Shanta et al., 2006), Ethiopia (Belihu et al., 2010), (Bersabeh, 1999) and Nigeria (Bala et al., 2011), who reported 70, 41.7, 6.9 and 65.33% prevalence, respectively.

In the present study, flea was the second most prevalent ectoparasite of poultry with overall prevalence of 26.6%. However, *E. gallinacean* (stick tight flea) was the only species of flea identified from the present study area. The current finding was higher than 6 and 8% of *E. gallinacean* reported from Eastern Ethiopia (Amede et al., 2011) and Iran (Mirzaei et al., 2016). However, higher prevalence rates were reported by most scholars from different countries. For instance, 56% (Maina, 2005) and 76.7% (Mungube et al., 2008) were reported in Kenya. Similarly, 76.7% (Permin et al., 2002) infestation of *E. gallinacean* was reported in Zimbabwe.

This study revealed that, mites were the least prevalent ectoparasite with overall prevalence of 10.9%. *C. mutans* (14%) and *D. gallinae* (7.8%) were the two species of mites identified in the current study area. The overall prevalence recorded in the current study (10.9%) was closer to the finding of Mungube et al. (2008) who reported 13.3% of mite infestation in poultry from Kenya. However, the current finding was lower as compared to the findings of Permin et al. (2002) and Mania (2005) who reported the prevalence of 32 and 24%, respectively.

Generally, variations in prevalence and types of poultry ectoparasites encountered in the present study and aforementioned studies may be due to a variation in agro-climatic and topographic conditions, species adaptability, management system and husbandry practices which account for the difference in finding. In addition, duration and season of study might show the seasonal prevalence pattern of the parasites compared to the shorter one. Larger sample sizes depict the true reflection of what is on the ground compared to smaller sample sizes, hence the variation encountered. Collecting ectoparasites within a relatively short period minimizes errors since parasites have their own biology and populations that can vary rapidly in both space and time (Clayton and Drown, 2001). Furthermore, hygiene practice in the farm and chicken houses as well as control measures towards such parasites has great attribution for variation in poultry ectoparasite. Ectoparasite tends to be more of a problem in household flocks than commercial flocks, as commercial breeders do not permit parent-offspring contact.

Regarding age of examined chickens, statistically significant variation was observed and adults were found more susceptible for ectoparasites than young chickens (OR=6.29; CI=3.745-10.587; P=0.000). The adult chickens had prevalence of ectoparasite 88.6% which was higher than that of growers (55.2%). This association agreed with the studies of Permin et al. (2002) and Biu et al. (2007), Sabuni et al. (2010) and Nnadi and George (2010) in which adult chickens were highly infested as compared to young chickens. However, the current result disagrees with the findings of Sabuni et al. (2010) were young chickens were found more infested by ectoparasites than adult chickens. The higher prevalence of ectoparasite in adult chickens than younger one may be due to longer exposure to the infested environment than the young grower, hence a higher prevalence and intensity rates.

In the current study a statistically significant variation was encountered between sexes of chickens as females were more infested than male chicken (OR=1.48; CI=1.277-2.242; P=0.040). Female birds had higher prevalence (58.3%) than male which has prevalence of 48.6%. Higher prevalence of ectoparasite in female chickens than male was in contrast with the finding of Mungube et al. (2008) and of Belihu et al. (2010) who reported that males had a higher rate of occurrence of ectoparasites compared to female chickens. Additionally, Sabuni et al. (2010) and Amede et al. (2011) reported almost similar prevalence between males and females. But in lining with the current finding several researchers like Biu et al. (2007), Mekuria and Gezahegn (2010) and Bala et al. (2011), reported that hens had a higher prevalence of ectoparasites than cocks.

One of the reasons could be the stationary state of hens during the incubation of their eggs, which makes them more susceptible to parasitic infestations. Not only this, bedding materials and premises used during the incubation period may host parasites and may facilitate parasite infestation. In addition, it is also suggested that the odor that hens emit during incubation may attract parasites (Bala et al., 2011). Furthermore, cocks may introduce more parasites to the hens during mating, since the male is forced upon the female for every mating.

Local breed (83.3%) of chicken was found more prone to ectoparasites than exotic breed (29.4%) with statistically significant variation (OR=12; CI=7.320-19.673; P=0.000). The higher prevalence of ectoparasite in local breed in comparison with the exotic agreed with several findings reported in Ethiopia by Bala et al. (2011), Tolossa and Tafesse (2013) and Tamiru et al. (2014) who reported higher susceptibility of local breed to ectoparasites than exotic breeds. The higher prevalence observed in local breeds may be due to difference in management, hygienic practice and health care facility provided to the flocks. More importantly, in the current study almost all the local breed of chickens examined were owned by small holder farmers, kept under extensive management system at the back yard and free range system with poor hygiene and minimal health care provision whereas exotic breeds were sampled from intensively managed poultry farms of Jimma University (JUCAVM) and Kito Furdisa campus with better hygiene and health care services. Additionally, in free-range system chickens are entirely released and stay out door thus becomes more vulnerable to ectoparasite than exotic breed, which are almost kept in door.

In this study, chickens kept under extensive management were found significantly prone to ectoparasites than those kept under intensive management system (OR=8.12; CI=5.012-13.164; P=0.000). The overall prevalence in intensive management system (35%) of poultry farms owned by Jimma University while in extensive management system (81.5%) the result agreed with the finding of Mekuria and Gezahegn (2010) who report high prevalence in back yard system than in intensive system.

This variation is due to better measures and practices related to good housing, feeding and husbandry system applied in intensive farms. Extensive management could be due to the free-range system practiced in the study areas, which exposes the chickens to poor hygiene on the farm and chicken houses thus, enabling them to contract a wide range of harmful ectoparasites. Arend (2003) noted that management could be a contributing factor to the type of ectoparasites that are predominating in chicken houses. The extensive system provides a more sustainable environment for the parasites that lack of control measures towards these parasites was a possible factor contributing to the high prevalence of the parasites, becoming vulnerable to ectoparasitism (Mungube et al., 2008).

Moreover, inappropriate environmental conditions such as extreme temperature encourage the abundance of ectoparasite in poultry (Mekuria and Gezahegn, 2010). According to Arends (2003), *M. gallinae* were frequently found in a hot humid climate rather than in a hot dry condition. To this effect, unhygienic poultry farming carried out by the farmers that neglect the sanitation and poor ventilation may pave a way to the increment of ectoparasite infestation in free range farming system. According to Zarith et al. (2017) unsuitable housing as well as no additional food supplement is the most unethical practice conducted in traditional backyard poultry that make the poultry vulnerable to ectoparasite infestation.

CONCLUSIONS AND RECOMMENDATIONS

Poultry provides a valuable protein to the diets of people world- wide and is an important source of egg production. Some of the ectoparasites of poultry like ticks, lice and mites play an important role in the transmission of certain pathogens which cause heavy economic losses to poultry industry in addition to direct effect of causing morbidity by sucking blood and causing irritation to the birds which adversely affects economic production of poultry. The present study demonstrated the high burden of ectoparasites of poultry in Jimma Town and its surroundings with overall prevalence of 65.6%. Mite, lice and flea were the common types of ectoparasites in the study area. The observed overall prevalence of lice infestation was higher than that of mites and flea. Among the four species of lice identified *L. caponis* and M. *gallinae* were the most common species in the study area. Regarding fleas infestation, *E. gallinacean* (stick tight flea) was the only species of flea identified. Concerning mite infestation, two species of poultry mites (*C. mutans* and *D. gallinae)* were identified from the current study area with *C. mutans* found more commonly infesting than *D. gallinae*.

The occurrence of parasitic infestations found was influenced by a number of factors like breed, sex, age, and management. Local breed, female chickens, adult and chickens kept under extensive management were found highly infested as compared to exotic breed, male chickens, young and intensively managed chickens. Notably, the occurrence of ectoparasites was highly influenced by production system, being higher in the free range system than the intensive one as observed in this study. Ectoparasites affect the chickens by causing irritation, loss of weight, skin lesions that may be site of secondary infection, sucking blood, hence leading to anemia and death at times. In addition, external parasites act as mechanical or biological vectors transmitting a number of pathogens.

This study revealed high ectoparasite burden in chickens of the current study area which demands serious efforts to curtail the problem. High infestation of parasites can be reduced by a well-planned management of poultry, emphasizing on hygiene and suitable environment around the poultry farm and awareness creation to the farmers and farm farms staffs. It was concluded that, use of specific chemicals in the approved manner may also help the poultry farmers in the control of ectoparasites. Therefore, control of these ectoparasites and enlightenment campaign to the chicken rearers on the dangers resulting from ectoparasitic infestation on chickens should be instituted. Based on the above conclusions the following recommendations are forwarded:

1. Awareness should be created in the community on the overall effect of ectoparasites on productivity of poultry

and farmers, and extension staff should be trained regarding on improved housing, feeding, disease control and improved productivity of local chicken.

2. Government should take responsibility to provide the control measure to the farmers like regular pesticide applications

3. The role of the ectoparasites on the outbreaks of concurrent parasitic infection as well as on bacterial and viral infections should be determined.

4. Further studies are needed to identify more species and genus of poultry ectoparasites circulating in this area and to the direct and indirect economic losses of ectoparasite infestation in the area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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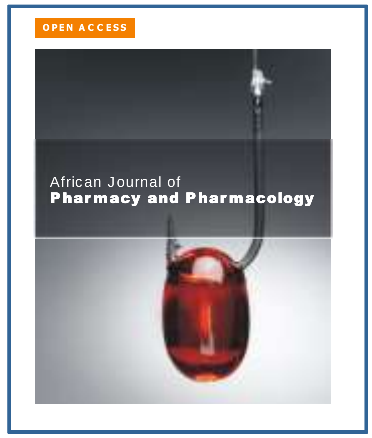

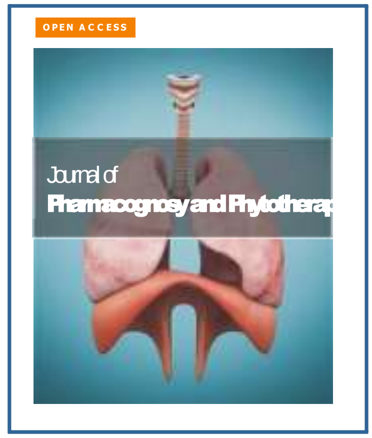

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