Laboratory and field tests of Carbaryl 5% against fleas in Lushoto district, Tanzania

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Abstract

Background and Objective: Lushoto district has been an active focus of plague disease since 1980 and many pesticides were used to control rodents and fleas from 1980 to 2003 when outbreaks occurred yearly. For over seven years ago commercial Carbaryl 5% powder has been used for controlling fleas in the area. However, there is no current research to substantiate its effectiveness either in the laboratory or in the field.

Methods: Immature stages of *Xenopsylla brasiliensis* were collected from two villages in Lushoto and transported to Sokoine University of Agriculture (SUA), Morogoro and reared in an insectary to stock colony. Known weights of commercial Carbaryl 5% powder were thoroughly mixed with known weights of clean fine sand as to obtain a final concentration of 0.05% (WW) of Carbaryl/sand mixture. The same concentration was suspended in 50cc distilled water and pieces of filter paper were soaked in the solution, left at room temperature until the suspension was fully adsorbed and the papers were left to dry. Adult fleas of mixed ages and of both sexes were obtained from the stock colony and exposed to both Carbaryl/sand mixture and Carbaryl-adsorbed filter papers for various periods of time. Field trials were carried out at Manolo and Viti villages where house and rodent flea indices were determined before and after dusting with commercial Carbaryl 5% powder.

Results: In the laboratory tests, 100% mortality occurred at 90 minutes exposure in Carbaryl/sand mixture experiments and at 35 minutes exposure to Carbaryl-adsorbed filter papers. LT_{50} in both sets of exposure was 48.2 min and 23.1 min in Carbaryl/sand mixture and Carbaryl-coated filter papers tests respectively. In field trials, *X. brasiliensis* and *Pulex irritans* were the most abundant flea species. Post-dusting flea populations were significantly lower in treated than in control houses (p=0.028). House flea indices dropped from 7.7 to 0.33 and 37.8 to 0 in Viti and Manolo villages respectively at 3 months post-dusting.

Conclusion: Commercial Carbaryl 5% powder in current use was still effective against potential flea vectors in Lushoto.

Keywords: Carbaryl, dusting, flea, flea index, plague, rodents, Tanzania

Introduction

Lushoto district in north-eastern Tanzania has been an active focus of plague for more than 20 years. However, there have been no reported cases of the disease in the district since 2003 (WHO, 2004). Many studies on the epidemiology and control of the disease have been carried out in the district since its emergence in 1980 (Kilonzo *et al.,* 1992). Through such studies, several species of rodents have been proven to be suitable reservoirs/hosts of the causative agent of the disease (*Yersinia pestis*) and several flea species have been incriminated as potential vectors, both in the sylvatic and murine cycles of the disease (Kilonzo & Mhina, 1982; Njunwa *et al.,* 1989; Kilonzo *et al.,* 2006; Laudisoit *et al.,* 2007; Pape *et al.,* 2008). The serologically proven rodent reservoirs/hosts include *Mastomys natalensis, Arvicanthis nairobe* and *Rattus rattus* (Kilonzo *et al.,* 2005) while the potentially efficient flea vectors include *Xenopsylla brasiliensis, X. cheopis, Dinopsyllus lypusus* and *Pulex irritans* (Kilonzo *et al.,* 2000)*.* About 5.5% of domestic dogs have also

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been demonstrated to be suitable carriers of the pathogen in the area (Kilonzo *et al.*, 2006). Basically all the examined dogs and cats were heavily infested with fleas, most of which were *Ctenocephalides canis* and *Ctenocephalides felis* respectively , which have been reported to be potential vectors of the disease elsewhere (Pape *et al.*, 2008; Dobler & Pfeffer, 2011; Dantas-Torres & Otranto, 2014).

Control measures that included improvement of hygiene in and around households, community based education about the disease and application of insecticides and rodenticides for controlling fleas and rodents, respectively in and around households, were always applied during and soon after outbreaks (Kilonzo *et al.,* 2005). Initially, DDT was used as the insecticide of choice but this was later on discontinued due to its detrimental effects on the environment (Kilonzo & Shemangale, 1991). Cyfluthrin (1% WP) was then substituted and used for about one year but discontinued due to its failure to effectively control flea populations and consequent recurrence of outbreaks of the disease every year (Kilonzo *et al.,* 1992). After this discontinuation, Actellic Super dust (1.6% pirimiphos methyl + 0.3% permethrin) was used. The decision for this substitution was based on the demonstrated effectiveness of Actellic Super dust against *X. brasiliensis* and *X. cheopis* in captivity (Kilonzo and Shemangale, 1991), easy availability in the country, and highly subsidized prices aimed at enabling farmers to acquire the pesticide for controlling the Larger Grain Borer (*Prostephanus truncatus*), a major storage crop pest in the country. Its effectiveness in the field however seemed to be temporary since plague transmission and consequent outbreaks of the disease continued to occur in the district every year despite using the pesticide for about ten years especially in villages where the rodent flea index was observed to be more than 0.5 fleas per animal (Kilonzo, 1997).

As a consequence of this failure, Actellic Super was substituted by Carbaryl (5%) as an insecticide of choice for controlling fleas in the district, and is being used to date. After applying the insecticide for about five years, it is desirable to determine its current effectiveness against the common flea vectors in the district, and consequently establish scientific evidence on its efficacy for controlling the insects in the area.

Despite the failure to demonstrate *Y. pestis* in fleas by PCR techniques carried out during quiescent periods (Laudisoit *et al.,* 2007), earlier observations in the district incriminated *X. brasiliensis* as the most potential plague vector in the area. Such incrimination was based on its abundance on rodents (including plague positive species) and ubiquitous feeding habits. Likewise, *P. irritans* was considered to be the most potential vector in inter-human transmission of the disease. There are higher abundances and higher population densities in residential houses in villages experiencing frequent plague outbreaks compared to rare or no outbreaks of the disease in the focus. Such potentiality was based on the fact that breeding and resting habitats are close to peoples sleeping sites and that is in an efficient vector of plague elsewhere (Kilonzo *et al.,* 1992; Laudisoit *et al.,* 2007; Dantas-Torres & Otranto, 2014; Hieronimo *et al.*, 2014).

In view of the above observations, it was considered that effective control of the two species of fleas would be a potentially important undertaking that could interrupt transmission of plague and consequently prevent its outbreaks in the area. Establishment of the current status of efficacy of the currently used insecticide against *X. brasiliensis* and *P. irritans* in the district was therefore essential. The objective of the current study was to determine the current status of the effectiveness of Carbaryl 5% dust on the flea vectors of plague in Lushoto district and provide appropriate recommendations to the public health authorities. The present paper reports results of laboratory tests of Carbaryl (5%) against Lushoto *X. brasiliensis* population and field tests of the same pesticide against rodent and house fleas in two selected villages in Lushoto.

Materials and Methods

Study site

This study was carried out in Lushoto District in north-eastern Tanzania. Two villages, Viti and Manolo were selected for the study (Figure 1). The selection was based on the fact that both of them are prone to plague outbreaks and that they had experienced frequent outbreaks of the disease during the past three decades.
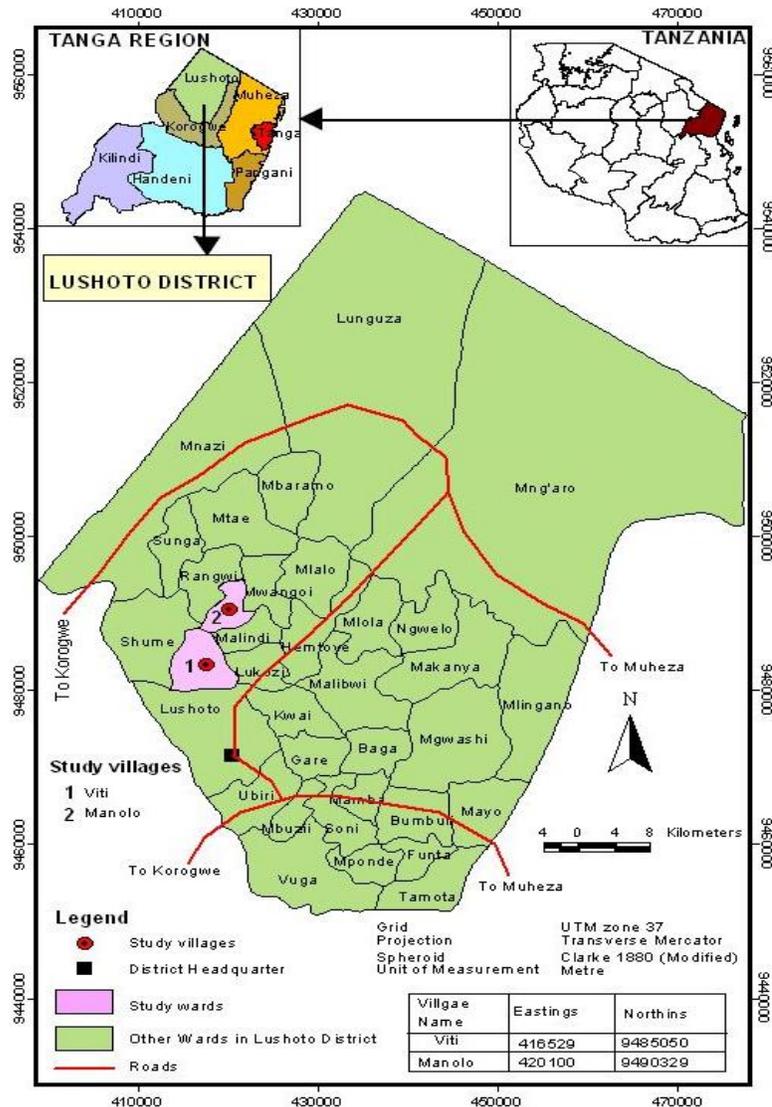

Figure 1: Map of Lushoto district showing study areas of Manolo and Viti Villages.

Collection and rearing of fleas

Live fleas were collected from live-trapped rodents in the study villages and transferred to a glass cage containing fine sand mixed with dry blood powder to serve as larval food, and white mouse confined in a wire mesh strainer, to serve as a source of blood meal for the adult fleas. They were then kept in a field laboratory in Lushoto until substantial numbers of larvae and cocoons appeared in the litter. All the larvae and cocoons in the cages were removed and transferred to fresh rearing jars and transferred to Sokoine University of Agriculture, Morogoro, where they were kept in a room maintained at 27±2.5°C and 80-85% relative humidity. Emerging adults were slightly anaesthetized with ether and identified under light microscope at 4X or 10X magnification

and all were found to be *Xenopsylla brasiliensis.* They were continually fed on white mice for four weeks when the colony (stock colony) expanded to about 1,000 adults and numerous larvae and cocoons.

Laboratory testing of Carbaryl and Carbaryl+sand mixture against X. brasiliensis

Commercial 5% Carbaryl powder (SAPA Chemicals Industries, Tanzania) was obtained from the stock being used in Lushoto and elsewhere in the country. Various mixtures of the powder were prepared by thoroughly mixing known weights of the chemical with known weights of sterile fine sand, so as to obtain the following concentrations (W/W) of pesticide: 0% (sand only - control), 0.5%, 1%, 5%, 10%, and 20%. Eighteen dry clean test tubes were prepared and serially numbered. Five grams of each mixture (concentration) were put in the tubes as follows: tubes 1-3 (0% -control), 4-6 (0.5%), 7-9 (1%), 10-12 (5%), 13-15 (10%) and 16-18 (20%). Ten adult fleas of both sexes and mixed ages were aspirated from the stock colony and put in each test tube. The tubes were stood at an insectary maintained at 27±2.5°C and relative humidity of 80-85% for 24 hours. At the end of this period, dead fleas in each tube were counted and recorded, considering moribund individuals as dead. The experiment was replicated three times; in each case, mortality rates in all tubes except the controls were 100%. The tests were repeated with lower concentrations of 0.01%, 0.05%, 0.1% and 0.3% of the mixture and replicated twice. A 0.05% Carbaryl/sand mixture was observed to be the lowest concentration producing 100% mortality at 24 hours exposures, and was hence used for determining periods of time exposure required to kill the target insect species.

To accomplish this, about 10g of 0.05% Carbaryl+sand mixture was put in each of 21 serially numbered, clean dry test tubes. Ten adult fleas of both sexes and mixed ages were aspirated from the stock colony and put in each tube. The tubes were kept in the same insectary conditions as for the tests above and left for various periods of time (24hr,15, 30, 45, 60, 75 and 90 minutes for tubes 1-3, 4-6, 7-9, 10-12, 13-15, 16-18 and 19-21, respectively). Numbers of dead/moribund fleas in each tube were recorded at the end of each corresponding period while live ones were transferred to holding tubes bearing corresponding numbers and partly filled with clean fine sand and kept at the same conditions for 24 hrs prior to determining final mortality rates. Five replicates were carried out and mortality rates for the various periods of exposure were determined (Figure 2).

Laboratory testing of filter paper – adsorbed Carbaryl against X. brasiliensis

Small pieces (20x 10cm) of filter paper (Whatman No. 1 product of Whatman International Ltd, England) were prepared and soaked in 50ml of 0.05% (W/V) Carbaryl+distilled water suspension in a flat-bottomed stainless steel tray. The tray was kept at room temperature overnight or until all the suspension was adsorbed onto the filter paper. Each piece of filter paper was put in a dry beaker and kept in a dark room maintained at room temperature until the paper dried completely. Treated (test) and untreated (control) papers were cut into small discs (2.5x1.0 cm) tapered at one end. Twenty four serially numbered clean dry test tubes (15cm long) were prepared as for the carbaryl+sand experiments above. Ten adult fleas of both sexes and mixed ages were aspirated from the stock colony and put in each tube. One untreated filter paper-disc was put in each of the tubes 1-3 (control) with the tapered end touching the bottom of the tube. The process was repeated with treated discs for tubes 4-24. The tubes were kept in a dark room at room temperature and humidity for varying periods of time ranging from 10 minutes to 24hr (Figure 3). Holding tubes numbered 4-24 were similarly prepared and provided with similar but untreated filter paper discs. At the end of each exposure period, fleas in tubes 4-24 were transferred to holding tubes bearing corresponding numbers and left for 24 hrs. Dead and moribund fleas in each tube were recorded and mortality rates were determined accordingly.

Field tests of Carbaryl (5%) against fleas

Twenty houses with muddy walls and dust floors were randomly selected at Viti and Manolo villages. Four Sherman and one local box traps baited with maize bran mixed with peanut butter and one light trap described by Kilonzo (1977) were set in each selected house. Fleas were collected from the captured animals and light traps every morning using the normal procedures described elsewhere (Kilonzo, 1976). The activity was continued for three consecutive days and mean numbers of fleas and their population densities (indices) were determined accordingly.

One fallow field was selected in each of the study villages and twenty Sherman traps baited as above, were set at each field. The traps were inspected every morning for three days consecutively, and fleas were collected from the captured animals and processed as above. All the captured rodents and their flea ectoparasites were identified and counted, and flea indices were determined accordingly

Houses with high flea infestation (at least 4 fleas per house) and those with low infestation (1-3 fleas/house) in each village were considered as test (T) and control (C) houses respectively. On the basis of these criteria, three houses in Viti and five houses in Manolo villages were targeted as test houses and hence dusted with commercial Carbaryl 5% powder while two and 4 houses in the two villages respectively were considered as control. Three days trapping and consequent collection of rodent and house fleas were repeated in all the houses and surrounding fallow fields after one week, one month and three months post-dusting. Flea indices were determined for each trapping period and compared with those of pre-dusting trapping periods.

Data analysis

All the collected data were entered in excel sheets and simple arithmetic was used to compute flea indices, and percentages were used to compute mortality rates of fleas tested in the laboratory. For data obtained from dusted houses (i.e. post-treatment data), statistical testing of the effect of Carbaryl (5%) was done using non-parametric bootstrapping, which was considered most appropriate due to unequal variance and non-normal distribution of the data. By using this test, with 10 000 interactions, the effect of treatment on the regression between weeks and numbers of fleas was tested, with village included as a co-variable.

Ethical consideration

This study received approval from Directorate of Research and Post-Graduate Studies of Sokoine University of Agriculture. All people whom participated in the study area were informed of the objective and methods of the study and voluntarily signed an informed consent form.

Results

Mortality rates of X. brasiliensis tested with Carbaryl+sand mixture and Carbaryl suspension adsorbed on filter papers

A total of 150 fleas were exposed for each selected period of time. Mean mortality rates ranged from 0% for control test (24h exposure) to 100% at 90 minutes exposure in Carbaryl/sand mixtures. Likewise, in tests with Carbaryl adsorbed on filter paper all the fleas died at 35 minutes exposure. The LT_{50s} in Carbaryl/sand mixture experiments and in adsorbed filter paper tests were 48.2 and 23.1 minutes respectively (Figures 2 and 3).

LT$_{50}$ = 48.2 minutes
LT$_{50:}$ Lethal time of exposure causing 50% mortality
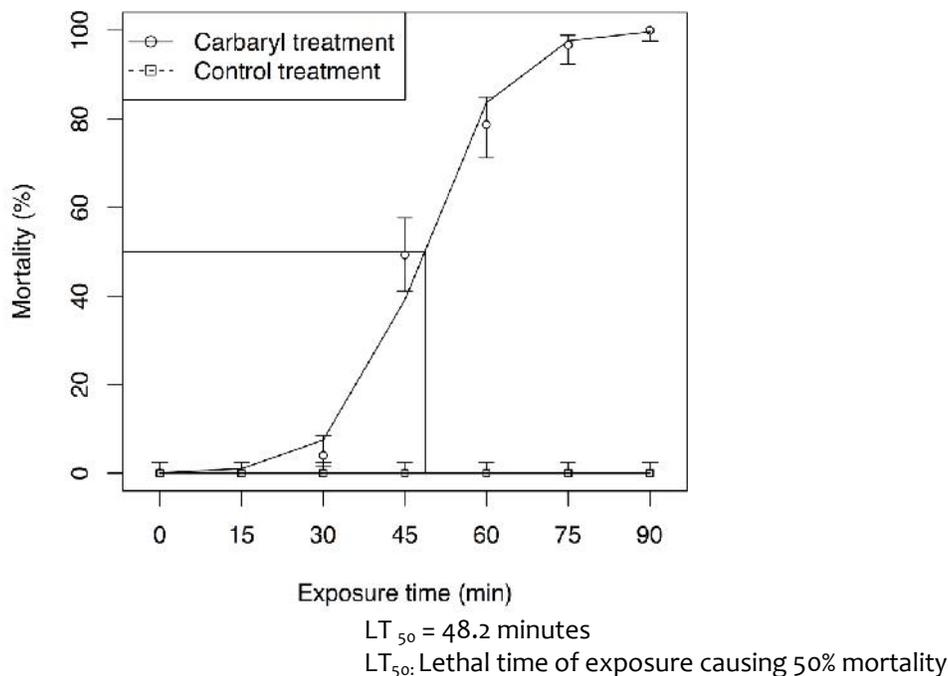
Figure 2: Mortality rates of fleas exposed to 0.05% Carbaryl+sand mixture

House flea populations at Viti and Manolo villages before and after dusting with Carbaryl 5%

Prior to dusting in Viti Village, 26 fleas were collected from five of the 20 trapped houses, making total and infested flea indices of 1.3 and 5.2 fleas per house respectively. Likewise 189 fleas were collected from nine of the 20 trapped houses in Manolo Village before dusting, making total and infested house flea indices of 9.5 and 21.0 respectively. After dusting however, flea populations in the treated houses in both villages dropped gradually after one week, one month and three months (Table 1). In Viti village for instance such populations dropped from 7.7 fleas per house to 0.33 fleas per house at 3 months post dusting. Similarly, in Manolo village, the populations in treated houses dropped from 37.8 fleas per house prior to dusting to zero three months post-dusting (Table 1).

LT$_{50}$ = 23.1Min
LT$_{50:}$ Lethal time of exposure causing 50% mortality
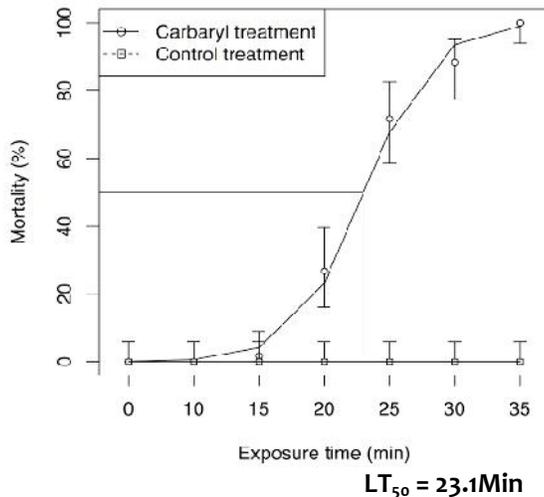
Figure 3: Mortality rate of fleas exposed to Carbaryl coated filter papers

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Table 1: Post-dusting flea populations in treated and control houses in Viti and Manolo Villages

Village	House categories	No of Houses	No of fleas collected	Flea index	After dusting					
					One week		One month		Three months	
					No of fleas	Flea index	No of fleas	Flea index	No of fleas	Flea index
Viti	Treated	3	23	7.7	2	0.67	0	0	1	0.33
	Control	2	3	1.5	0	0	2	1	1	0.5
Manolo	Treated	5	189	37.8	34	6.8	6	1.2	0	0
	Control	4	6	1.5	19	4.8	11	2.8	14	3.5

Species and numbers of rodents and their flea populations prior to dusting with Carbaryl 0.5%

A total of 37 house and field rodents (15 at Viti and 22 at Manolo) were live trapped and 62 flea ectoparasites were collected from rodents. Their total flea indices were 1.13 and 2.14 fleas per rodent for Viti and Manolo, respectively. As regards to species of captured rodents, all house-trapped rodents in both villages were *Rattus rattus* while the field-trapped rodents were *Mastomys natalensis*, *Grammomys spp* and *Lophuromys spp.* While *R. rattus* and *Lophuromys spp* were captured in both villages, *M. natalensis* and *Grammomys spp* were only captured in Manolo and Viti, respectively (Table 2).

Table 2: Species and number of fleas collected from house and rodents before and after dusting with Carbaryl 5%

Village	Flea host	Species and numbers of fleas collected						
		X. brasiliensis	*P. irritans.*	*D. lypusus.*	*E. gallinacea.*	*Ct. felis*	*Ct. canis*	No of fleas
Viti	Houses	14	10	-	-	-	4	28
	R. rattus	15	-	-	-	-	-	15
	Grammomys sp	-	-	1	-	-	-	1
	Luphuromys sp	-	-	1	-	-	-	1
	Total No of fleas on rodents	15	-	2	-	-	-	17
	Total fleas	29	10	2	-	-	4	45
Manolo	Houses	43	45	0	149	1		238
	R. rattus	24	4	3	-	-	-	31
	M. natalensis	1	-	12	-	-	-	13
	Luphuromys sp		-	3	-	-	-	3
	Total No of fleas on rodents	25	4	18	-	-	-	47
	Total fleas	68	49	18	149	1	-	285
	Total fleas in both villages	97	59	20	149	1	4	330

Post dusting flea populations

Only six *R. rattus* and one *M. natalensis* were captured in both treated and control houses at Viti and Manolo villages after dusting with Carbaryl 5%. Of the captured rodents, only one was caught in a treated house in Viti village and it had no fleas. However, in Manolo, three rodents with four fleas (Total index = 1.3) were caught in treated houses while 3 rats with no fleas were captured in control houses. Pre-dusting and post-dusting flea species collected from rodents in both villages comprised *Xenopsylla brasiliensis, Dinopsyllus lypusus* and *Pulex irritans.* Most fleas collected from house floors were *Echidnophaga gallinacea* and *P. irritans* followed by small numbers of *Ct. canis* and *Ct. felis* (Table 2). Carbaryl 5% powder was significantly effective on fleas in houses at the experimental villages (p=0.028). Flea populations in treated houses gradually decreased from the first week to the 12th week post-dusting (Figure 4). However, in the control houses such populations were more less the same during the same periods of time (Figure 4).
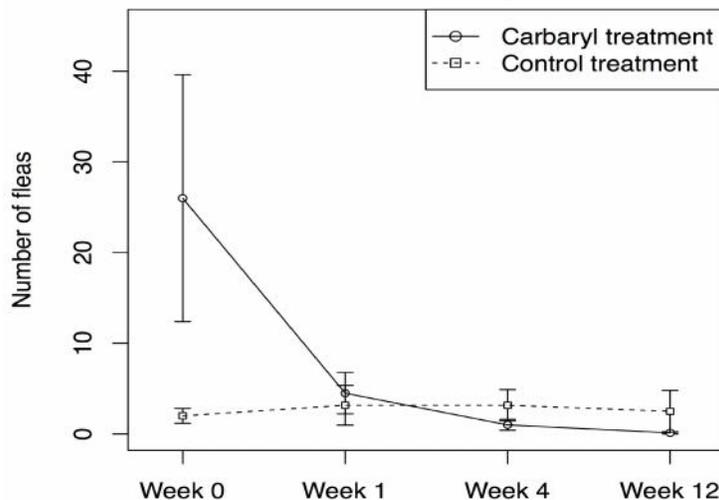

Figure 4: Numbers of fleas in control and Carbaryl-treated houses

Discussion

The current observation that *X. brasiliensis, D. lypusus* and *P. irritans* were the most abundant flea species in the study villages is consistent with several earlier observations in the district and elsewhere in Tanzania. This suggests that plague can easily spread if and when an outbreak of the disease occurs in the area (Kilonzo & Mhina, 1982; Kilonzo & Mtoi, 1985; Kilonzo *et al.,* 1992; Laudisoit *et al.,* 2007; Makundi *et al.,* 2008). The presence of large numbers of *E. gallinacea* in residential houses in Manolo village is likely to be attributed to the presence of local chickens in the experimental houses. Moreover this species of flea is mostly ectoparasitic on poultry and it is not important in plague epidemiology. The flea is considered a poor plague vector partly due to its "stick tight" behaviour on chickens (Laudisoit *et al.,* 2007) and limited biting habits on mammals. The fact that majority of *X. brasiliensis* were collected from *R. rattus* and residential houses, that most *D. lypusus* were collected from *M. natalensis*, and that the two rodent species are suitable plague reservoirs in the district and elsewhere in the country. The presence of the two rodent species further suggests potential transmission of the disease in the area, if and when favourable conditions prevail unless the fleas are effectively and promptly controlled (Kilonzo *et al.,* 1992, 2005; Makundi *et al.,* 2008; Katakweba *et al.,* 2012; Haule *et al.,* 2013; Ziwa *et al.,* 2013). Likewise, the observed abundances of *P. irritans* in houses is in consistence with several earlier observations (Kilonzo, 1976; Kilonzo *et al.,* 2000; Laudisoit *et al.,* 2007) suggests that effective control of the species is of utmost importance due to its potentially important role in plague epidemiology (Neerinckx *et al.,* 2008, 2010a; Dantas-Torres & Otranto, 2014).

The observations that 0.05% Carbaryl/sand mixture killed 100% of laboratory- reared adult *X. brasiliensis* at 90 minutes exposure, and that the same concentration killed all the fleas at exposure time of 35 minutes when suspended in water and adsorbed onto filter paper, suggests that the flea species in question is susceptible to the pesticide being tested, at least under laboratory conditions. These results suggest that effective application of Carbaryl in the field can result in substantial reduction of flea populations and consequent decrease in plague transmission since *X. brasiliensis* is the most abundant and most potential vector of the disease. Moreover, the species is the most ubiquitous in feeding habit and hence easily transmits disease pathogens between animal reservoirs and human hosts. Likewise the observed abundances of *P. irritans* and *D. lypusus* can be interpreted to suggest inter-human and inter-rodent transmission of the disease, respectively (Haule *et al.*, 2013).

The significantly higher pre-dusting house and rodent flea indices than post-dusting indices suggests that Carbaryl 5% is substantially effective against *X. brasiliensis* other flea species in the area. The gradual decrease of flea population densities from 1 to 12 weeks post-dusting suggests that more fleas, including those which were probably hiding in peoples beddings during and soon after dusting, got exposed to the pesticides when the latter remained on the floors of dusted houses for long periods. However, the flea populations did not drop to zero in Viti village after three months post-dusting, a fact which is probably attributable to the observation that some residents cleaned their houses prematurely, thus facilitating non-exposure of some fleas to the pesticide. Furthermore, the current observations revealed that pre-dusting flea indices were significantly higher in Manolo than in Viti villages while in the control houses such indices were similar. The observations that at 12 weeks post-dusting the flea indices in Manolo significantly dropped to zero in treated houses and increased to 3.5 in control houses further demonstrates current effectiveness of the insecticide on the fleas in the latter's natural environment and that it can be applied in plague control strategies.

It is broadly concluded on the basis of the current observations, that Carbaryl 5% is still effective for controlling common fleas in Lushoto district and possibly elsewhere in Tanzania. In view of the present observations therefore, its use as the insecticide of choice in controlling plague outbreaks as well as livestock infestation with fleas, is recommended. However, surveillance services on continued effectiveness of the pesticide against target pests/vectors should be established and sustained.

Acknowledgements

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