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Efficacy of Ivermectin for Control of Zoophilic Malaria Vectors in Pakistan*

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Abstract.- A voluminous body of research has reported that the therapeutic concentration of ivermectin in the blood/tissue of domestic animals can be lethal to zoophilic *Anopheles* mosquito species, when they ingest blood meal from treated animals. In Pakistan ivermectin is used for the treatment of endo and ecto-parasites in veterinary practices. Currently, no study is available in Pakistan showing the effect of ivermectin on the survivorship of malaria vector mosquitoes. These studies were undertaken to evaluate the insecticidal effect of ivermectin, on the survivorship of zoophilic malaria vectors *Anopheles culicifacies* and *A. stephensi* under field conditions of (district Okara) Punjab, Pakistan. *Anopheles* mosquitoes were sampled on cattle given ivermectin treatment. Insecticidal effect of ivermectin on the survivorship of *A. culicifacies* and *A. stephensi* was monitored for 12 days post blood feeding. In conclusion, ivermectin in a dose appropriate for cattle use led to a significant reduction in both *A. culicifacies* and *A. stephensi* survival when they fed on treated cattle, compared with controls. *A. stephensi* was found more susceptible than *A. culicifacies* to ivermectin after feeding on treated cattle and inseticidal effect was observed for 28 day post ivermectin treatment.

Key Words: *Anopheles culicifacies*, *Anopheles stephensi*, Ivermectin, zooprophylaxis, malaria vector, zoophilic macrolide antibiotic.

INTRODUCTION

In areas where *Anopheles* mosquitoes tend to be highly zoophilic, zooprophylaxis could be an effective strategy for controlling malaria (Mutero *et al.*, 2004; Mahande *et al.*, 2007; Mahande *et al.*, 2007; Simon *et al.*, 2008). Insecticide zooprophylaxis, is a method by which insecticide treatment is applied to domestic livestock, mosquitoes pick up a lethal dose when they attempt to bite a treated animal. In fact, Insecticide zooprophylaxis holds the added benefit of killing vectors immediately or over time.

Ivermectin, a semisynthetic marcrolide antibiotic is extensively used for control and treatment of parasitic infections in domestic animals and humans (Campbell *et al.*, 1983; Taylor and Greene, 1989; Ottesen *et al.*, 2008; Thylefors, 2008). The potential of ivermectin as systemic insecticide has been well recognized (Gabaldon, 1978) and is lethal to a wide variety of arthropods including mosquitoes (Jackson, 1989; Wilson, 1993).

Ivermectin agonizes the glutamate-gated chloride anion channels found in invertebrate postsynaptic neurons and neuromuscular junctions (Cully *et al.*, 1994; Kane *et al.*, 2000). This action hyperpolarizes the neurons and muscle fibers, leading to flaccid paralysis and insect death (Cully *et al.*, 1996; Bloomquist, 2003; Wolstenholm and Rogers, 2005). Ivermectin differs in its mode of action from the classes of insecticide (carbamates, pyrethroids, and organochlorines) currently used for ITNs and IRS (Hemingway and Ranson, 2005).

Numerous studies demonstrat that ivermectin reduced the daily survival rate of various mosquito species including Anophelines when they feed the drug in blood of man or variety of animals (Pampiglioni *et al.*,1985; Iakubovich *et al.*,1989; Nasci *et al.*,1990; Tesh and Guzman, 1990; Focks and Mclaughlin, 1991; Focks *et al.*,1991; Mahmood

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et al.,1991; Cartel *et al.*,1991; Jones *et al.*, 1992; Gardner *et al.*,1993; Bockarie *et al.*,1999; Fritz *et al.*,2009). Mosquito belonging to various species vary in their susceptibility to the insecticidal effect of ivermectin. Ivermectin is nontoxic to the environment because it neither retains nor accumulates in the environment.

In Pakistan, *A. culicifacies* and *A. stephensi* are malaria vectors (Jahan *et al.*, 2011) and are chiefly zoophilic. Ivermectin is also used for the treatment of endo and ecto parasites in veterinary practices. But no study is available showing the effect of ivermectin on the survivorship of malaria vector mosquitoes. Present study was designed to observe the toxic effect of ivermectin in cattle blood on the survivorship of wild population of *A. culicifacies* and *A. stephensi* under local field conditions.

MATERIALS AND METHODS

Eight cross bred calves weighing approximately 190-210 kg were randomly divided into two equal groups (four animals per group). In one group, animals were treated with ivermectin at the dose rate of 200 µg/kg of body weight subcutaneously. Before initiations of treatment, animals were weighed individually, so that appropriate treatment dose could be calculated. In other group animals received no treatment and served as control.

Wild *A. culicifacies* and *A. stephensi* mosquitoes feeding on ivermectin treated and control animals were sampled 1, 14 and 28-day post ivermectin treatment. For this purpose, treatment and control group animals were stationed at least 2.5 km apart from each other near known breading sites. Animals were confined under nets half-hour before sunset and one side of each bioassay net were raised 3□ above the ground. Animals were kept under those nets for the whole night and wild mosquitoes were allowed to feed on calves under nets. One collector entered into each net half-hour before sunrise and collected mosquito by mouth aspirator. In order to counter the collector's ability and site attractiveness animals were rotated between sites on each occasion.

Mosquitoes collected from ivermectin treated and control animals were placed in separate cages and brought to insectary maintained in a house in the study village Bhai Raokay of district Okara, Pakistan. From each cage blood engorged Anophline mosquitoes were sorted out and identified to species level.

After identification, blood engorged specimens of *A. culicifacies* and *A. stephensi* from control and treatment groups were transferred to separate cages and properly labeled. Mosquitoes were maintained at an ambient temperature for next twelve days. Mosquitoes were provided cotton wool pads soaked in 20% sugar solution. Mosquitoes were observed at the 12-hour intervals for survivorship. Dead mosquitoes were removed from each cage and counted. On sixth day of post feeding, remaining alive mosquitoes were offered blood feed from restrained rabbit for thirty minutes. After 24-hour of blood feeding pots for ovipositing were kept in cages. Records of death of *A. culicifacies* and *A. stephensi* from control and treatment groups were maintained on daily basis for twelve days of each post blood feeding.

Experiment was repeated thrice during March 2010 to October 2010. Every time new animals were used. Data from the three replicates were pooled for each species according to the post ivermectin treatment periods and processed for survivorship analysis.

Statistical analysis

For survival analysis mean, standard deviation (SD) and standard error (SE) of mean along its 95% confidence interval (CI) was made for descriptive analysis. Survival plots were made using Kaplan–Meier. Survival curves were compared using Log Rank test. Cox regression was also applied to predict the proportional hazards probability. Odd ratios (OR) and 95 % CI were also made.

All analyses were carried out using Microsoft Excel 2007 and statistical software, SPSS version 16 (SPSS Inc, Chicago, IL, USA).

RESULTS

Insecticidal effect of ivermectin on the survivorship of *A. culicifacies* and *A. stephensi* was

monitored for 28 days after the cattle were given ivermectin and sampled blood engorged mosquitoes were kept under observation for 12 days.

During the course of trials, a total of 2060 blood engorged mosquitoes, *A. culicifacies* (n=359) and *A. stephensi* (n=1701) were collected from ivermectin treated and control calves (Fig. 1).

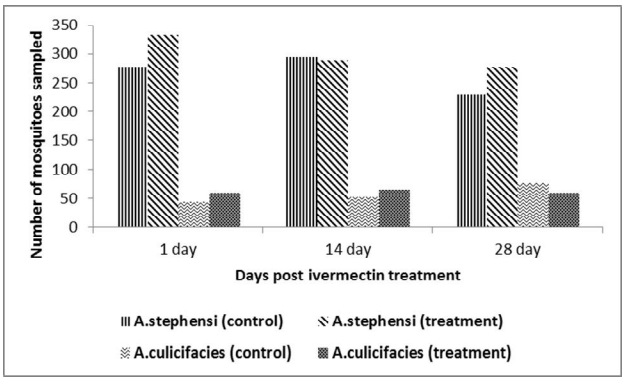

Fig. 1. Number of blood engorged *Anopheles culicifacies* and *A. stephensi* sampled on ivermectin treated and control calves at day 1, 14 and 28 after ivermectin treatment of calves.

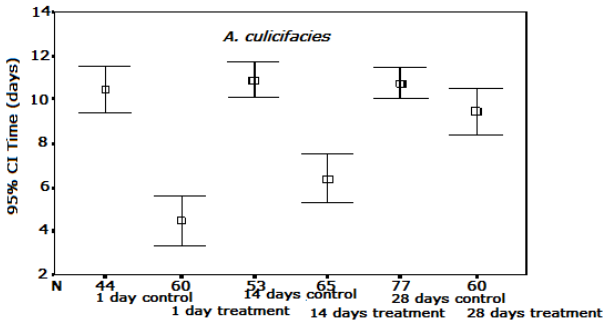

Fig. 2. Mean duration of survival of *A. culicifacies* blood engorged on control and ivermectin treated calves 1, 14 &28 days after ivermectin treatment. Along x –axis, N is number of blood engorged mosquitoes included in the study and sampling time. Along Y-axis is mean survival time in days with 95% CI.

Among blood engorged *A. culicifacies* sampled from ivermectin treated calves on 1, 14 and 28 day post ivermectin treatment 15%, 23.1% and 56.7% survived up to 12 days post blood feeding. Whereas 81.8%, 84.9% and 80.5% of *An. culicifacies* sampled from control calves on 1, 14 and 28 days survived up to 12 days post blood feeding. Mean duration of survival with 95% CI of *A. culicifacies* blood engorged on ivermectin treated and control calve at day 1,14 and 28 day post treatment are shown in (Fig.2). The shortest mean duration of survival of *A. culicifacies* was 4.48 days 95%CI (3.37-5.59) recorded in mosquitoes blood engorged on ivermectin treated calves at 1 day after treatment.

Cumulative survival rate of *A. culicifacies* for 12 days after blood feeding on ivermectin treated and control calves for sampling periods of 1,14 and 28 day after ivermectin treatment of calves are show in (Figs.3).

Among blood engorged *A. stephensi* sampled from ivermectin treated calves at 1, 14 and 28 day post ivermectin treatment 16.2%, 31.5% and 59.8% survived up to 12 days post blood feeding. Whereas 66.5%,73.1% and 72.9% of *A. stephensi* survived up to 12 days post blood feeding when blood engorged on control calves at 1,14 and 28 day respectively. The shortest mean duration of survival of *A. stephensi* fed on cattle 1 day after ivermectin treatment was 3.35 days with 95% CI (2.91-3.79) than the mean duration of survival of *A. stephensi* 10.23 days 95% CI (9.84-10.63) fed on control calves. Mean duration of survival with 95% CI of *A. stephensi* blood engorged on ivermectin treated and control calve at day 1,14 and 28 day post treatment are show in (Fig.4).

Cumulative survival rate of *A. stephensi* for 12 days after blood feeding on ivermectin treated and control calves for sampling periods of 1, 14 and 28 day after ivermectin treatment of calves are show in (Figs.5).

Pair wise comparisons of Kaplan–Meier survival curves (Figs. 3, 5) using log –rank test showed that daily probabilities of survival of blood engorged *A. culicifacies* and *A. stephensi* sampled from treated calves on day 1 and 14 after ivermectin treatment were lower and significant than their respective controls $p<0.05$. Daily probabilities of survival of *A. stephensi* blood engorged on calves 28 days after ivermectin treatment were lower and significant than their respective controls $p<0.05$. Whereas daily probabilities of survival of *A. culicifacies* blood engorged on calves 28 days after

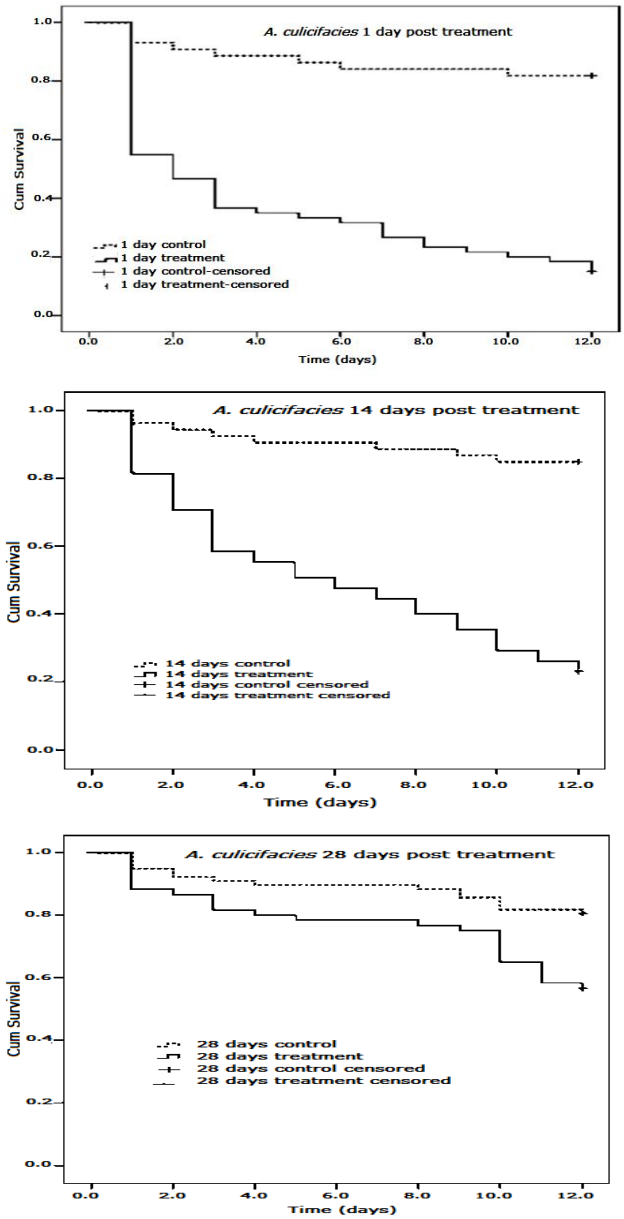

Fig. 3. Kaplan–Meier survival curves for *A. culicifacies* after feeding on ivermectin treated and control calves 1, 14 and 28 post ivermectin treatment of animals. X-axis post blood feeding survival time in days, Y-axis cumulative probability of survival.

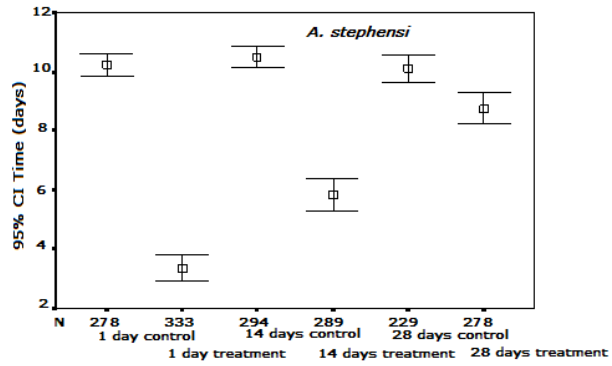

Fig. 4. Mean duration of survival of *A. stephensi* blood engorged on control and ivermectin treated calves 1, 14 & 28 days after ivermectin treatment. Along X–axis, N is number of blood engorged mosquitoes included in the study and sampling time. Along Y-axis is mean survival time in days with 95%CI.

Table I.- Kaplan-Meier pair-wise comparison of daily probabilities of survival of *A. culicifacies* and *A. stephensi* fed on treated and control calves on day 1, 14 and 28 after ivermectin treatment

Species	Control	Treatment (Days)		
		1	14	28
A. culicifacies	1day	.005*		
	14day		.020*	
	28day			.478
A. stephensi	1day	.000*		
	14day		.000*	
	28day			.000*

Log Rank (Mantel-Cox) p values level of significance 0.05

Table II.- Cox Regression Model.

Species	Time*	ß	SE	Wald
A. culicifacies	1	2.24	0.23	92.84
	14	1.88	0.23	66.17
	28	0.99	0.27	13.84
A. stephensi	1	1.79	0.09	381.86
	14	1.21	0.09	153.97
	28	0.37	0.12	9.86

*Time for blood feeding of mosquitoes in days with reference to post ivermectin treatment.

ivermectin treatment were lower than their respective controls but statistically non-significant $p>0.05$ (Table I). Cox proportional hazard ratios in (Table II) represent the hazards of *A. culicifacies* and *A. stephensi* to die when blood engorged from ivermectin treated calves at 1, 14 and 28 day post treatment as compared to *A. culicifacies* and *A. stephensi* engorged on control calves. Beta is

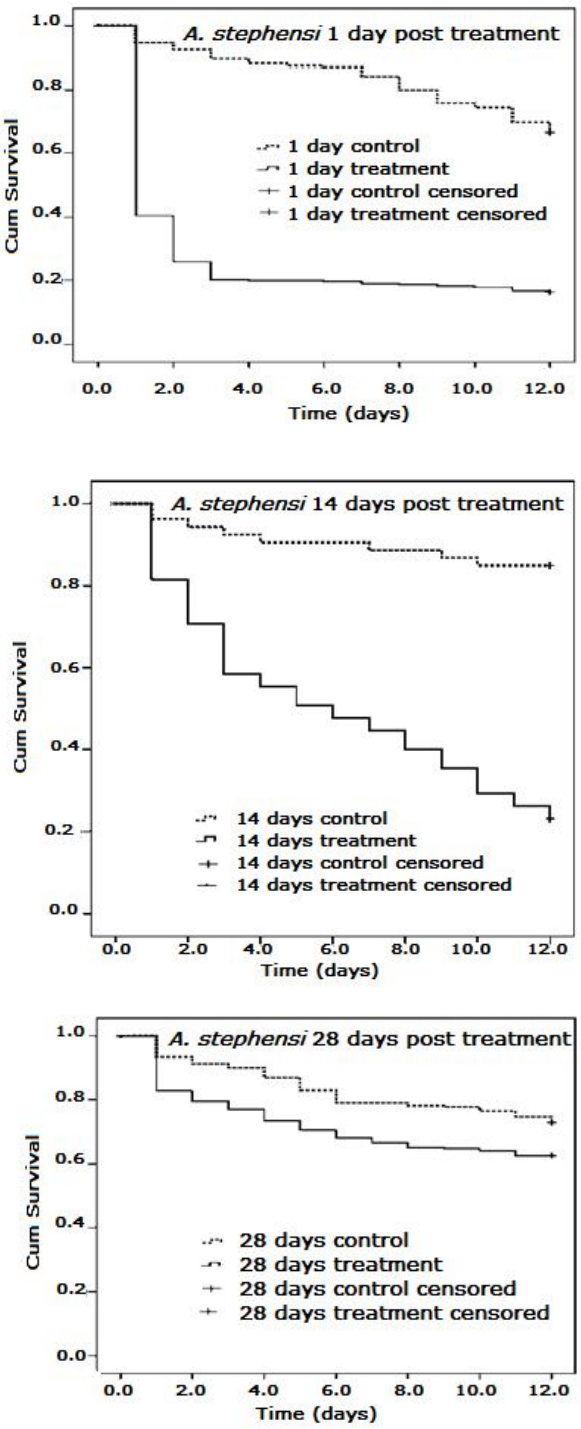

Fig. 5. Kaplan–Meier survival curves for *A. stephensi* after feeding on ivermectin treated and control calves 1, 14 and 28 post ivermectin treatment of animals. Along X-axis post blood feeding survival time in days, Y-axis cumulative probability of survival.

positive for all sampling periods which means that the log of the incidence rate for survival in *A. culicifacies* and *A. stephensi* when blood engorged from ivermectin treated calves at 1,14 and 28 day post treatment are significantly lower than *A. culicifacies* and *A. stephensi* blood engorged on control calves (p-value < 0.001).

DISCUSSION

Mosquito vectors of malaria in Pakistan are chiefly zoophilic, so the enhanced zooprophylaxis strategy of Bogh *et al.* (2002) for malaria control might be accomplished by administration of avermectin endectocides to livestock kept near domiciles.

In this study it was observed that, ivermectin in a dose appropriate for cattle use led to a significant reduction in both *A. culicifacies* and *A. stephensi* survival when they fed on treated cattle, compared with controls. The obtained results agree with previous studies. Ivermectin/ systemtic entectocide given to animal or human host significantly reduced the daily survival rate of many laboratory colonized mosquito species (Pampiglioni *et al.*, 1985; Jakubovich *et al.*, 1989; Tesh and Guzman, 1990; Jones *et al.*, 1992; Gardner *et al.*, 1993; Bockarie *et al.*, 1999; Fritz *et al.*, 2009; Foy *et al.*, 2011).

Ivermectin in cattle blood remained insecticidal to *A. culicifacies* and *A. stephensi* for 28 days.The effects of ivermectin in this study were of smaller duration than that of Foley *et al.* (2000) who in similar experiment found that daily survivorship of *A. farauti* significantly decreased when *A. farauti* blood fed on a person who ingested 250 µg/kg dose of ivermectin 44 days post-ivermectin administration. Whereas Chaccour *et al.* (2010) found that insecticidal effect of ivermectin in *A. gambiae* fed on human 14 days post ivermectin treatment was insignificant and loosely correlated to lower plasma concentrations of ivermectin in host at that time. This study differs from other published studies in that it specifically assesses the mosquitocidal effect of ivermectin on wild zoophilic, malaria vector mosquitoes following the treatment of cattle with a single standard therapeutic dose (200µg/kg) of ivermectin under field

conditions. The deviations of the results of present study from other studies may be due to a combination of factors including Anopheles species differences, wild versus laboratory mosquitoes and pharmacokinetics of ivermectin. Pharmacokinetics of ivermectin vary for animal species, body condition, age, and physiological status of the recipient, the route of administration and formulation (Changa *et al.*, 2007).

It was also found that *A. stephensi* was more susceptible than *A. culicifacies* to ivermectin after feeding on treated cattle. The varying susceptibility of the two species of mosquito is probably due to differences in the physiological characteristics of two species of mosquito. This agrees with the findings of Kobylinski *et al.* (2010) who found ivermectin levels that would be present in humans after MDA did not affect the survivorship and re-blood feeding behavior of *Ae. aegypti* but reduced *A. gambiae s.s.* survivorship and altered its re-blood feeding behavior. Overall effect of ivermectin on survival of Anopheline vectors may be even greater in field because low concentrations of ivermectin in a blood meal have been found to adversely influence on further breeding of surviving *Anopheles* by lowering their fecundity and egg viability (Gardner *et al.*, 1993; Fritz *et al.*, 2009).

In conclusion, this study revealed that blood feeding from ivermectin treated cattle reduced the survival of zoophilic malaria vectors. But the residual efficacy of ivermectin treated cattle was of short duration. So in a campaign style approach in which all the animals in the community are treated with ivermectin at the same time might have maximum impact on malaria transmission.

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