# BIONOMICS OF HYALOMMA TICKS IN THREE DISTRICTS OF PUNJAB, PAKISTAN

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## ABSTRACT

A survey of ticks as the vector in transmission of the disease was carried out in Rawalpindi, Multan and Lahore Districts by collecting 300 specimen of ectoparasites randomly from cattle in each district. The results showed highest prevalence (66.7%) of ticks in district Lahore while highest prevalence of lice (36.3%) and mange mites (4%) in district Rawalpindi and district Multan, respectively. The highest prevalence (12%) of *Hyalomma* ticks and lowest prevalence (3.1%) of *Rhipicephalus* was recorded in cattle. The bionomical study showed that the highest mean pre oviposition period was during spring while it was lowest in autumn .The mean oviposition period was also highest in spring . The incubation period of the ova of *Hyalomma* varied in different seasons. No oviposition was recorded at the temperature $10^0$C and 85% humidity. The maximum number of eggs was laid at $34^0$C and lowest egg production occurred at $15^0$C. The maximum number of eggs hatched at $32^0$C and 85% humidity. The specie wise prevalence of Theileria in the gut of ticks was confirmed by PCR test which showed highest prevalence (86.6%) for *Hyalomma a. anatolicum* while it was lowest (20.8%) for *Hyalomma m.marginatum.*

**Keyword:** *Hyalomma a. anatolicum*, *Hyalomma m. marginatum*, Theileria; bionomics; Punjab; Pakistan

## INTRODUCTION

Ticks were considered parasites of domestic animals as early as 400 B.C. Aristotle in his famous Historia Animalium" stated that the ticks were disgusting parasites and that were generated from grass. Despite this early realization little work was done until the later half of nineteenth century, when a number of parasitologists all over the world started working on taxonomy, prevalence, and bionomics, seasonal and regional occurrence of the ticks (Dobbelaere and Heussler,1999).Ticks are cosmopolitan in distribution, but occur principally in tropical and subtropical regions (Soulsby, 1982). Pakistan being a tropical country provides optimal climatic conditions for growth and multiplication of ticks. Tick fauna of Pakistan is rich in number of genera and species (Rasul and Akhtar, 1975). Despite this richness and suitability of the climatic conditions little systematic work has been done to investigate taxonomy, bionomics, seasonal and regional occurrence of ticks infesting the livestock in Pakistan. Durrani and Kamal (2008) have reported the highest prevalence (15%) of *Hyalomma* tick followed by *Boophilus* (12%),*Haemaphysalis* (5%) and *Rhipicephalus* (3%) in district Kasur, Pakistan. Ticks are the most important ectoparasites of livestock in tropical and subtropical areas and are responsible for substantial economic losses. Ticks not only cause direct effect on animals by sucking blood, they are also responsible for the transmission of several dreaded protozoan, rickettsial and viral diseases (Soulsby, 1982). The harm done by tick bites and blood sucking has been reduced by control measures taken to check the diseases transmitted by them (Kumar *et al*., 1992). The two forms of harm are themselves importance. Estimates of the amount of blood removed vary according to the species under consideration. A single adult female tick may remove 0.5 — 2.0 ml. of blood (Pegram and Chizyuka, 1990). If an animal carries numerous ticks a substantial loss of blood may occur. Heavy infections do occur in nature, it is more usual for animals to carry a few hundred ticks. These produce what is generally known as "tick worry". Systematic control of ticks always results in improved weight gains and yields. *Hyalomma* ticks, as vectors of tropical theileriosis are widespread in North Africa, southern Europe, Middle East, Central Asia and China (Preston, 2001). In Pakistan piroplasmosis, theileriasis and anaplasmosis, are common livestock diseases, which are transmitted by the ticks (Abdussalam, 1959). The information on the ticks of livestock in Pakistan is scanty. In the present study prevalence of different tick genera and bionomics of the species of genus *Hyalomma* was undertaken which help in control of various will tick borne diseases transmitted by ticks of this genus in Pakistan.

## MATERIALS AND METHODS

**Collection of ticks, lice and mange mites:** Surveillance studies of ticks as vector in transmission of the disease was carried out in Rawalpindi, Multan and Lahore Districts. Three hundred specimen of ectoparasites were

randomly collected from cattle in each district. Specimen were brought to the laboratory in wide mouthed ,screw capped ,glass jars which were carefully labeled at the collection site and identified by using key of morphological characters. All parts of the body of cattle were carefully examined. The ticks were removed from the body of the host with the help of fine and smooth forceps taking all necessary precautions to avoid damage to the mouth parts of the ticks and skin of host. Specimens were preserved in 1:1 solution of 10% formalin and chloroform for identification by using low power stereoscopic microscope. The collection was stored for record in screw capped specimen bottles. The live *Hyalomma* ticks were placed in tubes covered on top with a small piece of muslin cloth for bionomical studies.

**Rearing on the host:** For rearing on the host, adult ticks were attached to the rabbits. The animals were kept in small steel cages and provided with food and water .The cages were placed in trays containing grass. These trays were inturn placed in larger trays containing creosote solution to prevent escape of ticks. As the cages were entirely in open , the ticks were exposed to the same conditions, they encounter in nature, with the possible exception of the relative availability of the host. (Viseras *et al*.,1999). Piroplasms and sporozoites of *Theileria annulata* in the gut and salivary glands of engorged ticks were identified by PCR test according to method described by Ole.Moiyol *et al.,* (2000).

**Rearing in tubes:** For rearing in tubes 15 cm long tubes with bottom removed were taken and fixed in vertical position on the trays containing a mixture of moist sand and clay. Ticks at various stages of development were placed in these tubes so that each tube contained only one tick. A small piece of muslin cloth was tied on top of each tube in order to prevent the escape of ticks and to allow aeration. For obtaining outdoor records the tubes were kept under two different conditions. In one lot the tubes were exposed to direct sunlight and rainfall as well as variation of temperature, humidity and air circulations. The tubes of other lot were placed under trees so that they were sheltered from direct sunlight and rain fall but exposed to all variations of temperature, humidity and air circulation (Ahmed *et al*., 1999).

**Effect of Temperature and Humidity:** Influence of temperature and humidity on the rate of development of *Hyalomma* was studied by confining different developmental stages of the ticks in small, wide mouthed bottles covered with muslin cloth in four seasons of the year i.e summer (May, June, July, August), autumn (September, October), winter ( November, December, January, February) and spring (March, April).

## RESULTS AND DISCUSSION

The survey results showed highest prevalence (66.7%) of ticks in district Lahore while highest prevalence of lice (36.3%) and mange mites (4%) in district Rawalpindi and district Multan respectively. The highest prevalence of *Hyalomma* (12%) ticks followed by *Boophilus,* 8.1% *Haemaphysalis,* 5% and *Rhipicephalus* 3.1% was observed.

**Pre oviposition period:** The mean pre oviposition period recorded during spring season varied from 10 to 30 days. In mid summer it was 15-25 days while in autumn it was observed to vary from 6-8 days.

**Ovi position Period:**The mean oviposition period during the study period varied from 15-20 days in spring, 10 to 15 days in summer and 10 to 18 days in autumn.

**Incubation period:**The incubation period of the ova of *Hyalomma* was found to vary in different seasons. In spring under natural conditions it was found to be 19 to 30 days; in summer, 15 to 25 days and in autumn, 10 to 20 days. The eggs were oblong in shape and measured 0.475 X 0.424 mm in size and weight 0.047 mg on an average. Total number of eggs laid by a single female under natural conditions varied seasonally. It was observed that the maximum number of eggs laid by a single female tick in spring varied from 3735 to 3920, in summer from 2615 to 2970 and in autumn from 2425 to 2610.

**The larva:** For larvae during spring, maximum longetivity without food was observed to vary from 35 to 90 days.It was observed that the larvae engorged between 8-15 days in spring, 8 to 19 days in summer and 7 to 10 days in autumn.The weight of unfed larva was found to vary from 0.037 to 0.048 mg and of engorged larvae from 0.160 to 0.175 mg. The amount of blood sucked during this period was found to vary from 0.130 to 0.133 ml.The moulting period varied from 8 to 28 days in spring, 8 to 20 days in summer and autumn.

**Nymph:** The maximum longetivity without food during spring was found to vary from 49 to 90 days.It was observed that nymph engorge between 6 to 15 days in spring, 4 to 10 days in summer, 6 to 10 days in autumn. The weight of unfed nymph was found to vary from 0.106 to 0.126mg and of engorged nymph from 0.895 to 1.528 mg. The amount of blood sucked varies from 0.790 to 1.407mg. The fully engorged nymphs were placed in rearing tubes and exposed to natural environmental conditions during different seasons. The moulting period varied from 10 to 30 days in spring, 8 to 20 days in summer and 9 to 15 days in autumn.

**The Adult:** It was observed that females engorged between 8 to 18 days in spring, 7 to 19 days in summer

and 5 to 8 days in autumn.The weight of unfed female was found to vary from 1.30 to 2.15 mg and of engorged nymph from 181.09 to 240.09 mg. The amount of blood sucked varies from 180.75 to 239.00mg. It was recorded that 0.132 mg of blood was sucked by first instar, 1.1mg of blood was sucked by the second instar and 209.87 mg of blood was sucked by the third instar. The total amount of blood sucked by single female during lifespan was found to be 211.102mg.It was observed that *Hyalomma* preferred rabbit although they could be attached to rats. Ticks were maintained on rabbits successfully for several generations.

**Influence of temperature and humidity on the rate of development of *Hyalomma* tick:** The mean duration of 6 days of pre-oviposition period of *Hyalomma* was recorded at constant temperature of $30^0$C with variable humidities at 52%, 72%,79%,83%,91% and 100%. At a constant humidity of 85% and duration of pre-oviposition period of Hyalomma found to be highest at $15^0$C (25 to 35 days) while it was shortest at $25^0$C (6 to 8 days). The variation of relative humidity has no appreciable effect on oviposition period. The longest oviposition period noticed at $15^0$C and 85% humidity while the shortest oviposition period noticed at $34^0$C and $36^0$C at 85% humidity. No oviposition occurred at $10^0$C at 85% humidity .It was observed that the number of eggs laid varied with the rise in temperature. The maximum number of eggs laid at $34^0$C and lowest egg production occurred at $15^0$C. The maximum number of eggs hatched at $32^0$C and 85% humidity. The protozoa in the gut of engorged infected tick identified as *Theileria* on microscopic examination and confirmed as *Theileria annulata* by PCR test. The specie wise prevalence of Theileria in the gut of ticks showed higher prevalence (86.6%) for *Hyalomma a. anatolicum* while it was lower (20.8%) for *Hyalomma m. marginatum.*

During the present study highest prevalence (12%) of Hyalomma ticks was observed followed by Boophilus (8.1%), Haemaphysalis (5%) and Rhipicephalus (3.5%). The findings are in accordance with Durrani and Kamal (2008) who also reported infestation of similar genera of ticks on Friesian cattle in district Kasur, Punjab. The results of present study also coincides with Rasul and Akhtar (1975) who found *Hyalomma, Boophilus, Rephicephalus* and *Haemaphysalis* genera infesting domestic animals. The species of *Hyalomma* were also reported by Khan *et al.,* (1996) who studied prevalence of tick infestation 28.2% (1269/4500) in cattle and 14.7% (662/4500) in buffaloes in Faisalabad district of Pakistan. Seven species of ticks i.e. *Rhipicephalus sanguineus, Boophilus microplus, B. annulatus, Hyalomma a. anatolicum, H. marginatum marginatum, Hyalomma aegyptium* and *Dermacentor marginatus* were identified by them. All species of ticks were found infesting buffaloes. Ali (1986) studied the incidence of ectoparasites in cattle, sheep, goats and poultry in Northern areas of Pakistan. The species recorded were *Hyalomma aegypticum, Hyalomma a. anatolicum, Boophilus microplus* and *Hypoderma lineatum* in cattle. The results of present study are not in accordance with Wahid *et al.,* (2004) who reported five species of Ixodidae ticks infesting different animals (cow, buffaloe, goat and sheep) in their study. They reported that *Haemaphysalis* (H) *sulcata* and *Hyalomma anatolicum anatolicum* occurred throughout the year; however the infestation of Haemaphysalis (H) *sulcata* was more common than that of *Hyalomma anatolicum anatolicum*. They also reported that high density of ticks were recorded in the months of August, September, and October, when the mean temperature was ($27^0$C) and relative humidity as 84%. The prevalence of blood protozoa in the salivary gland was also studied by Razmi *et al., (*2003) by using staining technique. They recorded the prevalence of ticks infesting cattle as *Hyalomma anatolicum excavatum* (92.35%), *H. marginatum marginatum* (5.14%), *H. asiaticum asiaticum* (1.17%) and *Rhipicephalus sanguineus*., (1.32%) They also examined 510 tick salivary glands that revealed 51% of *H. a. excavatum* and 1.3% of *H. a. asiaticum* infected with sporozoites of *T. annulata*. The rearing of ticks on rabbits was also reported by Walker and McKellar (1983) who reported that adult *Hyalomma anatolicum anatolicum* ticks infected with *Theileria annulata* (Hissar strain) were incubated at 36°C or fed on rabbits. They stained tick salivary glands with methyl green pyronin, ground up and deposited on microscope slides and stained with Giemsa's solution. According to them the *Theileria* in the salivary glands of the fed ticks matured more completely and rapidly than in the incubated ticks. They confirmed that fed ticks will be more suitable for sporozoite production for infection of cattle and production of stabilate. The effect of temperature as observed in the present study was in accordance with Boulter and Hall (2000) who reported the effect of temperature on the transtadial transmission of *Theileria annulata* in *Hyalomma anatolicum anatolicum* They reported that variation in temperature (4–40°C) had a significant effect on moulting rate of the ticks and transmission of theilerial parasites from nymphs to resultant adults. The temperatures above 40°C and below 12°C prevented moulting. The infection rate in salivary glands was assessed using a methyl green pyronin technique. The effect of temperature and humidity on various developmental stages of *Hyalomma* was studied and it was found that moderate temperature and humidity greatly enhanced the developmental stages (Viseras, *et al*., 1997).

**Acknowledgement:** The authors are thankful to Higher Education Commission, Government of Pakistan for providing funds under the research project "Epidemiology of Theileriosis in Bovine".

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