



**Experimental infection of native chicken with cattle tick  
*Rhipicephalus (Boophilus) annulatus* (Acari: Ixodidae)**

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**Abstract:** *Rhipicephalus (Boophilus) annulatus* is considered as one of the most widespread tick species of the world on several host including cattle. The current study has been conducted to evaluate infestation ability of larval *R. annulatus* on chicken growth and development. Indicated very few larvae quested or fed on indigenous adult chickens when compared with the number of larvae which used in this study, and that the larval and nymph feeding period of these ticks was very long. The number of larvae feeding shows in hollows wings, while the number of feeding nymphs investigated on head, wings and above of thigh, both larvae and nymphs described on the same individual adult chicken, the engorged adult female not observed while the adult male noticed on chickens. Designated the weight of feeding larvae and nymphs stage is  $0.32 \pm 0.04$  mg and nymph's  $2.73 \pm 1.22$  mg. The weight of larvae and nymph stage under mattress and feeder's chicken  $0.27 \pm 0.21$  and  $2.52 \pm 1.75$  mg respectively, shows not significantly when compared between the weight of feeding on chickens and the weight under mattress and feeder's chicken by larvae and nymphs stage at ( $P < 0.05$ ) values according chi-square. Experimental study shows not transmissions pathogens from incubating larvae, which got from engorged female infested with *Babesia* spp., to adult chickens when examined their blood by Giemsa stain.

**Keywords:** *Rhipicephalus (Boophilus) annulatus*, Infected engorged female, *Babesia* spp., Native adult chicken.

## Introduction

Hard ticks (Acari: Ixodidae) have a significant impact on public health and the rural economy in many parts of the world (Parola & Raoult, 2001; Jongean & Uilenberg, 2004). *Rhipicephalus annulatus* (formerly *Boophilus annulatus*) is a hard tick found most often on cattle, heavy tick burdens on animals can decrease production and

damage pelts (Peter *et al.*, 2005). *Rhipicephalus annulatus* is an economically important tick which transmitted parasitic diseases for a variety of livestock; it is known as the cattle tick or southern cattle tick, the most important vectors of disease-causing pathogens in domestic and humans. It is transmitted many babesiosis pathogens like *Babesia bigemina*, *B. bovis*,

and *Anaplasma marginale* (Antunes *et al.*, 2012).

There are a number of species of ticks infesting poultry, the recently of these include; *Haemaphysalis punctata*, *Hyalomma lusitanicum* and *Ixodes frontalis* (Calvete *et al.*, 2003), *Amblyomma americanum* and *Ixodes brunneus* have been found on wild turkeys *Meleagris gallopavo* (Scott *et al.*, 2010). Among the Ixodidae, described *Ixodes* species are strictly parasitic on birds, *Amblyomma* species have immature that feed on birds. *Haemaphysalis* strictly parasites of ground-feeding birds and in nesting birds and no *Hyalomma* are dependent on birds, although immature *Hyalomma* species frequently feed on them as well as on mammals, although a few *Rhipicephalus* species occasionally are found on large ground-feeding birds such as ostrich. None of the other Rhipicephaline genera parasitise birds (Oliver, 1989). The infected birds may transmit a tick-borne disease to the ticks, and the alternative where ticks may transmit pathogens to each other by co-feeding (Hubalek, 2004). Transovarial pathogens passage of *Babesia* spp from female *R. annulatus* during oviposition. Kinetes of *Babesia* were present in eggs laid at a suitable temperature (Ouhelli & Schein, 1988).

The aim of current study to evaluate *Boophilus* spp., experimentally for growth and ability this genus to transmit pathogens on native adult chickens.

## Materials and Methods:

### Study area

The current study was conducted at Bkrajo farm- Animal Sciences Department, College of Agriculture, University of Sulaimani.

### Ticks collection and identifications

The engorged female of *R. annulatus* removed by using tweezers and alcohol from different part of cattle body from various flock, collected ticks stored in sterile glass (Bijou tube) for isolation and identifications. (Walker *et al.*, 2003).

### Hemolymph (Hemocyt slide) Smear

Fifty adult female of *Rhipicephalus* species have been used for detection of pathogens (Piroplasmosis), collected by severing the forelegs at the coxal-trochanteral joint and drawing hemolymph into a glass micropipette. For the purpose of light microscopy of hemocytes, hemolymph was smeared on a glass slide, immediately dried, and fixed in methanol for 10 min. The specimens were stained with Giemsa stain 10% for 10 min. Examination under a light microscope with high was followed 100X (Mustafa, 2011).

### Incubations stage

The pathogenic females (10 engorged females of *R. annulatus*) were allowed to oviposit for 14 days, the weight of engorged females and mass of deposited eggs was done by electronic analytical balance (Mettler-Toledo, Switzerland), and returned to the incubator at  $27\pm 1.6^{\circ}\text{C}$  with relative humidity (RH%)  $85\pm 1.4\%$  (Mustafa, 2011).

### Chicken preparation to study

Chickens were used as a laboratory model to determine the conditions that ability of infection with larvae /ticks. Using only 30 native chickens non-infested with ectoparasites (depending on clinical signs and examination) putting in the room  $3\text{m}^2$  during the study to experimental infestation with a proximally 10000 larvae (as average 800-1150 larvae/ engorged female) which was got by incubation 10 engorged female *R. annulatus* and placed on the room's floor to estimate of feeding status.

### Larvae and nymph stage weight

Isolation 30 of adult chickens infested experimentally with *R. annulatus*, for each stage for weighting; larvae and lymph which collected randomly on body chicken during the feeding period and engorged larvae and nymph found under mattress and feeder's chicken by using tweezers and alcohol.

### Blood smear preparation

Using one drop of blood was drawn from wing vein to prepare blood smear for examining and investigating the presence of

piroplasmosis by light microscope use oil immersion.

### Statistical Analysis

In this study, statistical tests were performed according to chi-square test between two different stage; larvae and nymph weights of *R. annulatus*.

### Results

Three genera of Ixodidae family have been isolated and identified from local cattle at different areas in Sulaimani province; these genera were *Rhipicephalus* with the highest isolation rate, followed by the genus *Haylamma*. *R. annulatus* has been identified and used in artificial infestation of adults chickens (30 adults chicken have been applied with approximately 10000 larvae. Detected very few larvae quested or fed on native adult chickens when comparison with the number of larvae which used in this study. The attachment period of larvae in common was less than 12 hours, the number of chickens infested with unfed larvae increased in the first day and reached the top in 8 days and starts to decline, while the feeding larval starts to increase until 11 days and reached and lost in 16 days, the number of feeding larvae investigate in hollows wings site. The number of unfed larvae on the chicken body was higher than the number of fed larvae; this may be that the blood of chicken wasn't preferable for *R. annulatus*, as shown in Fig. (1). Results showed that the number of

nymphs was lower than the number of larvae which feeding on chicken. Fed and unfed larva in age 17 days disappeared may be because some of these larvae enter the dispose stage.

Observed lowest number of nymph quested or fed on indigenous adult chickens when comparison with the number of larvae which used in this work, the cycle of nymph feeding on chicken reached to 15 days (16-30 days as shown in the diagram), the number of feeding nymph appeared on head, wings and above of thigh, the different site of feeding on the chickens may interfere with tick development. Observed the number of infested chickens with unfed and feeding nymph starts in 16 days and reaches the top in 23 days, the cycle of this stage reaches to 14 days (in 17 days to 30 days). Both feeding larvae and unfed nymph described on the same individual adult chicken, the number of unfed nymph on the chicken body was higher than the number of fed nymph. The number of infested chickens was higher than the unfed and feeding nymph. The engorged adult female was not observed while the adult male noticed on chickens. Presence of a number of engorged larvae and nymphs stage under mattress and feeders chicken to enter the dispose stage as shown in Fig. (2).

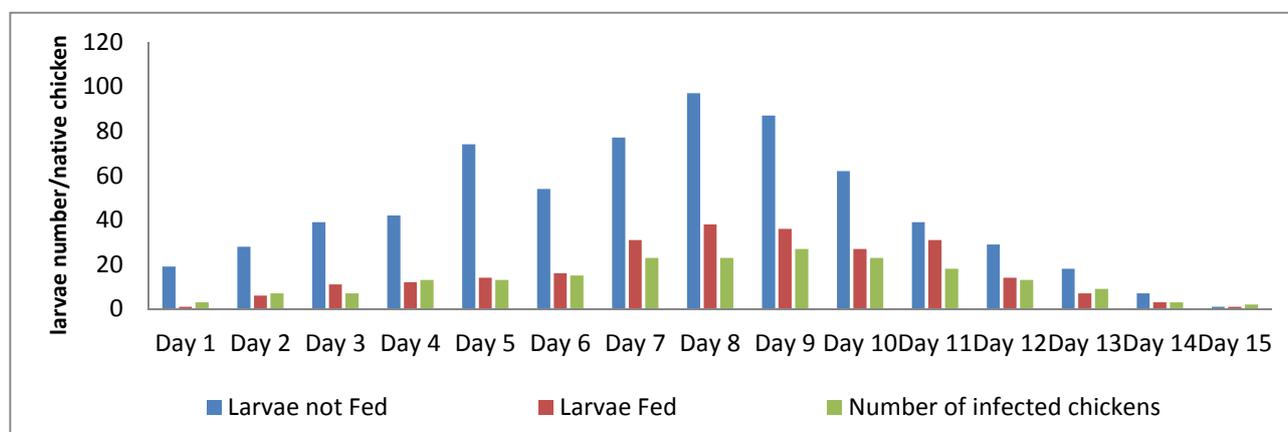


Fig. (1): Biological generation of *R. annulatus* larvae (fed and unfed stages) on adult chickens infested experimentally.

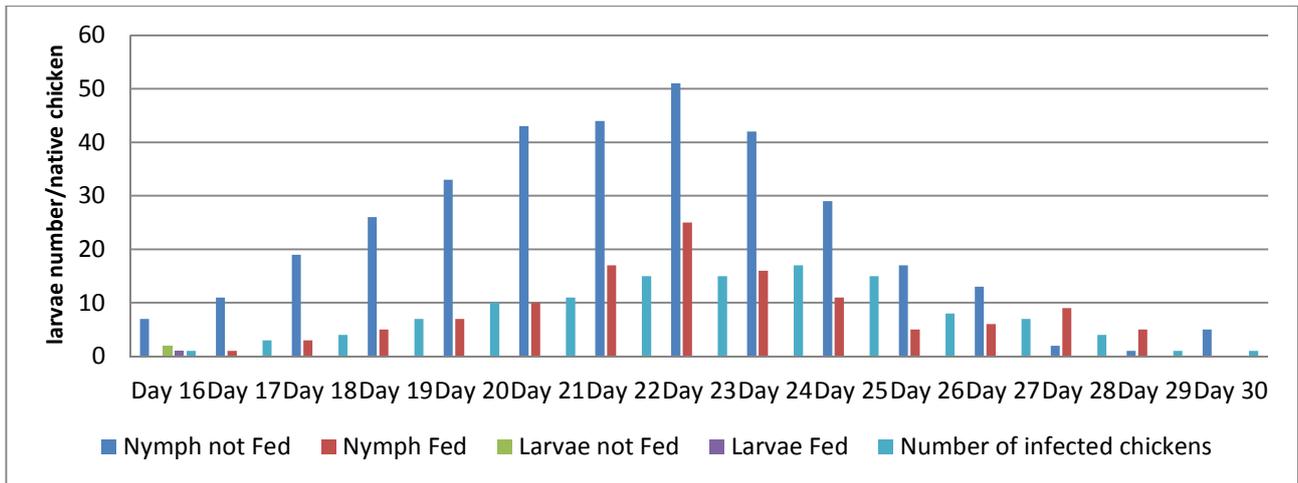


Fig. (2): Biological generation of *R. annulatus* nymph (fed and unfed stages) on adult chickens infested experimentally.

During the study showed the weight of feeding larvae reached to  $0.32 \pm 0.21$  mg along the feeding period on adult chickens and  $0.27 \pm 0.04$  mg when examined under mattress and feeders chicken. In nymph stage the weight of feeding nymph reached to

$2.73 \pm 1.75$  mg, while the weight of nymph appeared under mattress and feeders  $2.52 \pm 1.22$  mg, without any significant differences-according chi-square between two stages weight; larvae and nymph as shown in Fig. (3).

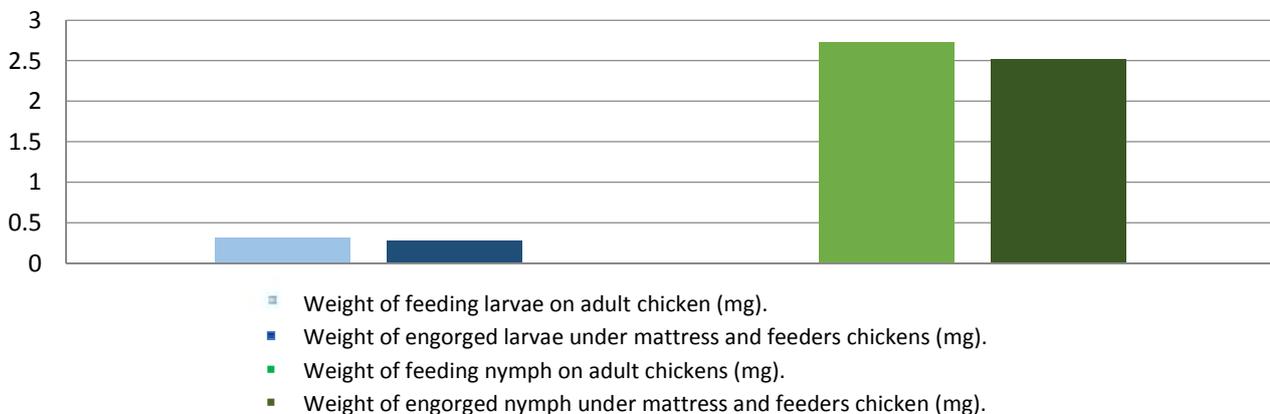


Fig. (3): Weighting of larvae and nymph feeding with *R. annulatus* on adult chicken.

### Discussion

The current research showed the high occurrence of *R. annulatus* than other species at different regions in Sulimania province; this result was in a good agreement with the results of Hasson (2012) when they proved that the species of *R. annulatus* was higher than *B. kohlsi*. Results showed that adult chicken were unsuitable hosts (very low infestation to larvae and nymph to *R. annulatus*, although showed feeding on the blood chickens. These results in contrast with

Tuama *et al.* (2007) results which proved the isolation of 30% of hard ticks infested on chicken and 3.3% infested with *R. annulatus*. The prevalence of tick infestation on the birds showed a variation between the different studies. The different bird species feeding habits in ground feeding or not (Mehl *et al.*, 1984. Hasle *et al.*, 2009), age of the bird (Scharf, 2004) and season (Hoogstraal *et al.*, 1963; Olsen *et al.*, 1995) have an influence on the prevalence of tick infestation. Feeding

period in larvae and nymph stage was longest; 16 and 15 days respectively and isolation randomly larvae feeding stage from chicken for weighing, showed the weight of feeding larvae and nymph were  $0.27 \pm 0.04$  and  $2.52 \pm 1.22$  mg respectively, while the weight of larvae and nymph when examined under mattress and feeders chicken were  $0.32 \pm 0.21$  and  $2.93 \pm 1.75$  mg respectively, the difference between the values were not significantly. The weight of larvae and nymph in rabbits were  $0.58 \pm 0.09$  and  $15.8 \pm 1.4$  mg respectively when infested experimentally with *Hyalomma truncatum* (Rechav & Fielden, 1997). The larvae and nymph period fed on the rabbits were  $4.7 \pm 1.2$  and  $9.6 \pm 2.2$  days respectively. Oliver (1989) indicated that the larval and nymphal ixodids feeding on warm blooded animals usually require 3-7 and 4-8 days respectively.

At the same time, the higher number of feeding larvae showed on wings, whereas feeding nymph examined on head, wings and above of thigh, the different site of feeding on the chickens may interfere with tick development (suitable condition including temperature and humidity, Hasson, 2012) infestation percentage of hard ticks on chicken were 30 %; 3.3 % of them were *R. annulatus*, the engorged adult female wasn't observed while the adult male noticed on chickens and investigate a number of engorged larvae and nymphs stage under mattress and feeders chicken this could be attributed to the fact that the male nymphs didn't require blood for molting and feeding on skin crusts for their growth and development. Experimental study showed no transmission of trans-ovarially of *Babesia* spp. to adult chicken, this results agreement with Hasle, (2013) & Hamsíkova *et al.* (2016) when they didn't show any *Babesia* spp. positive in birds. Birds contribute to the geographic distribution of various tick-borne pathogens and serve as their hosts. In Europe, *B. microti*, *B. venatorum*, and *B. divergens* have been found in ticks infesting birds (Toma, *et al.*, 2014). The occurrence of *Babesia* spp. in ticks (especially in larvae) from birds suggested that birds may be able to infect ticks (Hasle, 2013). Although studies

on this topic are lacking in Iraq and especially in Kurdistan region, birds may act as carriers for *Babesia*-infected ticks contributing to the dispersal of the hard ticks

## Conclusion

The study revealed that native poultry (local Adult chicken) that feed on the ground, are particularly prone to carry ticks and a risk source for the presence of a number of engorged larvae and nymphs stage, subsequently which infect the final host such as sheep and goats and as especially cattle. The ability of *R. annulatus* larvae on the feeding of chicken blood. These results suggest investigations on epidemiological aspects of tick infestation in poultry in the Kurdistan region as the specialist and Iraq as a general should be carried out to provide an effective to control, which is considered the intermediate stage for the growth to the final host.

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