ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

ORIGINAL ARTICLE

Korean J Parasitol Vol. 54, No. 3: 301-305, June 2016
http://dx.doi.org/10.3347/kjp.2016.54.3.301

Biological Parameters of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) Fed on Rabbits, Sheep, and Cattle

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Abstract: In order to determine the effect of various hosts on feeding performance of *Rhipicephalus* (*Boophilus*) *microplus*, we used 3 mammalian species as hosts, cattle (Qinchuan), sheep (Tan), and rabbits (Japanese white rabbit) for infesting ticks. Five hundreds of *R. microplus* larvae were exposed to each animal (3 animals/host species). Tick recoveries were 11.0%, 0.47%, and 5.5% from cattle, sheep, and rabbits, respectively. The averages of tick feeding periods were not significantly different on cattle, sheep, and rabbits, 28.8, 25.3, and 26.7 days, respectively. The average weights of individual engorged female from cattle, sheep, and rabbits were 312.5, 219.1, and 130.2 mg, respectively and those of egg mass weights each to 85.0, 96.6, and 17.8 mg. The highest egg hatching rate was in the ticks from cattle (96.0%), followed by those from rabbits (83.0%) and sheep (19.2%). These data suggest that rabbits could be as an alternative host to cultivate *R. microplus* for evaluating vaccines and chemical and biological medicines against the tick in the laboratory, although the biological parameters of ticks were less than those from cattle.

Key words: *Rhipicephalus* (*Boophilus*) *microplus*, biological parameter, cattle, rabbit, sheep

INTRODUCTION

Ticks are obligate hematophagous ectoparasites of vertebrates, and transmit varieties of pathogenic microorganisms of humans and animals, i.e., protozoa, rickettsiae, spirochaetes, and viruses [1,2]. *Rhipicephalus* (*Boophilus*) *microplus* is a one-host hard tick species, normally considered with high host-specificity for bovine animals, and can transmit bovine babesiosis and anaplasmosis. It originally comes from south and southeast Asia. To date, it is widely present in the south and east Asia, east and south Africa, central and south America, and north and east Australia due to animal transportation and global climate warming. It became one of the most widely distributed tropical and subtropical tick species and can cause huge economic losses to the cattle industry [3]. In China, *R. microplus* is the most widespread tick species distributed in 23 provinces [4] and the vector of *Babesia bovis*, *B. bigemina*, *Anaplasma marginale*, and *Coxiella burnetii* [2,5,6]. Consequently, there are lots of papers which reported the control techniques of *R. microplus*, such as medicine treatment [7-9], bio-control (*Beauveria bassiana* and *Metarhizium anisopliae*) [10-13], and vaccination [14].

The tick cultivation usually used cattle as the experimental animal due to high host-specificity of this tick species, and it increased research cost and practice difficulty. However, in the literature, *R. microplus* can also infest sheep, goats, camels, horses, donkeys, and rabbits [15,16]. Thus, we herein compared the biological parameters of *R. microplus* fed on cattle, sheep, and rabbits under laboratory conditions so as to evaluate the suitability of *R. microplus* on sheep and rabbits, as experimental animals for cultivating the ticks, which will improve the feasibility and decrease the cost of experiment. It will also provide additional information on potential host species and life cycle of *R. microplus* in China.

• Received 12 January 2016, revised 5 May 2016, accepted 7 May 2016.
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MATERIALS AND METHODS

Ticks

Ticks used for experimental infestation were from a laboratory colony of *R. microplus* originated from cattle (*Bos taurus*) in Guilin of Guangxi province, China. Ticks were reared and maintained as previously described by Chen et al. [17] and Ma et al. [18] on cattle (Qinchuan). Briefly, ticks were restricted in a chamber (muslin cloth bag, ø 9 cm) glued on the back of cattle for feeding in parasitic phase. Engorged ticks were collected into glass tubes sealed with hydrophilic cotton and maintained at 28˚C in an incubator with 70-80% relative humidity (RH) and daylight cycle for laying eggs and hatching in free-living phase. The ticks in 4th generation were used in the experiment.

Animals

One-year-old Qinchuan cattle (*Bos taurus*), 6-month-old Tan sheep (*Ovis aries*), and 3-month-old Japanese white rabbits that were never exposed to ticks were kept in tick-free folds with food and water available in the Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science. The animal experiments were approved by the Gansu Provincial Science and Technology Department and in accordance with the Animal House of Lanzhou Veterinary Research Institute Instructions (license no: SYXK2010-0001).

Experimental infestation

Tick infestations were performed in September (Autumn) under laboratory conditions (12-29˚C, 50% RH) as mentioned above. Three animals of each species (total 9 animals) were used to infest ticks. Each animal was infested with 500 *R. microplus* larvae. Feeding chambers were inspected every day, and the feeding periods were recorded from infestation with larvae to detachment of engorged females on animals. The daily engorged ticks were collected, counted, and weighed on an analytical scale. The engorged females dropped from each animal species were individually collected into a glass tube and kept in an incubator for oviposition as above. The egg masses of each female were weighted, and the numbers were estimated using the number per milligram eggs at the end of oviposition process (until the engorged female died). The reproductive efficiency index (REI) was calculated by using the formula: REI = [egg mass weight (mg)/tick weight (mg)] × 100 [19]. In addition, 3 engorged females randomly selected, dropped from each animal species, were used to evaluate the weight of eggs daily laid by each female. Finally, in total, 500 eggs were used for observing the incubation period. After hatching, the hatchability was calculated. Data of biological parameters from ticks fed on various host species were submitted to 1-way analyses of variance using SPSS 19.0, with 5% significance ($P < 0.05$).

RESULTS

A total of 254 engorged females were collected from 9 animals. Tick recoveries on cattle, sheep, and rabbits were 11.0% (165/1,500), 0.47% (7/1,500), and 5.5% (82/1,500), respectively. Most larvae died during 5 days after exposure to sheep, which was responsible for the low tick recovery on sheep. In addition, 57.1% (4/7) of females engorged on sheep were dead (no laying eggs) during the period of incubation, and those of cattle and rabbits were each to 15.8% (26/165) and 22.0% (18/82). Repletion degrees of females from the 3 host species were quite different. Almost all ticks from cattle were fully replete. Ticks from sheep and rabbits were just partially engorged compared with those from cattle, and the repletion degree was significantly different between ticks (Fig. 1).

The parasitic period from flat larvae to engorged females was 24-33 days on cattle (average 28.8 ± 0.22 days) and rabbits (25.3 ± 0.29), and that was 25-27 days on sheep (26.7 ± 0.28). The weights of females engorged on cattle, sheep, and rabbits were 312.5 ± 10.0, 219.1 ± 62.4, and 130.2 ± 9.1 mg, respectively. This was in accordance with the smaller females taking fewer days for sucking blood. Meanwhile, 85.0 ± 4.7 and 17.8 ± 1.4 mg of eggs laid by individual female each engorged on cattle (139 engorged females) and rabbits (64 engorged females) were also consistent with the weight of engorged females, bigger females laying more eggs. However, the weight of eggs laid by individual female (3 engorged females) from sheep was 96.6 ± 19.0, more than that of females engorged on cattle, although its average weight of the 7 engorged females was less than that of cattle. It was possible that most of the small engorged females from sheep were dead and did not lay eggs. That was the reason for that the reproductive efficiency index (REI) of females from sheep was 46.1 ± 8.1, quite higher than those of cattle and rabbits (Table 1). Three engorged females dropped from each animal species were used to evaluate the weight of eggs daily laid by each female. Results showed that on the first day of oviposition, the egg weights were almost similar by females engorged from the 3 host species.

However, the peaks of weight of daily laid eggs by females

Fig. 1. Engorged females of *Rhipicephalus* (*Boophilus*) *microplus* on rabbits (A), cattle (B), and sheep (C).

Table 1. Biological parameters of *R. microplus* on 3 host species (cattle, sheep, and rabbits) in parasitic phase, free-living phase at 28°C, 70-80% relative humidity and daylight cycle in incubator

Parameters	Rabbits	Cattle	Sheep
No. of exposed ticks per animal	500 larvae	500 larvae	500 larvae
No. of engorged females per animal	27.3 ± 15.9^{b}	55.0 ± 11.4^{c}	2.3 ± 1.2^{a}
Feeding period (day)	26.7 ± 0.28^{a}	28.8 ± 0.22^{b}	25.3 ± 0.29^{a}
Weight of each engorged female (mg)	130.2 ± 9.1^{a}	$312.5\pm10.0^{b,c}$	$219.1\pm62.4^{a,b}$
Weight of eggs laid by each female (mg)	17.8 ± 1.4^{a}	$85.0\pm4.7^{b,*}$	96.6 ± 19.0^{b}
No. of eggs laid by each female	516.8 ± 61.9^{a}	$2,061.8\pm114.8^{b}$	$2,293.8\pm451.7^{b,*}$
Hatchability (%)	83.0 ± 3.7^{b}	96.0 ± 4.0^{b}	$19.2\pm8.2^{a,*}$
Reproductive efficiency index (REI)	14.3 ± 8.6^{a}	26.4 ± 17.2^{b}	$46.1\pm8.1^{c,*}$

Means within a row followed by different letters are significantly different ($P<0.05$).
*Data were just from 3 engorged ticks as other 4 engorged ticks were died.

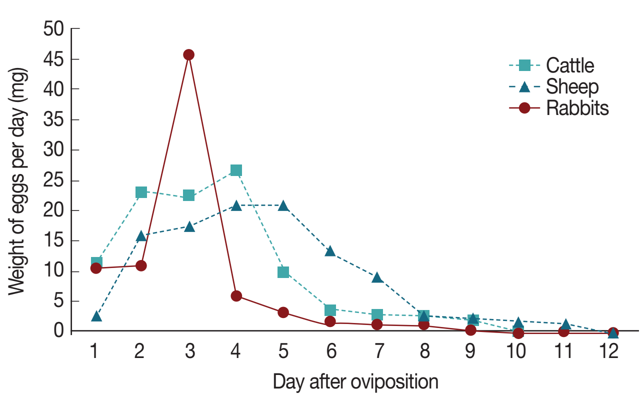

Fig. 2. Weight of eggs daily laid by each *Rhipicephalus* (*Boophilus*) *microplus* female engorged on rabbits, sheep, and cattle (based on 3 females).

engorged on each host species were different in time. Those of ticks dropped from cattle and sheep peaked from 2nd-4th and 2nd-6th day after oviposition, respectively. However, that of ticks from rabbits had a sharp peak on the 3rd day. Then, the weights of daily laid eggs were declined gradually (Fig. 2). The hatchability of 500 eggs laid by engorged females from sheep was considerable low, just up to 19.2±8.2% but those of cattle and rabbits were 96.0±4.0 and 83.0±3.7, respectively (Table 1). On the basis of these data, rabbits were more suitable as an alternative host to cultivate *R. microplus* for experiments of evaluating vaccines, chemical compounds, and biocontrol agents, rather than sheep.

DISCUSSION

The question of whether ticks have host specificity was much debated over the last half-century as it is important for understanding their evolution, population, and prevalence of diseases that they transmit. Recently, the viewpoint, ticks being global generalists but local specialists, is generally accepted by scientists [20]. It is considered that *R. microplus* has high specificity for cattle and is only found on other animals that usually appear with infested cattle, such as goats, sheep, camels, horses, donkeys, pigs, dogs, and some of wildlife deer and rodents [15,16,21]. Biological parameters of *R. microplus* fed on goats, horses, and rabbits were also analyzed in some literatures [22-25]. However, few of them compared the biological parameters of *R. microplus* fed on different hosts at the same conditions, which is important for evaluating the suitability of them as experimental animals in the research of *R. microplus* control techniques.

In the present study, we used 3 mammal species, i.e., cattle, sheep, and rabbits, for comparing the suitability as the hosts of *R. microplus* at the same conditions in the laboratory. On sheep, only 7 females were engorged (0.47% recovery). This result is slightly lower than that of goats, 0.68-1.9% tick recovery previously described by Daemon et al. [24]. They demonstrated that the low female recovery was due to animals grooming and scratching at the walls for riding larvae biting and caprine immune reaction against *R. microplus* infestation. However, on the internal region of the thigh, hindleg, and udder of goat body, infestation rates reached 12.6-39.0% [24]. In this experiment, we restricted ticks in a cloth chamber glued on the back of animals, which can ensure tick safety during larvae biting. However, most of ticks died in 5 days. Thus, low tick recovery might be responding to host species or infestation region. In addition, 4 of 7 engorged females died during the period of incubation and the average hatchability of eggs laid by the other 3 living females was just 19.2 ± 8.2 although the weight of eggs from single female and REI was quite high (96.6 ± 19.0 mg and 46.1, respectively). Daemon et al. [24] reported that the hatchability of eggs from goats was up to 93.6-94.3%. This suggests that, compared to goats, the sheep is not suitable for cultivating *R. microplus* as a host in the laboratory. However, sheep and goats might be the optional host for *R. microplus* in the field, especially when there is no other preferential host species available. For example, investigation of the prevalence in the field showed that the infestation rates of *R. microplus* in goats were 25.5% in Bangladesh [26] and 32.4% in Mozambique [27].

Do Amaral et al. [22] infested 4 rabbits with *R. microplus* in/on the ear and back. The recovery of engorged female ticks from backs was 4.9%, similar to that of our study (5.5%), but it was 10.7% from ears, similar to that of cattle in the present study (11.0%), which suggests that tick recovery is significantly associated with the infestation region on the host. Ears might be the preferential region for *R. microplus* larvae biting. The weight of replete females was 34.4-36.3 mg that is lower than those of the present study, 130.2 mg, but the REI was 17.4, slightly higher than that of the present study (14.3). The REI of replete females from cattle was also only 26.4 in the present study, although the weight of replete females was up to 312.5 mg. The possible reason is that the maintaining conditions of engorged ticks in the present study, such as temperature, humidity, light duration, or cultivation equipments, affect the quantity of eggs laid by engorged females. The hatching percentage of eggs laid by females engorged on rabbits was 83.0%, slightly low than that of cattle (96.0%). These data indicated that *R. microplus* could successfully complete its lifecycle on rabbits.

Generally, goats and rabbits can be as the alternative hosts of *R. microplus* for completing the lifecycle although bovine is its natural host. Sheep might be occasionally infested by *R. microplus* under certain conditions. Comparison of biological parameters of ticks on rabbits, goats, and cattle showed that rabbits could be used as an alternative host to cultivate *R. microplus* in experimental evaluation of vaccines, chemical compounds, and biocontrol agents, although the biological parameters of ticks from rabbits were less than those from cattle, which will improve the feasibility and decrease the cost of experiments. However, normally experimental rabbits have a diverse genetic background, which lead to deviation of data between experimental groups. Further researches should be carried out for testing the suitability of *R. microplus* on mice or rats that have homogenous genetic backgrounds, which is helpful for obtaining better results during evaluating efficacies of preparations against ticks or useful for performing other kinds of experiments.

ACKNOWLEDGMENTS

This study was financially supported by the Supporting Program (2013BAD12B03), NSFC (no. 31372432, 31201899, 31272556, 31402189, 31471967), ASTIP, FRIP (2014ZL010), CAAS; Creative Research Groups of Gansu Province (no. 1210RJIA006); “948”(2014-S05), NBCIS CARS-38, Special Fund for Agro-scientific Research in the Public Research (no. 201303035, 201303037), MOA; 973 Program (2015CB150300, 2010CB530206), MOST, China; Jiangsu Co-Innovation Center Programme for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, State Key Laboratory of Veterinary Etiological Biology Project. The research was also facilitated by CRP no. 16198/R0 IAEA.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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