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Effect of extrinsic incubation temperature on borrelial infection in various organs of *Ornithodoros (O.) savignyi*

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ABSTRACT

Dissemination levels of *Borrelia* sp. isolated from a natural population of *Ornithodoros savignyi* in Egypt were assessed in various organs of the infected female tick reared at three different temperatures. Results of the present investigation showed that the extrinsic incubation temperature at which the infected tick was reared (EI) was crucial in affecting borrelial dissemination levels in different organs including gut, salivary glands, coxal organ, ovaries and hemolymph. The increase of EI from 17°C to 27°C and 37°C increased the infection rates (IRs) and mean number (no.) of spirochetes localized in different organs. Also, it enhanced the appearance, prolonged persistence and delayed disappearance of spirochetes in most of the organs tested throughout the period of study (90 days after infective meal).

INTRODUCTION

The temperature at which the infected tick has been reared may influence the tick competence as a vector of disease and modify the course of infection with pathogens (Injeyan *et al.*, 1971; Young *et al.*, 1979; Young and Leitch, 1981; Dalgliesh and Stewart, 1982).

Few studies have shown that the extrinsic incubation temperature (EI) affects survival growth and transmission of lyme disease spirochete, *Borrelia burgdorferi*, and hence the competence of its ixodid tick vector (Shih *et al.*, 1995). Infection with *B. burgdorferi* was reduced and undetectable in the gut of *Ixodes scapularis* nymphs kept at 33°C and 37°C, respectively, as compared to moderate temperature at 27°C. Furthermore, Barbour (1984) found that lyme disease spirochete *B. burgdorferi* did not grow when cultured in BSK II medium at temperature in excess of 37°C. Also, Reisinger (1996) demonstrated that growth of two strains of *B. burgdorferi* was impaired when cultured at 37°C and inhibited at 39°C and 40°C.

In argasid ticks, no studies have been done on the effect of EI or borrelial infection and competence of the vector tick since Hindl investigation (1911) on distribution of *B. duttoni* in organs of *O. moubata* kept at 21°C and 36°C where spirochetes were found only in organs of ticks kept at 36°C.

Ornithodoros (O.) savignyi is one of seven ornithodorine tick species which had been recorded in Egypt (Hoogstraal & Kaiser, 1958) and was found to be naturally infected and able to transmit a specific *Borrelia sp.* to mammals (Shanbaky and Helmy, 2000). In evaluating vector competence, knowledge about dissemination and intensity of borrelial infection in the tick organs is essential. Studies on infection of organs involved in transmission to mammals (salivary glands and coxal organs) and to tick offspring (ovaries) are of major importance.

The aim of the present study is to evaluate the effect of rearing *O. (O.) savignyi* at three constant temperatures (17°C, 27°C and 37°C) on infection rates (IRs) with *Borrelia sp.* and mean numbers (no.) of the spirochetes in different organs and hemolymph of the adult female.

MATERIALS AND METHODS

Ticks and source:

Ornithodoros (O.) savignyi (Audouin), Argasidae, was collected from sand near cattle rearing places in Dahshore, Giza governorate, Egypt. The sand was sieved through paired large and small mesh metal sieves (Gaber *et al.* 1984).

Uninfected and infected tick colonies:

Field collected uninfected adult ticks were used to start uninfected colonies and were maintained at 27±1°C, 75% RH and 16 hrs light/day. Ticks were fed on hamster, *Mesocricetus auratus* as described by Helmy (2000).

Infected colonies started by feeding clean ticks on laboratory infected hamsters which had been fed upon by naturally infected *O. savignyi*. Infected colonies were kept at three different temperatures (17°C, 27°C and 37°C).

Detection of spirochetes in ticks and hamster:

Blood smears from tick and hamster (Helmy, 2000) were stained with Fontana stain (Conn *et al.*, 1960) or with direct (Piesman *et al.*, 1986) and indirect immunofluorescence technique (Lane and Manweiler, 1988), respectively.

For IDF antigen slides were prepared from spirochetes obtained from ticks and cultured in modified Kelly's medium (Barbour *et al.*, 1983). The spirochetes were centrifuged at 9000 xg for 20 min. at 22°C and the spirochete pellet was washed six times in sterile pbs adjusted to pH 7.4 and supplemented with 5 mM magnesium chloride. The spirochete pellet was diluted in pbs and applied to wells in scored slides for IDF test. The slides were incubated at 37°C until dry and stored at -70°C until used.

Spirochetes localization and number in tick organs:

Newly moulted uninfected females of *O. savignyi* were fed on infected hamsters. Counts were made in hemolymph (l µl) and different organs of each of 10 females daily for 10 days and then every 5 days for 60 days after feeding (daf) and then every 10 days till 90 daf. Hemolymph was collected and blood smears were prepared on slides. Gut, salivary glands, coxal organ and ovaries were dissected in a saline solution, squashed on slides, stained and examined microscopically and spirochete number in each organ was counted in ticks at each tested temperature.

The percentage of infection (IR) and mean number (no.) of spirochete in hemolymph and each organ was calculated. Data were analyzed using Chi-square test with the aid of Statistical Package for Social Science (SPSS) version 8.0 for Windows.

RESULTS AND DISCUSSION

The spirochetes infection rate (IR) and mean number (no.) in various organs and HL of the female *O. savignyi* are illustrated in Figures 1-5.

Localization of *Borrelia sp.* in the gut:

Spirochetes were observed in the gut contents of all adult females of *O. savignyi* on the first daf on hamster infected with *Borrelia sp.* Spirochetes persisted in the gut lumen for 80 and 90 daf of ticks kept at 17°C and 27°C or 37°C, respectively (Figs. 1 a & b). During these periods, (IRs) and (no.) of spirochetes per gut were relatively high at the beginning (IR = 90-100%; no. = 96±10.02, 103.9±14.3 and 111.4±11.55, respectively) and gradually decreased ($P < 0.05$) in subsequent days after feeding, starting on day 8 (IR = 80%, no. = 29.7±5.29), 25 (IR = 80%, no. = 39.1±7.39) and 35 (IR = 80%, no. = 33±6.15), at the three tested temperatures, respectively, to reach minima (IR= 10%, no. = 0.4±0.39, 0.3±0.29 and 1±0.67) at the end of their persistence period.

The present findings conform to those reported by Burgdorfer (1951), Teravsky (1959) and Nikita (1964 & 1965) where numbers of the spirochete *B. duttoni, B. sogidiana* and *B. anserim* greatly decreased in the gut lumen few days after infection in *O. moubata, O. papillipes (tholozani)* and *Argas persicus*, respectively, until disappeared. Migration of the spirochetes from the gut to the hemolymph may contribute to the observed drop in no. of spirochetes in the gut. Furthermore, Balashov (1972) suggested that later disappearance of spirochetes from gut lumen in ticks is probably associated with the unfavorable changes as ingested blood is gradually digested.

In accordance with the present findings are those of Helmy (2000) where *Borrelia sp.* IRs in field collected nymphs and adults of *O. savignyi* were high in summer months and low in winter months during which the highest and lowest temperatures were recorded, respectively.

Fig (1 a) The percentage infection with *Borrelia sp.* in the gut of female *O.savignyi* at different days after feeding on infected hamster at different temperatures

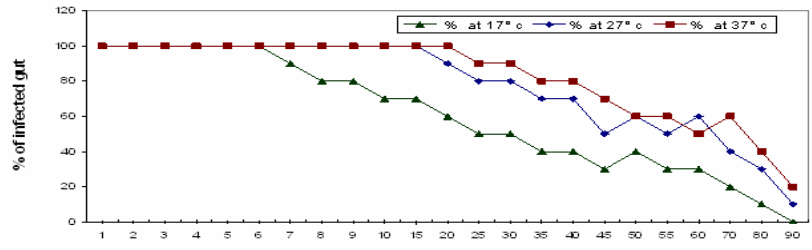


Fig (1 b) The mean number of *Borrelia sp.* in the gut of female *O.savignyi* at different days after feeding on infected hamster at different temperatures.

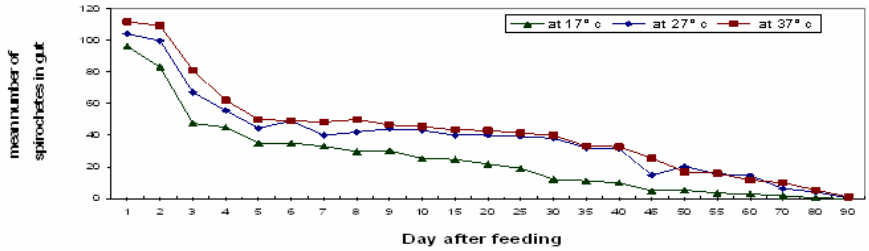


Fig (2 a) The percentage infection with *Borrelia sp.* in the hemolymph of *O.savignyi* at different days after feeding on infected hamster at different temperatures.

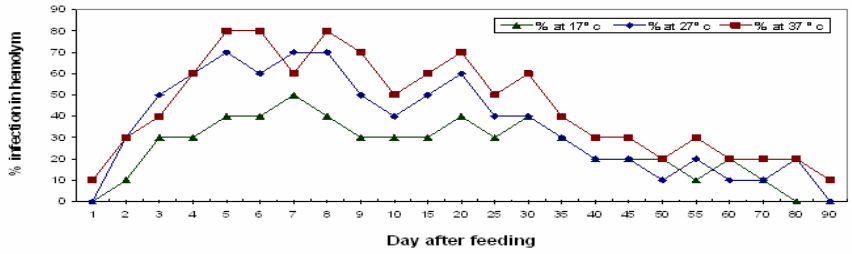


Fig (2 b) The mean number of *Borrelia sp.* in the hemolymph of female *O.savignyi* at different days after feeding on infected hamster at different temperatures.

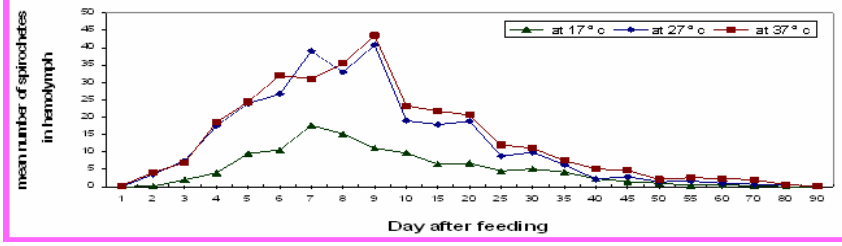


Fig (3 a) The percent of infected salivary gland with *Borrelia sp.* in female *O.savignyi* at different days after feeding on infected hamster at different temperatures.

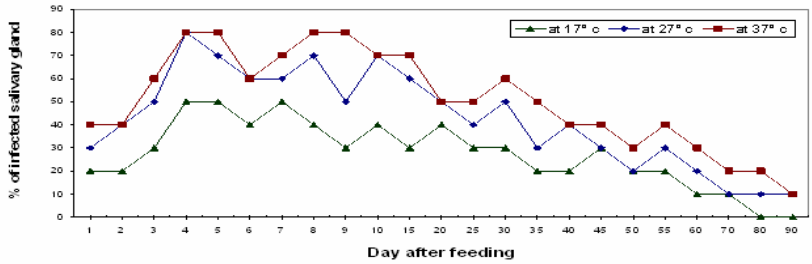


Fig (3 b) The mean number of *Borrelia sp.* in the salivary gland of female *O.savignyi* at different days after feeding on infected hamster at different temperatures.

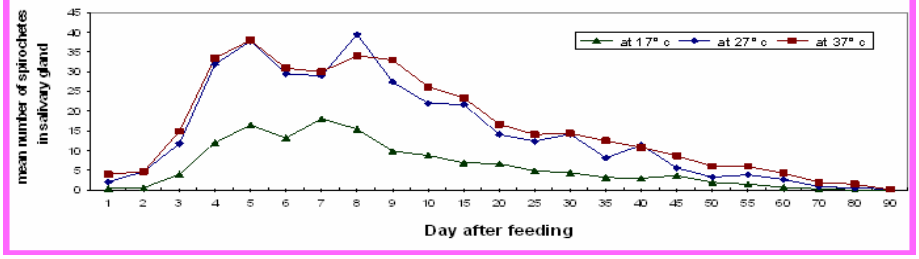


Fig (4 a) The percent of Infected coxal organ with *Borrelia sp.* In female *O.savignyi* at different days after feeding on Infected hamster at different temperatures.

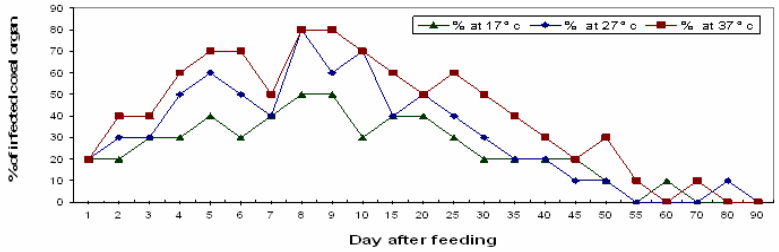


Fig (4 b) The mean number of *Borrelia sp.* in the coxal organ of female *O.savignyi* at different days after feeding on infected hamster at different temperatures.

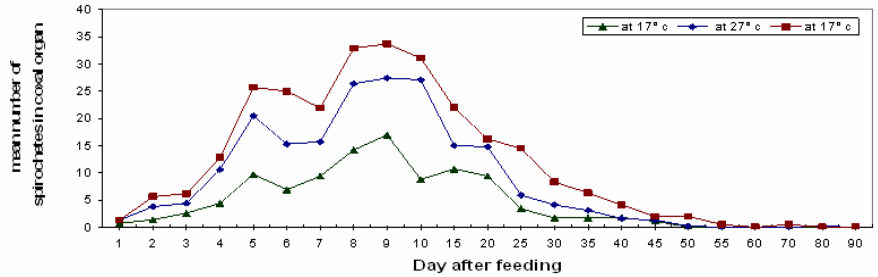


Fig (5 a) **The percent of infected ovary with *Borrelia sp.* in female *O.savignyi* at different days after feeding on infected hamster at different temperatures.**

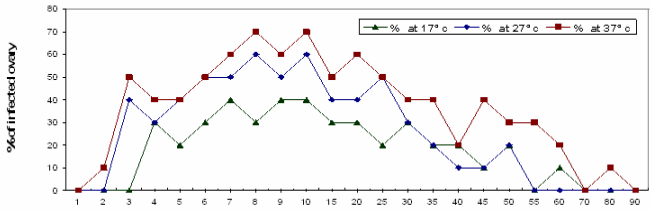
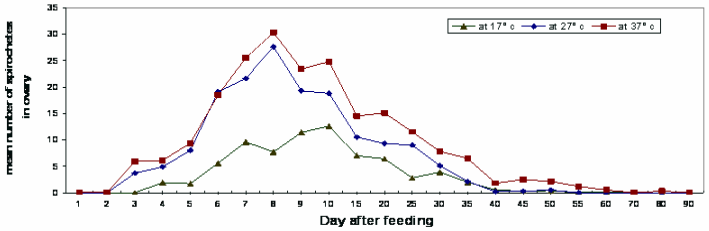


Fig (5 b) **The mean number of *Borrelia sp.* in the ovary of female *O.savignyi* at different days after feeding on infected hamster at different temperatures.**



The relatively high temperature, among other factors prevailed in summer might have contributed to the increased IR's reported in the aforementioned study. Temperature is one environmental factor known to affect persistence of spirochetal infection inside the tick. Shih *et al.* (1995) demonstrated that the persistence of spirochetal infection of the lyme disease *B. burgdorferi* in the gut of the nymphal deer tick, *Ixodes dammini* was reduced or became undetectable by incubation of the ticks at temperatures higher than 27°C (33°C and 37°C, respectively), for longer than two weeks. Although findings of the present study agreed with those of Shih *et al.* (1995) in the existence of an effect of EI on the level of borrelial infection inside the tick, our data pointed to a general increase of IR and no. of spirochetes and prolongation of persistence of infection inside the gut of the infected *O. savignyi* by increasing EI of the tick from 17°C to 27°C or 37°C but not from 27°C to 37°C. This difference might be attributed to probable biological and physiological differences of borreliae and tick species used in both studies.

Localization of *Borrelia sp.* in hemolymph:

In hemolymph of adult female *O. savignyi*, spirochetes were detected in the second and first daf of the tick and persisted for 70, 80 and 90 daf at 17°C, 27°C, and 37°C, respectively (Fig. 2 a & b). During these periods, IR's and no. of spirochetes in the hemolymph showed an initial period of increase ($P < 0.05$) in their levels to reach maxima on 7-8 daf (IR = 40-50%, no. = 15.2±7.14 – 17.7±8.87), 5-9 daf (50-70%, no. = 23.9±6.61 – 40.8±15.47) and 5-9 daf (IR = 70-80%, no. = 24.4±4.91 – 43.5±10.80) at 17°C, 27°C and 37°C, respectively. This was followed by a gradual decrease ($P < 0.05$) of the levels to reach minima (IR = 10, 20 & 10%, no. = 0.2±0.19, 0.7±0.52 & 0.1±0.09 at 17°C, 27°C & 37°C, respectively) at the end of their persistence period.

It is well documented that borreliae as well as other pathogens ingested during tick feeding on infective blood penetrate the gut wall and enter hemolymph to be distributed to other organs (Balashov, 1972 & Hoogstraal, 1985). In the present study, the initial increase of spirochete number in HL could be attributed to their migration from the gut to HL as was reported in other tick species (Gaber *et al.*, 1984 & Helmy *et al.*, 1996). However, multiplication of *Borrelia sp.* in HL might have contributed to their increase. Hodzic *et al.* (2002) reported that feeding stimulates spirochetes multiplication and their number may increase markedly after tick feeding on either infected or uninfected

host.Generally, IR of ticks with spirochetes in HL is dependent on the species of the vector and agent and also by the physiological state of the tick (Balashov, 1972). Hemolymph of argasid tick is known to be rich in important nutrients, fatty acids and amino acids which provide a favorable condition for multiplication of spirochetes (Burgdorfer *et al.*, 1989). Furthermore, tick HL has been reported as a favorable medium which allows survival and intracellular multiplication of other pathogenic organisms such as rickettsiae (Balashov and Daiter, 1965) and piroplasm (Markov & Abromov, 1958). The aforementioned investigations support the present suggestion that migration from the gut and multiplication in HL of *Borrelia sp.* may explain the initial increase of the IRs and no. of the spirochete in Hl during the first few days after the tick infective meal at the three tested temperatures in the present study.

Localization of *Borrelia sp.* in salivary glands and coxal organ:

Spirochetes were detected in salivary glands and coxal organ of female *O. savignyi* infected with *B. sp.* as early as the first daf at all temperatures tested (Fig. 3 & 4). Generally in each organ (salivary and coxal) there was a period of a gradual increase ($P < 0.05$) of IR and no. of spirochetes per organ to reach maxima where the risk of transmission of *B. sp.* from the tick to host is anticipated to increase (Piesman *et al.*, 2001). These maxima were reached in the salivary glands on 4-8 daf (IR = 40-50%, no. = 12±4.49 – 18.1±6.53), 4-15 daf (IR = 60-80%, no. = 21.7±6.79 – 37.8±9.17) and 4-15 daf (IR = 60-80%, no. = 23.4±5.43 – 38±7.31) at 17°C, 27°C & 37°C, respectively. In coxal organ maximal infection levels were reached on 8-9 daf (IR = 50%, no. = 14.2±5.27 – 17±6.45), 8-10 daf (IR = 60-80%, no. = 26.4±7.13 – 27.4±8.69), and 8-10 daf (IR = 70-80%, no. = 31.1±7.41 – 33.7±6.76) at 17°C, 27°C & 37°C, respectively. The increase of infection levels was followed by a period of a gradual decrease ($P < 0.05$) of the spirochetal infection until disappeared from the salivary glands on 80 daf at 17°C or reached minima at the end of the persistence period in the gland (90 daf) at 27°C and 37°C. Spirochetes nearly disappeared from coxal organs on 55-80 daf of the three tested temperatures.

The early appearance of spirochetes (first daf) in the salivary glands might be attributed to contamination of the gland during feeding on infective blood meal or dissemination from hemolymph. De-Silva and Fikrig (1995) demonstrated that the spirochete, *B. burgdorferi* had disseminated to the salivary glands in the majority of nymphs of *Ixodes scapularis* 36-48 hrs after attachment. Piesman *et al.* (2001) found that spirochete, *B. burgdorferi* in the tick salivary glands increased more than 17 fold from 1.2 per salivary gland pair before feeding to 20.8 at 72 hrs post attachment. The period of the most rapid increase in number of spirochetes in the salivary glands occurred from 48 to 60 hrs post attachment. This time period coincided with maximal increase of transmission risk during nymphal feeding. Furthermore, heavy infections with *B. duttoni*, *B. sogidiana* and *B. anserina* were observed in the salivary glands of *O. moubata* (Giegy, 1951), *O. tholozani* (Teravsky, 1959) and *Argas persicus* (Nikitina, 1965), respectively. Varma (1956) reported that the transmission of relapsing fever spirochetes by *Ornithodoros* ticks was mainly by inoculation of infective saliva and coxal fluid into the bite wound. In the present work, early appearance of spirochetes in the coxal organs of female *O. savignyi* can be correlated to the production of the coxal fluid which is discharged during feeding and shortly after detachment of the argasid tick species (Balashov, 1972).

Localization of *Borrelia sp.* in the ovary:

Spirochetes showed their first appearance in the ovary of female *O. savignyi* on the fourth, third and second daf on infected hamsters at 17°C, 27°C, and 37°C, respectively (Fig. 5 a & b). The initial appearance was followed by a gradual increase ($P < 0.05$) of IR and no. of spirochetes in ovaries up to 10 daf at the three-tested temperatures (Fig. 5 a & b). Maximal infection levels in ovaries reached on 7-10 daf at 17°C (IR = 30-40%, no. = 7.7±4.54 – 12.6±5.55), and on 6-10

daf at 27°C (IR = 50-60%, no. = 18.8±6.19 – 27.6±8.50) and 37°C (IR = 50-70%, no. = 18.5±6.7 – 30.3±7.41). Infection levels decreased ($P < 0.05$) in subsequent days until spirochetes nearly disappeared on 55, 55 and 70 daf at 17°C, 27°C and 37°C, respectively.

Heavy infections of the reproductive organs of *O. moubata* with *B. hispanica* (Grun 1950) and *B. duttoni* (Giegy, 1951) and of *Ixodes pacificus* with *B. burgdorferi* (Burgdorfer, *et al.*, 1989) were reported. Zhenqin (1998) stated that *B. burgdorferi* could be found intracellularly within ovaries of *I. ricinus*. We suggest that migration and penetration of spirochetes into ovaries and oocytes of the tick vector are prerequisites of transovarial transmission. Also, it could be considered as a physiological adaptation increasing the potential and competence of the tick as a reservoir and vector of a pathogen (Hoogstraal, 1985). Furthermore, localization of the spirochete in the ovaries may help in recognizing the specificity of the tick species as a vector of a certain *B. species* particularly in ornithodorine and some argasid ticks (Balashov, 1972 & Hoogstraal, 1985). Experimental infection of some argasid (Diab & Soliman, 1977 and Zaher *et al.*, 1977) and of some ornithodorine (Gaber *et al.*, 1984 and Shanbaky & Helmy, 2000) tick species with borreliae species isolated from other tick vectors resulted in failure of localization of the spirochetes in the gonads and transovarial transmission in the unnatural recipients.

In general, there was no significant difference ($P > 0.05$) between IR and no. of spirochetes in each organ of *O. savignyi* adult females kept at 27°C and 37°C at different daf. However, there was a significant increase ($P < 0.05$) of infection levels in each organ of females kept at 27°C and 37°C than females kept at 17°C at different daf.

The results and discussion of the present study show that the appearance, persistence, IR and no. of the spirochete varied in the different organs examined which conform with other previous investigations (Varma, 1962; Balashov, 1972; Diab & Soliman, 1977; Zaher *et al.*, 1977, and Gaber *et al.*, 1984). It was suggested (Burgdorfer, 1951), and experimentally demonstrated (Sarsian, 1959, and Grun & Blatter, 1960) that chemotropic factors induce micro-organisms to migrate and to localize in tick tissues.

The present work, showed that the increase of the extrinsic incubation temperature (EI) at which the female *O. savignyi* infected with *Borrelia sp.* was kept, crucially affected dissemination levels of the spirochete in organs of the tick vector. Increasing EI temperature from 17°C to 27°C or 37°C significantly increased the IR and no. of spirochetes in different organs. Also, it enhanced the initial appearance and prolonged persistence or delayed disappearance of spirochetes in most of the organs tested throughout the period of study (90 daf after infective meal).

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ARABIC SUMMARY

تأثير درجة الحرارة بالبيئة المحيطة على معدلات الإصابة بالبورليا في الأعضاء الداخلية المختلفة لقراد *أورنيثودوروس سافيجنياى*.

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تم تعيين معدلات إنتشار البورليا (المعزولة من مجموعات القراد المصابة فى الطبيعة بمصر) فى الأعضاء الداخلية المختلفة لإنثى القراد المصابة بعد تربيتها عند ثلاث درجات حرارة مختلفة وذلك بعد تغذيتها على حيوان الهامستر المصاب بالبورليا. وقد وجد من الدراسة الحالية أن درجات الحرارة بالبيئة المحيطة لها تأثير فعال على معدلات الأصابة بالبورليا فى الأعضاء الداخلية للقراد مثل المعى والغدد اللعابية والسائل الحرقفى والمبايض والدم. وقد وجد أنه بإزدياد درجة الحرارة بالبيئة المحيطة من 17 م إلى 27و 37م تزداد معدلات الإصابة وعدد السبيروكيت (البورليا) فى الأعضاء الداخلية المختلفة. وكذلك فإن زيادة درجات الحرارة بالبيئة المحيطة تعمل على اطاله مدة بقائها وتأخير إختفائها فى الأعضاء الداخلية المختلفة التى تم إختبارها خلال فترة الدراسة (90 يوم بعد تغذية القراد على وجبة دم مصابة).