BIONOMICS OF *OTOBIUS MEGNINI*, DUGES,

(ACARI: ARGASIDAE)

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PREFACE

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ABBREVIATIONS

24-h	24 hours
R. H.	Relative humidity
T	Temperature
24 h DD	24 hours darkness
R. E. I.	Reproductive Efficiency Index
C. E. I.	Conversion Efficiency Index
L. S. D.	Least Significant Difference
Wt.	Weight
Lab.	Laboratory
C. V.	Coefficient of variation
12 D: 12 L	12 hours darkness, 12 hours lightness
S. D.	Standard Deviation
S. E.	Standard Error
%	Percent
°C	Degree Celsius
h	Hours
mg	Milligrams
µg	Micrograms
eggs/mg	Eggs per milligram
µg/mg	Microgram per milligram
p	Probability
r	Correlation coefficient
r^2	r-square

m^2	Square meters
cm^3	Cubic centimeters
g	Grams
10-ml	10 milliliter
EDTA	Ethylene diamine tetraacetic acid
W. B. C.	White blood cells
IgG	Immunoglobulin G
/	Per
P_2O_5	Phosphorous pentoxide
$MgCl_2$	Magnesium chloride
NaCl	Sodium chloride
$K_2Cr_2O_2$	Potassium dichromate
O. S. U.	Oklahoma State University
$p < 0.05$	5% probability level

GENERAL INTRODUCTION

Otobius megnini (Duges) is a cosmopolitan, one host tick with a broad host range. It is present in north and south America (Hooker, et al. 1912; Becklund and Mitchell, 1958; Duges, 1884; Gregson, 1953; Cooley and Kohls, 1944; Kemper and Peterson, 1953; Dios, 1930; Keirans, 1972; Rich, 1957), Africa (Theiler and Salisbury, 1950; MacLeod, et al. 1970; Schoenaers, 1950) and Asia (Ramanujachari and Alwar, 1955; Sen, 1937; Chellappa, 1973; Hoogstraal and Klein, 1979).

Otobius megnini has been found only on mammals probably because it lives in the external auditory meatus (Hooker, et al. 1912; Herms, 1917) which is an anatomic structure present only in the vertebrate class mammalia. The orders and species of mammals used by O. megnini as hosts are: 1) Artiodactyla; Bos taurus, Odocoileus hemionus, Ovis aries, Antilocapra americana, Tayassu tajacu, Oreamnos americanus, Ovis canadensis, Odocoileus virginianus, Cervus elaphus and Capra hircus (Rich, 1957; Chellappa and Alwar, 1972; Schad, 1958; Allen, 1955; Meleney and Roberts, 1970; Meleney, 1975). Among these species Ovis aries (Koshy, et al. 1979; Herrero and Barker, unpublished)and Bos taurus (Herrero and Barker, unpublished; Wanchinga and Barker, 1986) have been used in the laboratory to rear O. megnini, 2) Perissodactyla; Equus caballus and Equus asinus (Townsend, 1893; Cooley and Kohls, 1944), 3) Lagomorpha; Sylvilagus floridanus, Lepus californicus and Lepus walda (Rodriguez, 1977; Cooley and Kohls, 1944), Oryctolagus

cuniculus have been used in the laboratory to rear O. megnini (Herrero and Barker, unpublished; Wanchinga and Barker, 1986), 4) Carnivora; Canis familiaris, Canis latrans and Felis catus (Cooley and Kohls, 1944), 5) Primates; Homo sapiens (Eads and Campos, 1984).

Base on the world distribution of O. megnini host species, it is possible to suggest a broader geographic range for this tick. However, more systematic and current surveys are needed to fully understand the actual range of O. megnini.

In Oklahoma, O. megnini is a common pest of horses and cattle. Other domestic and wild hosts may also be attacked (Hair, et al. 1979). No studies have quantified the effect of O. megnini on the health of host animals. However, it is possible to suggest a direct effect on livestock production (milk or beef) due to annoyance, irritation, damage to skin tissues and loss of blood (Rich, 1957; Hooker, et al. 1912; Stiles, 1944). Nervous alterations and tick paralysis of hosts have been associated with infestations of O. megnini (Gregson, 1953; Ramanujachari and Alwar, 1955). Biological transmission of pathogens has not been demonstrated, but Bacillus anthracis (Stiles, 1944) and Coxiella burnetti (Jellison, et al. 1948) have been isolated from this tick.

The life cycle of Otobius megnini includes a parasitic and a free living phase (Hooker, et al. 1912; Herms, 1917); the immature stages larva and nymphs, are the parasitic stages, with the adult being non parasitic. Little is known about the bionomics of this tick and only a few notes related to its molting and reproductive behavior have being published. The objectives of this thesis are: a) to evaluate the reproductive potential of O. megnini under constant conditions; b) to compare the

reproductive efficiency and egg output in two different weight groups of O. megnini females; c) to compare molting parameters among nymphs obtained from three different hosts and d) to evaluate, under field conditions, the reproductive efficiency, egg output and oviposition/eclosion dynamics of O. megnini. Each part of this thesis is a separate and complete manuscript to be submitted for publication. Each part is in the format of the Journal to which it will be submitted.

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PART I

REPRODUCTIVE POTENTIAL OF FEMALE *OTOBIUS MEGNINI* DUGES, (ACARI: ARGASIDAE) UNDER LABORATORY CONDITIONS

ABSTRACT

Otobius megnini (Duges) reproductive potential was studied in a group of one-month-old, post-ecdysis females of similar weight under constant conditions in the laborabory (75% R.H., 20°C, 24 h DD). The mean initial weight of this group of females was 125.6 ± (S.D.) 13.3 mg, and they were obtained from a laboratory colony fed on cattle.

Oviposition dynamics were characterized by a preoviposition period of 3.2 ± (S.D.) 1.4 days and an oviposition period of 105.4 ± (S.D.) 18.1 days with 20.0 ± (S.D.) 4.8 ovipositions separated by 19.4 ± (S.D.) 4.0 regular intervals of 4.9 ± (S.E.) 2.1 days.

Total number of eggs laid per female was 856 ± (S.D.) 225 with a total mass weight per female of 34.6 ± (S.D.) 8.9 mg. The relationship between quantity of eggs (numbers or mass) and oviposition number was analyzed. The mean individual egg weight was 43.9 ± (S.E.) 11.0 μg; incubation period was 18.1 ± (S.E.) 2.5 days with 38.95 ± (S.D.) 15.85 percent hatch.

Mean nutrient weight was 58.9 ± (S.D.) 15.4 mg; nutrient index was 59.6 ± (S.D.) 9.8%; reproductive efficiency index was 6.8 ± (S.D.) 1.6 eggs/mg; and conversion efficiency index was 277.3 ± (S.D.) 67.4 μg/mg. A positive correlation between egg quantity (numbers and mass) and nutrient weight was demonstrated.

A negative correlation between postoviposition female weight and oviposition number existed; both males and females lost weight during oviposition.

INTRODUCTION

Facultative autogeny occurs in the argasid ticks Argas persicus (Oken), Ornithodoros tartakovskyi (Olenev), Ornithodoros papillipes (Birula) (Balashov, 1972), Ornithodoros tholozani (Laboulbène and Mégnin) and Ornithodoros parkeri (Cooley) (Feldman-Muhsam, 1973). In Otobius and Antricola species autogeny has been reported to be obligatory (Balashov, 1972).

Only a few notes on the reproductive biology of Otobius megnini (Duges) have been published. The period from nymphal molting to the beginning of female oviposition, total number of eggs produced by females, duration of oviposition and percent hatching have been studied. These studies indicated the intermittent nature of the oviposition behavior and the inability of O. megnini to lay eggs without mating (Hooker, et al. 1912; Herms, 1917).

Parish (1949) reported that a female O. megnini must mate only once in order to be able to lay eggs throughout her productive life. He also, indicated that under natural conditions the incubation period of eggs is dependent on environmental factors such as temperature and that the initial egg mass contained the most eggs and the last clusters usually contained fewer eggs.

Recently, pre-mating period, frequency and duration of intermission periods, pre-oviposition period, oviposition period, egg incubation period, egg output, conversion efficiency index (C.E.I.) and

reproductive efficiency index (R.E.I.) have been evaluated for females reared in the laboratory (Wanchinga and Barker, 1986).

The objective of this study was to evaluate the reproductive potential of O. megnini under constant conditions in the laboratory.

MATERIALS AND METHODS

Otobius megnini adults less than one-month-old, post-ecdysis, were obtained from a laboratory colony fed on cattle maintained in the Medical Entomology Laboratory at Oklahoma State Unviersity.

Nine females and nine males were weighed individually, paired, and placed in vials maintained in a chamber at a temperature of 20°C, photoperiod 24 h DD and a relative humidity of 75%. Temperature and relative humidity were monitored using a thermometer and an Airguide Hygrometer (Airguide Instr. Corp., Chicago, Ill., U.S.A.).

The number of days between pairing and the day that the first batch of eggs was observed was defined as the pre-oviposition period. Daily observations were made of each pair and for each oviposition the following data were noted: a) date on which the eggs were laid, b) female weight, c) egg number and mass, d) the day in which the first larvae eclosed in each batch of eggs (incubation period), and e) the number of larvae that eclosed within 30 days of the first larval eclosion (hatching number). The eggs were counted under a stereomicroscope and their weight was measured with a Mettler H 51 balance sensitive to 0.1 mg (Mettler Instr. Co., Heighstown, N.J.). The oviposition period was defined as the period between the first and the last oviposition and initial and final weights were compared. Nutrient weight and nutrient index were calculated as for Boophilus microplus (Canestrini) by Bennett (1974a) and the reproductive efficiency index (R.E.I.) and

conversion efficiency index (C.E.I.) as for Amblyomma maculatum (Koch) by Drummond and Whetstone (1970).

Regression analysis was used to establish the relationship between the following: 1) egg quantity (mass and numbers) and nutrient weight, 2) egg quantity (mass and numbers) and number of ovipositions, 3) postoviposition female weight and oviposition number, 4) oviposition period and nutrient weight, 5) number of ovipositions per female and oviposition period and 6) nutrient weight and initial weight.

Egg quantity (mass and number), incubation period, postoviposition female weight and duration of intermission periods from each oviposition were analyzed as a randomized complete block design and a Least Significant Difference (L.S.D.) test was used to compare means when significant differences were found (Steel and Torrie, 1960).

RESULTS

Oviposition dynamics of O. megnini were characterized by a pre-oviposition period of 3.2 ± 1.4 days and an oviposition period of 105.4 ± 18.1 days. There were 20.0 ± 4.8 ovipositions separated by 19.4 ± 4.0 regular intervals of 4.9 ± 2.1 days (S.E.). No significant differences ($P < 0.05$) were found among the durations of intermission periods. The number of females that laid eggs, the percent of eggs laid and percent larval eclosion decreased as oviposition number increased.

Total number of eggs laid per female was 856 ± 225 with a total mass of 34.6 ± 8.9 mg. There was a negative correlation between egg mass and oviposition number ($Y = -0.32x + 0.01x^2 + 3.91$, $r^2 = 0.70$, ($P < 0.01$) and also between egg number and oviposition number ($Y = -7.32x + 0.17\ x^2 + 95.23$, $r^2 = 0.81$, $P < 0.01$). There were significant differences ($P < 0.05$) in egg mass weights and numbers of eggs produced during the first six ovipositions after which no significant difference existed. In the first egg batch the greatest quantity of eggs was produced. The number and mass of eggs produced in this oviposition was 13.7% and 14.3%, respectively, of the totals.

The mean and standard error for individual egg weight of 43.9 ± 11.0 µg, incubation period of 18.1 ± 2.5 days and percent hatching of 38.95 ± 15.85% were determined. No significant differences ($P < 0.05$) were observed among incubation periods of eggs produced in different ovipositions.

Mean nutrient weight was 58.9 ± 15.4 mg, and the total egg mass produced by a female represented 57.3 ± 11.1% of this weight. Nutrient index was 59.6 ± 9.8%, R.E.I. was 6.8 ± 1.6 eggs/mg and C.E.I. was 277.3 ± 67.4 μg/mg. A positive correlation was found between egg mass and nutrient weight ($Y = 2.70x + 0.03\ x^2 + 126.81$, $r^2 = 0.98$, $P < 0.01$) and also between egg number and nutrient weight ($Y = 47.88x - 0.29\ x - 910.89$, $r^2 = 0.89$, $P < 0.01$).

There was a negative correlation between postoviposition female weight and oviposition number ($Y = -3.21x + 0.03x^2 + 126.81$, $r^2 = 0.98$, $P < 0.01$). There were significant differences ($P < 0.05$) among post-oviposition female weights determined after different ovipositions.

A reduction in the weight of males during the oviposition period was observed. This reduction represented 36.3% of the initial weight and was significantly different than the female weight loss ($P < 0.05$). The initial weight of males was 54.4 ± 5.3 mg. The weight loss by females represented 46.9% of the initial weight which was 125.6 ± 13.3 mg. No significant correlations were found between oviposition period and nutrient weight ($r^2 = 0.0337$), number of ovipositions per female and oviposition period ($r^2 = 0.1065$) or nutrient weight and initial weight ($r^2 = 0.2862$).

DISCUSSION

Obligatory autogeny makes the genera Otobius and Antricola unique among argasid ticks (Balashov, 1972). Little is known about reproduction in these genera; less is known about the mechanism of blood meal conversion into eggs in O. megnini. In this paper we evaluated the reproductive potential of O. megnini under constant conditions.

Factors that affect the quantity of eggs produced by ticks are: origin of the blood meal (Galun, et al. 1978), abiotic factors such as temperature, relative humidity and photoperiod (Bennett, 1974b; Fujisaki, et al. 1975), the initial weight of the females (Honzakova, et al. 1975) and mating (Leahy and Galun, 1972; Aeschlimann and Grandjean, 1973). In our study all these conditions were controlled.

The total egg mass produced by a female represented 57.3 ± 11.1% of nutrient weight. The loss of weight not explained by conversion to eggs could have been a result of energy spent on cellular respiration (Obenchain and Oliver, 1973), loss of water (Lees, 1947; Browning, 1954), and excretion (Bassal and Hefnway, 1972). Reproductive efficiency in O. megnini evaluated by the nutrient index was lower than that of ixodid ticks (Bennett, 1974; Gray, 1981).

The relationship between egg quantity (mass or number) and nutrient weight was better estimated by quadratic than by linear equations which seems to indicate that no matter how much energy is available there is a limit for egg production.

When females of similar weight were compared, nutrient weight did

not correlate significantly ($P < 0.05$) with initial weight, which indicates that other factors such as metabolic (Galun and Warburg, 1968; Aboul-Nasr and Bassal, 1972) or neuroendocrine (Shanbaky and Khalil, 1975) differences among females may be involved in energy use.

A positive correlation between the quantity of eggs and initial weight was established for O. megnini when the R.E.I. was calculated. This compared to R.E.I.'s of the following female hard tick species: Ixodes ricinus (L.) (8.96 eggs) (Graaf, 1978), Hyalomma aegyptium (L.) lay (9 eggs) (Sweatman, 1968), Rhipicephalus sanguineus (Latreille) (11 eggs) (Sweatman, 1967), and Amblyomma maculatum (9.72 eggs) (Drummond and Whetstone, 1970) which indicates that female ixodid ticks are more efficient at producing eggs than is O. megnini. Also, the C.E.I., as an index to estimate the ability of females to convert body weight into egg weight was lower for O. megnini than those for ixodid ticks (Drummond and Whetstone, 1970).

The quantity of eggs produced in ovipositions I thru VI represented 51.4% of the total egg number and 51.5% of the total egg mass. The decline after oviposition VI could be an effect of aging (Aeschliman and Grandjean, 1973), the consequence of a reduction in the quantity of blood meal reserve available (Kitaoka, 1961) or due to a decrease in the quantity of sperm available (mating frequency or sperm stored) (Aeschlimann and Grandjean, 1973). Also, there was a decrease in the number of females that laid eggs over time.

Percent larval eclosion was lower than reported by Wanchinga and Barker (1986) possibly because of the physiological state of the eggs laid and relative humidity and temperature conditions.

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PART II

EFFECT OF INITIAL WEIGHT ON REPRODUCTIVE EFFICIENCY AND EGG OUTPUT IN FEMALE *OTOBIUS MEGNINI* DUGES (ACARI: ARGASIDAE)

ABSTRACT

The means and standard deviations of Otobius megnini (Duges) reproductive efficiency in the laboratory was compared in two groups of females of different weights. Initial weight in group 1 was 38.9 ± 5.7 mg and in group 2 was 118.9 ± 12.8 mg.

In group 1, preoviposition period was 17.4 ± 6.9 days, oviposition period was 56.8 ± 22.6 days; ovipositions per female were 13.4 ± 5.0; intermissions per female were 12.4 ± 5.0; total number of eggs per female was 222.6 ± 48.8; total egg mass per female was 9.8 ± 2.1 mg; nutrient weight was 16.6 ± 5.7 mg; nutrient index was 60.8 ± 10.6%; reproductive efficiency index was 5.7 + 1.1 eggs/mg and conversion efficiency index was 251.9 + 46.3 µg/mg.

In group 2, preoviposition period was 9.0 ± 4.6 days; oviposition period was 98 ± 15 days; ovipositions per female were 31.3 ± 6.9; intermissions per female were 30.3 ± 6.9; total number of eggs per female was 985 ± 412; total egg mass per female was 43.2 ± 18.1 mg; nutrient weight was 63.3 ± 17.9 mg; nutrient index was 65.5 ± 13.5%; reproductive efficiency index was 8.1 ± 2.8 eggs/mg and conversion efficiency index was 355.5 ± 124.3 µg/mg.

All ovipositional parameters measured were significantly greater in the heavier female group ($P < 0.05$) except for the nutrient index, and reproductive and conversion efficiency indexes.

When final weight, weight loss, egg weight and nutrient weight

were calculated as a percentage of female initial weight, there were no significant differences ($P < 0.05$) between groups.

INTRODUCTION

A positive correlation between engorged female weight and the quantity of eggs produced during oviposition has been found in anautogenous ixodid ticks (Koch and Dunn, 1980; Gladney and Drummond, 1970; Drummond et al., 1969; Honzakova et al., 1975; Bassal and Hefnawy, 1972; Drummond and Whetstone, 1970) and argasid (Hafez et al., 1972; Mango and Galun, 1977ab) tick species. In facultative autogenous species engorged female weight has a positive effect on production of eggs (Feldman-Muhsam, 1973; Feldman-Muhsam and Havivi, 1973) and also on the expression of autogeny (Pound et al., 1984). In obligatory autogenous species no study has been conducted to evaluate initial weight effect of females on reproductive efficiency and egg output.

Otobius megnini (Duges) is an obligatory autogenous specie (Balashov, 1972). Weights of female O. megnini obtained from nymphs fed on different hosts under laboratory and field conditions vary. Wanchinga (1986) observed different rates of engorgement by nymphs on cattle and rabbits, with newly molted female weights what ranged from 53 to 118 mg.

Female O. megnini reproduction has not been studied to evaluate the effect of initial female weight on reproductive efficiency and egg output. Therefore, two different weight groups of females were studied in the laboratory to compare this relationship.

MATERIALS AND METHODS

A comparison of the reproductive efficiency and egg output of two different weight groups of O. megnini females was conducted. Adults less than one week old post-ecdysis were obtained from a laboratory colony of nymphs collected from cattle and maintained at the Medical Entomology Laboratory at Oklahoma State University.

Eleven females were assigned to one of two groups of similar weight. One group of 5 females had an initial weight of 38.9 ± 5.7 mg, and the other group of 6 females had an initial weight of 118.9 ± 12.8 mg. A group of 11 males had an initial mean weight of 27.1 ± 5.7 mg. Females and males were paired and placed in vials and labeled by group.

During the study pairs were maintained at 85% relative humidity, 21°C and total darkness in a humidity chamber. Relative humidity and temperature were monitored using a thermometer and an Airguide Hygrometer (Airguide Instr. Co. Chicago, Ill, U.S.A.).

Preoviposition period was defined as the number of days between pairing and the day that the first batch of eggs was observed. Each pair of adults was observed daily for 5 months and for each oviposition the following data were noted: a) date on which the eggs were laid, b) female weight, c) egg number, and d) egg mass. The eggs were counted using a stereomicroscope and their weight determined. Oviposition period was defined as the period between the first and the last oviposition. Females were weighed after the last oviposition and initial and final weights were compared.

Nutrient weight and nutrient index were calculated as for Boophilus microplus (Canestrini) by Bennett in 1974 and the reproductive efficiency index (R.E.I.) and conversion efficiency index (C.E.I.) as for Amblyomma maculatum (Koch) by Drummond and Whetstone in 1970.

Final weight, egg weight, nutrient weight and weight loss were calculated as percentages of the initial weight. The means for each parameter, from both groups, were compared using Student's t tests (Steel and Torrie, 1960).

RESULTS

In group 1, of lower mean weight, mean preoviposition period was 17.4 ± 6.9 days, mean oviposition period was 56.8 ± 22.6 days, mean number of ovipositions per female was 13.4 ± 5.0 and mean number of intermissions per female was 12.4 ± 5.0. In group II mean preoviposition period was 9.0 ± 4.6 days, mean oviposition period was 98.0 ± 15.0 days, mean number of ovipositions per female was 31.9 ± 6.9 and mean number of intermissions per female was 30.3 ± 6.9. When a comparison between both groups was made, all these parameters were significantly different ($P < 0.05$). It is clear that heavier females have a shorter preoviposition period, longer oviposition period and a greater number of ovipositions per female.

In group I the mean egg number was 222.6 ± 48.8 and the mean egg mass was 9.8 ± 2.1 mg, and in group II the mean egg number was 985 ± 412 and the mean egg mass was 43.2 ± 18.1 mg. The differences observed between both groups were significant ($P < 0.05$). The minimum number of eggs produced by the lightest female (initial weight of 31.2 mg) was 170 with an egg mass weight of 7.5 mg and the maximum number of eggs produced by the heaviest female (initial weight of 137.0 mg) was 1524 with an egg mass of 66.9 mg.

Reproductive efficiency was evaluated in both groups; in group I mean nutrient weight was 16.6 ± 5.7 mg, mean nutrient index was 60.8 ± 10.6%, mean R.E.I. was 5.7 ± 1.1 eggs/mg and mean C.E.I. was 251.9 ± 46.7 µg/mg, and in group II mean nutrient weight was 63.3 ± 17.9 mg,

mean nutrient index was 65.5 ± 13.5%, mean R.E.I. was 8.1 ± 2.8 eggs/mg and mean C.E.I. was 355.5 ± 124.3 µg/mg. No significant differences ($P < 0.05$), were observed between both groups in nutrient index, R.E.I. and C.E.I.; nutrient weight differences were significant ($P < 0.05$).

Final weight, nutrient weight, eggs weight and weight loss were expressed as percentages of the initial weight. In group I, final weight represented 57.5 ± 11.0, nutrient weight 42.7 ± 11.1, egg weight 25.2 ± 4.7 and weight loss 17.5 ± 3.1 percent of the initial weight, which was 38.9 ± 5.7 mg. In group II, final weight represented 47.4 ± 11.1, nutrient weight 52.6 ± 11.1, egg weight 35.6 ± 12.4 and weight loss 17.1 ± 3.1 percent of the initial weight which was 118.9 ± 12.8 mg. No significant differences ($P < 0.05$) were observed in the initial weight mean percentages between both groups.

DISCUSSION

A linear relationship between initial weight of the females and the quantity of eggs produced has been found in Amblyomma americanum (L.) (Koch and Dunn, 1980; Gladney and Drummond, 1970; Drummond, et al. 1971), Boophilus microplus (Canestrini) (Kitaoka and Yajima, 1958), Hyalomma aegyptium (L.) (Sweatman, 1968), Dermacentor variabilis (Say), Rhipicephalus sanguineus (Latreille) (Nagar, 1968), Anocentor nitens (Neumann) (Drummond, et al. 1969), Ixodes ricinus (L.) (Honzàkova, et al. 1975), Hyalomma dromedarii (Koch) (Bassal and Hefnway, 1972), and Amblyomma maculatum (Koch) (Drummond and Whetstone, 1970). All these tick species are anautogenous ixodids and their production of eggs is dependent on the quantity of blood ingested during the adult stage.

In argasid ticks, two types of gonotrophic strategies may be found: anautogenous or autogenous. In anautogenous species the relationship between the amount of blood imbibed and number of eggs produced is often obscured by individual variation in amounts of undigested blood reserves from previous blood meals and the strong tendency to conserve reserves for further development (Balashov, 1972). However, in Argas arboreus (Kaiser) (Hafez, et al. 1972) and Ornithodoros moubata (Mango and Galun, 1977ab). A linear relationship also exists between egg number and tick engorged weight. In autogenous species, egg development relies on food reserves acquired in larval and nymphal stages (Balashov, 1972).

Autogeny may be facultative or obligatory. In Ornithodoros tholozani (Laboulbène and Mégnin), Ornithodoros tartavkovsky (Olenev) and Ornithodoros parkeri (Cooley), all facultatively autogenous, a positive correlation was found between the weight of the female at ecdysis and the number of eggs in the first autogenous oviposition (Feldman-Muhsam, 1973; Feldman-Muhsam and Havivi, 1973). Body weight is also a factor in the expression of autogeny in Ornithodoros parkeri (Pound, et al. 1984).

Currently, no systematic study has been reported that compares the relationship between body weight and egg output or reproductive efficiency in obligatory autogenous species. In this study we compared the egg production and reproductive efficiency of O. megnini females of different initial weights.

In previous studies when the reproductive efficiency and egg output of O. megnini of similar initial weight (125.6 ± 13.3 mg) were evaluated in the laboratory nutrient weight was 58.9 ± 15.4 mg, nutrient index was 59.6 ± 9.8%, R.E.I. was 6.8 ± 1.6 eggs/mg, C.E.I. was 277.3 ± 67.4 µg/mg, total number of eggs laid per female was 856 ± 225 and total mass weight per female was 34.6 ± 8.9 mg which are values close to those determined in this study for the heavier group (Herrero and Barker, unpublished). Also, a R.E.I. of 9.64 ± 2.08 and a C.E.I. of 560 ± 120 µg/mg have been reported in the literature for females of 86.17 ± 32.7 mg (Wanchinga and Barker, 1986). The last study did not control the initial weight variation and reported greater values for the R.E.I. and C.E.I.

The results presented in this study suggest that the quantity of eggs (number and mass) produced by females depends on initial weight.

However, the reproductive efficiency is independent of initial weight and no significant differences in R.E.I., C.E.I. and nutrient index were found when lighter and heavier females were compared.

When final weight, weight loss, egg weight and nutrient weight were calculated as a percentage of initial female weight, there were no significant differences in these percentages between groups which strongly indicates that the basic mechanism of conversion is equally efficient in both groups. However, the specific mechanism of blood conversion into eggs remains unknown for many tick species including O. megnini. Because the variation within each group was found to be high, further studies will be needed with greater sample sizes to verify our results.

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PART III

NYMPHAL MOLTING OF *OTOBIUS MEGNINI* DUGES (ACARI: ARGASIDAE) FED ON THREE DIFFERENT HOSTS UNDER CONTROLLED CONDITIONS

ABSTRACT

A total of 48 Otobius megnini were obtained from cattle (21 nymphs), sheep (16 nymphs) and rabbits (10 nymphs) under experimental conditions. The engorged weights of nymphs obtained from these hosts were significantly different ($P < 0.05$) after 25 days feeding.

In the cattle sample, the mean weight of nymphs that molted was 53.6 ± 14.7 mg and nymphs that failed to molt was 20.6 ± 8.8 mg. The mean adult weight was 46.0 ± 14.1 mg, mean exuvial weight 1.2 ± 0.4 mg and mean weight loss during molting 6.5 ± 1.2 mg. Molting percent in this sample was 90.5%.

In the sheep sample, the mean weight of nymphs that molted was 44.4 ± 8.7 mg, the mean weight of nymphs that failed to molt was 14.4 ± 6.0 mg. The mean adult weight was 32.2 ± 7.1 mg, mean exuvial weight 1.7 ± 0.5 mg and mean weight loss during molting 10.5 ± 4.5 mg. Molting percent in this sample was 62.5%.

In the rabbit sample, the mean weight of nymphs that molted was 21.7 ± 11.8 mg, mean weight of nymphs that failed to molt was 7.2 ± 0.9 mg. The mean adult weight was 15.8 ± 10.0 mg, mean exuvial weight 2.0 ± 0.9 mg and mean weight loss during molting 5.9 ± 2.9 mg. Molting percent in this sample was 80°. A linear relationship between adult weight and nymphal weight was found for ticks from all host species.

These data suggest that nymphal engorgement rate, as weight gain per day, is different among ticks feeding on rabbits, sheep and cattle successful molting of partially fed nymphs was observed.

INTRODUCTION

Otobius megnini (Duges), the spinose ear tick, is a cosmopolitan one host tick, with a broad host range (Harwood and James, 1979). Several authors have reported on the life history of O. megnini (Davis, 1934; Herms, 1917; Hooker, 1908; Hooker et al. 1912; Koshy et al. 1979 and Loomis, 1961) but a detailed study of its molting biology is lacking.

During the life cycle of O. megnini, molting occurs twice; molting from larva to nymph occurs on the host and molting from nymph to adult occurs of the host. Molting from nymph to adult also results in a significant change in habits and habitat (from a parasitic stage on a host to a free living non parasitic, reproductive stage off the host). Recently, Wanchinga and Barker (1986), published the first report on the colonization and laboratory development of O. megnini. They reported that nymphs fed on cattle and rabbits lost 20.4% of their body weight before molting to the adult stage. During the second molt, they are susceptible to environmental conditions and predators.

In this paper we compared pre- and post-engorgement nymphal weights, nymphal weight loss during molting, newly molted adult weights, molting time and % molting success in ticks obtained from three different hosts.

MATERIALS AND METHODS

Two stanchioned Suffolk sheep (Ovis aries), two Holstein calves (Bos taurus) and two caged domestic rabbits (Oryctolagus cuniculus) were individually infested with 100 larvae (50 per ear). Larvae were allowed to feed on the host for 25 days. After this period, partially engorged nymphs were removed from ears, transported to the laboratory and placed in a humidity chamber (Precision Scientific Instruments, Chicago, Ill.) under constant conditions (85% R.H., 24 h DD, 21°C). In order to monitor individuals, each nymph was placed in a separate vial (18 ml).

After removal from the hosts, nymphs were cleaned, counted and weighed in a Mettler electronic balance sensitive to 0.1 mg (Model AC 100). Nymphs were maintained in the humidity chamber until they molted; then adults were sexed and weighed. At the same time the exuvium for each new adult was weighed.

The parameters determined for each individual tick were a) nymphal, b) adult, c) exuvium weights; d) adult sex, and e) nymphal molting time. From these data tick weight loss during molting and molting percent were calculated. To calculate tick weight loss during molting the following equation was used: Wt. loss = Nymphal Wt. - Adult Wt. - Exuvium Wt. Adult weight, exuvium weight and weight loss were determined as a percentage of the engorged nymphal weight. Engorged nymphal weight was defined as the weight of nymphs able to molt and become

adults. The relationship between adult weight and nymphal weight was evaluated by regression analysis.

To compare mean weights of nymphs that fed and molted from each host, a one-way classification analysis of variance and a Fisher's Least Significant Difference (L.S.D.) test were used (Steel and Torrie, 1960).

RESULTS

When rabbits, sheep and calves were infested with equal numbers of larvae, 5, 8, and 10.5%, respectively, were collected after 25 days. Mean engorged nymphal weights were shown to be significantly different ($P < 0.05$) among hosts (Table 1).

Table 1. Nymphal, adult (males and females), and exuvial weights of Otobius megnini fed on cattle, sheep and rabbits and weight loss during molting.

	Host					
	Cattle		Sheep		Rabbit	
Stage	n	mean ± S.D. (mg)	n	mean ± S.D. (mg)	n	mean ± S.D. (mg)
Nymphs	21	50.5 + 17.3	16	32.8 + 16.3	10	18.9 + 12.0
low Wt.*	2	20.6 + 8.8	6	14.4 + 6.0	2	7.2 + 0.9
high Wt.**	19	53.6 + 14.7	10	44.4 + 8.7	8	21.7 + 11.8
Adults	19	46.0 ± 14.1	10	32.2 ± 7.1	8	15.8 ± 10.0
females	8	52.2 ± 16.6	2	28.8 ± 11.8	2	12.1 ± 1.7
males	11	41.5 ± 10.6	8	33.1 ± 6.4	6	17.0 ± 11.5
Exuvium	19	1.2 ± 0.4	10	1.7 ± 0.5	8	2.0 ± 0.9
Wt. loss***	19	6.5 ± 1.2	10	10.5 ± 4.5	8	5.9 ± 2.9

* Low Wt. = unengorged nymphal wt.
** High Wt. = Engorged nymphal wt.
*** Wt. loss = Nymphal Wt. - Adult Wt. - Exuvium Wt.

In the cattle sample, a linear relationship was found between adult weight and nymphal weight ($Y = 0.54x + 16.87$, $r^2 = 0.9945$,

$P < 0.01$). The adult weight represented 84.9 ± 3.5% of the engorged nymphal weight, exuvium weight represented 2.2 ± 0.6%, and the weight loss was 9.2 ± 3.7%. The molting time of nymphs was 13.1 ± 1.0 days and the molting success, 90.5%.

In the sheep sample, a linear relationship was found between adult weight and nymphal weight ($Y = 0.69x + 1.63$, $r^2 = 0.7044$, $P < 0.01$). The adult weight represented 72.6 ± 9.5% of the engorged nymphal weight; exuvium weight represented 3.7 ± 0.7%, and the weight loss was 23.7 ± 9.5%. The molting time of nymphs was 13.0 ± 0.8 days and the molting success, 62.5%.

In the rabbit sample, a linear relationship was found between adult weight and nymphal weight ($Y = 0.83x - 2.13$, $r^2 = 0.9556$, $P < 0.01$). The adult weight represented 71.9 ± 11.1% of the engorged nymphal weight, exuvium weight represented 9.7 ± 3.3%, and weight loss was 20.9 ± 6.6%. The molting time of nymphs was 13.2 + 1.1 days and the molting success, 80%.

DISCUSSION

Otobius megnini has been reported as an ectoparasite for sheep (Chellappa and Alwar, 1972), rabbits (Rodriguez, 1977), and cattle (Becklund and Mitchell, 1958) under natural conditions; however no experimental study has been published to compare O. megnini development on these hosts.

The data presented in this paper suggest differences in engorgement rate among nymphs obtained from different hosts. Variation in the weights among nymphs obtained from the same host was obvious as well, which suggest different engorgement rates among ticks on the same host.

According to Balashov (1972), an experimental study of correlations between ingested blood volume and ability to molt showed that argasids have extensive regulatory powers. Incompletely engorged nymphs of Ornithodoros papillipes (Birula) removed from a guinea pig are capable of molting if they have ingested 30-35% of the average blood volume ingested in this stage. In Ornithodoros tartakovskyi (Olenev) the critical index, 20-25%, is much lower. When ticks ingest an insufficient amount of blood they are incapable of molting and require additional bloodmeals.

Our data indicate that even when the feeding period was short, most of the ticks reached the minimum weight required to molt and only a small part of the nymphs recovered did not molt. It is possible that in nymphs removed from their host a decrease in juvenile hormone analogues (Ioffe and Uspensky, 1979) and an increase in ecdysteroid

(Delbecque, et al. 1978; Mango and Moreka, 1979; Germond, et al. 1980) levels occurred and triggered the physiological changes needed to molt. The fact that partially engorged nymphs were able to molt to the adult stage suggests that a population of underweight adults may occur in the field if detachment is produced by factors such as the grooming behavior of the host.

Based on our observations, nymphs obtained from cattle lost a smaller percentage weight during molting (9.2 ± 3.7% of engorged nymphal weight) than did nymphs from sheep or rabbits. This weight loss may be explained by high metabolic activity (Balashov, 1972) and water loss (Lees, 1947) during molting.

Sample size in this study varied considerably. The number of nymphs obtained from each host may be related to the number of larvae used to infest, the capacity of O. megnini larvae for attachment, the microenvironmental conditions represented by the host ear, the grooming behavior of the host, the quality of the bloodmeal obtained from each host and the environmental conditions in which the infestation experiment was conducted. Additional studies will be needed to estimate the relative importance of these conditions in different hosts.

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PART IV

SURVIVAL AND REPRODUCTION OF *OTOBIUS MEGNINI* DUGES, (ACARI: ARGASIDAE) UNDER FIELD CONDITIONS AT STILLWATER, OKLAHOMA

ABSTRACT

A study on the field biology of Otobius megnini was conducted in Stillwater, Oklahoma from September 1984 to August 1985. Adults and larvae survived the winter, but no reproductive activity was observed during the first seven months of the study. Oviposition began in mid April and continued through the third week of July.

Mean preoviposition time was 9.2 ± 3.6 weeks; oviposition period was 5.2 ± 4.5 weeks; number of ovipositions per female was 5.0 ± 2.6; nutrient weight was 40.0 ± 14.2 mg; total egg number per female was 430.3 ± 215.6; total egg weight per female was 21.7 ± 11.7 mg; R.E.I. was 4.5 ± 1.6 eggs/mg; and C.E.I. was 230.0 ± 80.0 μg/mg.

During this study oviposition and eclosion seemed to be related to temperature and/or photoperiod which increased in the study area during the reproductive activity of O. megnini.

INTRODUCTION

Otobius megnini (Duges) is present in north and south America (Hooker et al., 1912; Becklund and Mitchell, 1958; Duges, 1884; Gregson, 1953; Cooley and Kohls, 1944; Kemper and Peterson, 1953; Dios, 1930; Keirans, 1972; Rich, 1957), Africa (Theiler and Salisbury, 1950; MacLeod et al. 1970; Schoenaers, 1950) and Asia (Ramanujachari and Alwar, 1955; Sen, 1937; Chellappa, 1973; Hoogstraal and Klein, 1979). Based on the geographic distribution of O. megnini it is apparent that it is able to adapt to a wide variety of environmental conditions.

Otobius megnini has been reported in the neartic zone from the Southwestern, the Pacific coastal and other warm regions of the United States (Hooker et al. 1912; Becklund and Mitchell, 1958), British Columbia (Gregson, 1953; Rich 1957) and Mexico (Duges, 1884). However, no systematic field study on the seasonal biology of O. megnini has been reported.

Otobius megnini is an important pest of cattle, horses and other domestic and wild animals in Oklahoma (Hair, et al. 1979). Understanding its field biology is necessary in order to determine how an effective control program should be conducted. This study was designed to determine the survival potential of overwintered life stages, the time of the year in which reproduction occurs and the ovipositional biology of this tick in the field.

MATERIALS AND METHODS

This study was conducted near Stillwater, Oklahoma. A 25- m^2 area of pasture was fenced. The ticks used were less than one month old and included: 100 eggs, 190 larvae, five engorged nymphs, five unmated adults of each sex (separated) and nine pairs of adults.

In order to simulate a natural habitat where these life stages might overwinter, a weather station was placed on the ground within the study area and all life stages were located beneath the floor of the station. Each individual was placed in a vial (18 cm^3) which was placed inside a glass jar (220 cm^3). Soil was placed around the vials in the jars to minimize extreme temperature effects through the glass surfaces. A total of 14 jars that contained the life stages were placed in the study site on the following dates: eggs (10/13/84), larvae (12/16/84), engorged nymphs (10/19/84), unmated adults (11/3/84) and paired adults (9/29/84). Ticks were observed weekly to note possible activity (i.e. hatching, molting, reproduction and mortality) until 3/16/84. Surviral of all life stages were noted.

After overwintering, (beginning 3/16/85) females that survived were paired with males, placed in vials (one pair per vial) and placed in the same area as above. All the females used and four of the males were from the group that survived the winter in the field, but two males of similar age from the laboratory colony were used to complete pairing. Observations were made weekly between 3/16/85 and 8/31/85 to minimize

disturbing the reproductive pairs. Six pairs were located in the field, but only four females produced eggs. Initial weight of the females was measured on a Model 200 Ainsworth balance sensitive to 0.01 g. After pairing all the vials were transported to the laboratory each week to monitor oviposition. Observations were conducted until all females died.

Eggs were counted under a microscope and weighed with a Mettler H 51 electronic balance sensitive to 0.1 mg (Mettler. Instr. Corp., Heighstown, N.J.). Then each batch of eggs was placed in a different vial and labeled. The reproductive efficiency index (R.E.I.) and the conversion efficiency index (C.E.I.) were calculated as for Amblyomma maculatum (Koch) by Drummond and Whetstone (1970), and the nutrient weight (initial weight - final weight) determined. Data on temperature and photoperiod were obtained from the Agricultural Experiment Station at Oklahoma State University about 1.5 miles from the study area.

RESULTS

Although 46.4% of adults and 23.7% of larvae survived the winter, no hatching, molting, and reproduction occurred between the initiation date and the recovery date (3/16/85).

Table 1. Percent survival of Otobius megnini life stages under field conditions during the winter of 1984-1985 in Stillwater, Oklahoma.

Stage	N	No. Survived	Survival (%)
Adults	28	13	46.4
Females	14	6	21.4
mated	9	2	7.1
unmated	5	4	14.3
Males	14	7	25.0
mated	9	3	10.7
unmated	5	4	14.3
Nymphs	5	0	0.0
Larvae	190	45	23.7
Eggs	100	0	0.0

Mean preoviposition period was 9.2 ± 3.6 weeks, the oviposition period was 5.2 ± 4.5 weeks, ovipositions per female were 5.0 ± 2.6, total number of eggs per female was 430.3 ± 215.6, total egg mass per female was 21.7 ± 11.7 mg, nutrient weight was 40.0 ± 14.2 mg, R.E.I. was 4.5 ± 1.6 eggs/mg and C.E.I. was 230.0 ± 80.0 µg/mg.

It was observed that 53.2 ± 24.3% of the nutrient weight was converted to eggs. Female nutrient weight was 43.6 ± 10.7% of the initial weight. The individual egg weight was estimated as 49.9 ± 2.8 μg; hatching of eggs was very high (of 20 egg batches, 19 hatched). The incubation period was 2.0 ± 1.6 weeks.

Reproduction began in April (4-17-85 to 4-20-85) and a total of 1721 eggs were oviposited by four females during 105 days. This represented an egg mass production of 86.8 mg. Maximum egg production and egg hatching occurred during July when 41.3% of the eggs were laid and 45% of the egg batches hatched (Fig. 1).

DISCUSSION

Larval and adult Otobius megnini were able to survive the winter of 1984 in Stillwater, Oklahoma. They were inactive in the colder months during this period, i.e., January (range: -10.81 to -0.63°C) and February (range: -11.41 to 4.19°C). Eggs and engorged nymphs did not survive the winter period. Adults that survived during the winter months reproduced the following spring and summer when activity of larvae may facilitate infestation of cattle and other hosts.

Reproductive diapause has been reported for many ticks species (Belozerov, 1982). In many cases, this type of diapause has been related to photoperiod (Khalil, 1976; Belozerov, 1973). This study shows that O. megnini reproductive activity did not occur from September 1984 to April 1985 when females were in the continuous presence of the males and that the same females reproduced from April 1985 to July 1985 when photoperiod and temperature increased in the Stillwater area (Agricultural Experiment Station, Division of Agriculture, Oklahoma State University, Research Report p-821, 1982). These data suggest that ovipositional diapause occurred in this tick and that oviposition was triggered by environmental signals (photoperiod and/or temperature).

A comparison of oviposition under field conditions and our previous studies under laboratory conditions (Herrero and Barker, unpublished) show that under field conditions the mean preoviposition period was longer and the oviposition period shorter than in the laboratory. This was probably because of difference in the environmental conditions.

However, R.E.I. and C.E.I. values were close to those found in the laboratory. Replications of this study is necessary to verify our conclusions.

Otobius megnini reproductive potential was low (range: 214.7 - 645.9 eggs per female) compared with ixodid ticks such as Ixodes ricinus (L.) that produce more than 1000 eggs per female (Gray, 1981), but was not low when compared with argasid ticks such as Argas (P.) arboreus that produce a mean number of eggs per female of 681 (Guirgis, 1971). The lower reproductive potential in argasid ticks may be related to the habit of living in sheltered microbiotypes and the ability to have several gonotrophic cycles (Balashov, 1972).

It was clear that oviposition and eclosion did not take place at the same time and that larvae were active in the field after eclosion occurred (Fig. 1). Based on this study, an active larva population would be expected all year round in the Stillwater area since larvae overwinter with an increase in density during the summer months (June - August). More detailed studies on larval longevity are needed to understand O. megnini population dynamics.

Variation in the weather conditions of the Stillwater area may produce differences in O. megnini population behavior from one year to another. Therefore, replication of this experiment may be useful to describe patterns of survival and reproduction under field conditions.

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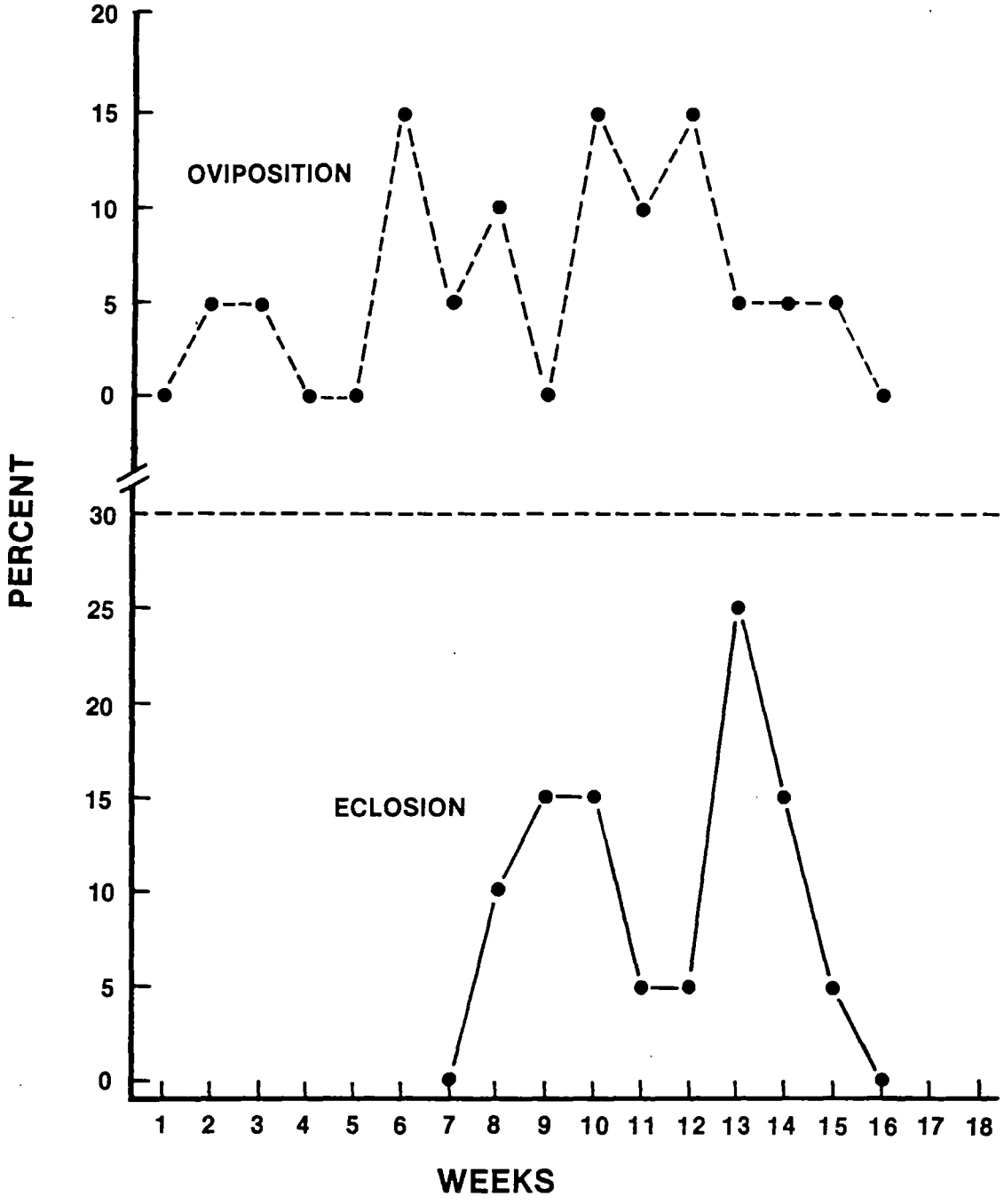
Figure 1. Oviposition (- - -) and eclosion (=-=-) dynamics of Otobius megnini under field conditions at Stillwater, Oklahoma.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WEEKS

PART V

APPENDIXES

APPENDIX A

THE EAR CANAL OF CATTLE AS A MICROENVIRONMENT FOR LARVAL AND NYMPHAL *OTOBIUS MEGNINI*

APPENDIX A

THE EAR CANAL OF CATTLE AS A MICROENVIRONMENT FOR LARVAL AND NYMPHAL *OTOBIUS MEGNINI*

Otobius megnini is very specific in its attachment site. *O. megnini* has been reported from many different hosts including cattle (Becklund and Mitchell, 1958), mountain sheep (Allen, 1955), sheep (Chellappa and Alwar, 1972), pronghorn antelope (Meleney and Roberts, 1970), collared peccary (Meleney, 1975), horses (Ramanujachari and Alwar, 1955), hares and rabbits (Rodriguez, 1977), goats, hogs and sometimes man (Chellappa, 1973, Townsend, 1893) and its attachment site is generally the inner fold of the ear canal.

Few exceptions to this site have been found; unusual attachment patterns were found when larvae attached on the outside of the ear, on the host face or even beneath the eyelids of rabbits (Wanchinga, 1983), and, in cattle, unusual feeding sites for the immatures such as under the tail and other protected areas have been reported (Bulman and Walker, 1979). *O. megnini* attachment to the conjunctiva of a child's eye was noted (Jenzen, et al. 1982).

In the research period 1983-1986 no unusual attachment site was observed. We conducted observations of some abiotic conditions (relative humidity and temperature) in the ear canal of cattle and looked for different attachment sites on the host body.

a) Relative humidity and temperature in the ear canal of cattle:

Temperature and relative humidity were measured in the ear canal of cattle using a thermometer and a psychrometer. Three sets of observations were made: 1) Both parameters were recorded for 10 Hereford cows maintained out of doors at the Medican Entomology Laboratory at O.S.U. Four times during the winter and two times during the summer host conditions were compared with ambient conditions (Table 1), 2) Daily variations were studied in two Holstein calves during a period of 24 hours in the winter (January, 1983). Both parameters were recorded every 4 hours and compared with ambient conditions (Table 2), 3) Temperature and relative humidity were also monitored in an infested Hereford calf to observe any change during the infestation period (July, 1984), (Table 3).

Table 1. Mean temperature and relative humidity in the ear canal of 10 Hereford cows vs. ambient conditions during two seasons, winter and summer, at Stillwater, Oklahoma.

Season	Winter*				Summer**			
N	R. H. (%)		T (°)		R. H. (%)		T (°)	
	cow	ambient	cow	ambient	cow	ambient	cow	ambient
1	36.9	71.0	28.6	13.0	52.6	25.0	35.4	36.1
2	23.2	39.0	31.7	16.7	37.7	26.0	37.2	34.4
3	21.0	73.0	27.3	5.0				
4	47.8	13.0	26.4	2.0				
Mean	32.2	49.0	28.5	9.2	45.2	25.5	36.3	35.3
S.E.	4.0	28.6	0.7	6.8	3.3	0.7	0.4	1.2
C.V.	12.3	58.4	2.5	74.7	7.4	2.8	1.1	3.3

* Winter (1983-1984) was colder than usual for Stillwater area.
** Summer (1984).

Table 2. Mean temperature and relative humidity in the ear canal of 2 Holstein calves during a 24-h period in the winter (1983-1984) at Stillwater, Oklahoma.

PARAMETER	RELATIVE HUMIDITY(%)		TEMPERATURE (°C)	
HOUR	calves	ambient	calves	ambient
08:50	18.5	nd*	16.67**	3.89
12:50	30.0	62.0	23.62	15.00
16:50	38.5	57.0	23.88	14.72
20:50	58.5	92.0	23.34	7.78
0:50	59.0	91.0	18.34	9.00
04:50	47.0	95.0	21.67	1.67
08:50	28.0	88.0	18.14	5.00
Mean	39.9	80.8	20.82	8.15
S.D.	15.6	16.7	3.03	5.18
C.V. (%)	39.0	20.7	14.55	63.56

*nd = no data
**based on one animal

Table 3. Mean temperature and relative humidity in the ear canal of an infested Hereford calf compared with ambient conditions during the summer of 1984.

	RELATIVE HUMIDITY (%)		TEMPERATURE (°C)	
DAY	calf	ambient	calf	ambient
1	53	nd*	38.33	38.89
2	52.5	47.0	38.61	40.00
3	76.5	74.0	38.06	39.44
4	41.0	36.0	38.19	40.50
5	48.5	63.0	37.22	35.00
6	45.0	45.0	37.92	35.56
7	45.0	38.0	38.19	35.56
8	62.0	76.0	36.67	32.22
9	44.5	44.0	37.64	37.22
10	27.5	26.0	37.36	36.11
Mean	49.55	49.89	37.82	37.06
S.D.	9.22	17.34	0.59	2.65
C.V. (%).	18.61	34.76	1.56	7.14

* nd = no data

b) Larval movement after infestation: One Hereford calf was infested with an unknown number of larvae on the right ear; larvae were allowed to move freely on the host and the host was observed daily to look for larval attachment sites.

c) Other attachment sites: Four animals, two sheep and two calves, were located in stanchion at the Efaw farm. One cell, made with panty hose to prevent larval movement out of the cell and covered with cloth, was attached on the back of each animal with adhesive cement. Each animal was infested with 100 larvae and observed daily during a period of three weeks to record larvae attachment site.

The inner fold of the ear canal was the only attachment place observed to be used by O. megnini. Few larvae moved from the right to the left ear after day 4 of infestation. The number of larvae increased from 0 to 9 before day 4 of infestation in the left ear. After day 4 no more larvae dispersed. Other areas of the host body were inspected, but no larvae attached in other sites. When the movement of larvae was prevented on the back of sheep and calves, no attachment occurred.

The inner fold of the ear canal of many animals represents a microenvironment in which tick development takes place. A study of the conditions, abiotic or biotic, in this microenvironment will help to elucidate attachment site specificity of O. megnini. The main conclusion that could be based on our data is that the relative humidity in the ear canal was more stable than the ambient relative humidity. The C.V. associated with ear canal temperature was smaller than the ambient temperature C.V. in the three sets of observations (Tables 1, 2, and 3), which indicated that the ear canal offers a more stable microenvironment for the tick.

APPENDIX B

DEVELOPMENT OF *OTOBIUS MEGNINI*

EGGS TO ENGORGED NYMPHS

APPENDIX B

DEVELOPMENT OF *OTOBIUS MEGNINI* FROM EGGS TO ENGORGED NYMPHS

Otobius megnini's life cycle includes eggs, larvae, nymphs and adults. Larvae and nymphs are parasitic stages (Hooker, 1908). General morphology and ultrastructure of all the stages have been described recently at O.S.U. (Wanchinga, 1983). Our observations of variability among individuals observed during the *O. megnini* life cycle are reported in this note.

Variation in egg viability was observed. Several eggs from a female that were the same age did not hatch during the same incubation period. Some of them never hatch.

Larvae are the first parasitic life stage. Attachment and development of larvae are needed to complete the life cycle. Differences in larval age affect survival on the host; thus we have observed that older larvae are less likely to survive.

It is clear that variation among individuals occurs in the nymphal stage. color, size, and weight variations (Table 4) are the most obvious. In our studies only one cast skin was observed at the end of the larval stage. During the nymphal stage no cast skin were found which suggests that only one nymphal stage occurred. However, the existence of two nymphal stages during *O. megnini* life cycle is commonly reported in the entomological literature (Teel, 1985).

Table 4. Nymphal weight change during Otobius megnini development on cattle.

WEEKS	STAGE	N	Wt. Range (mg)
1	Engorged larvae	2	10-20
2	nymph	3	10-35
3	nymph	5	40-90
4	nymph	3	90-100

To observe development of parasitic stages, a Hereford calf was infested twice and the following observations on development were made under different conditions. The first observations were made of a stanchioned calf in a temperature controlled room during the winter and the others of a calf outdoors during the summer; in both cases an unknown number of larvae were placed on the right ear of a calf and observations were made daily.

First infestation: 1/10/84 an unknown number of larvae were located on the right ear of a calf, 1/10/84-1/14/84 movement of larvae between both ears occurred, 1/14/84 attachment of larvae, 1/15/84 engorged larvae were observed, 1/20/84 nymphs were observed, first cast skins were collected from ear of cattle, 2/16/84-3/1/84 nymphs were removed from cattle.

Second infestation: 6/5/84 an unknown number of larvae were located in the right ear of a calf, 6/5/84-6/12/84 movement of larvae between ears occurred, attachment occurred, 6/13/84 engorged larvae were observed, 6/14/84 cast skins were collected, nymphs were observed, 7/2/84 engorged nymphs were removed from calf.

Nymphs removed from the calf were transported to the lab, and left in a humidity chamber under constant conditions (75% R.H., 20°C, 24 h DD) until molting. Adults were paired and used to maintain the colony.

APPENDIX C

ACQUIRED RESISTANCE OF CATTLE TO MULTIPLE INFESTATIONS OF *OTOBIUS MEGNINI*

APPENDIX C

ACQUIRED RESISTANCE OF CATTLE TO MULTIPLE INFESTATIONS OF *OTOBIUS MEGNINI*

In order to study the effect of multiple infestations of *O. megnini* on cattle, two calves were infested successively with an increasing number of larvae, from 0 to 1000, in successive additive infestations of 200 larvae each two weeks during a period of 10 weeks. The calves were bled weekly from the jugular vein using a 10-ml syringe with a #21 needle. Blood was collected in tubes in such a way that at least one tube with coagulated and one with uncoagulated (anticoagulant EDTA) blood was collected. The blood samples were sent to the laboratory in the Veterinary Hospital at O.S.U. to determine the hematocrit value, white blood cells (W.B.C.) count, and albumin:globulin ratios. As a control during the experiment blood from a third calf treated in the same way, but without larvae was analyzed (Table 5). At the end of this part of the study, serum from the three calves was obtained and an Oüchterloney test was run against cattle IgG, using the standard methods on the Medical Entomology Laboratory at O.S.U. The Oüchterloney test was negative; no systemic response was observed during this study.

All the values, except the albumin:globulin ratio in the control animal, were in the normal range expected for bovines (Mitruka and Rawnsley, 1977) which indicates that no host resistance could be determined.

Table 5. Mean blood parameters of Hereford calves exposed to consecutive infestations with Otobius megnini.

TREATMENT	INFESTED		NONINFESTED
PARAMETER/UNIT	Calf 1	Calf 2	Calf 3
W.B.C. (X 1000)	8.04	8.54	7.94
Hematocrite (%)	37.35	40.98	36.94
Albumin:Globulin Ratio	0.92	0.84	0.61

APPENDIX D

SURVIVAL OF *OTOBIUS MEGNINI* LARVAE UNDER DIFFERENT RELATIVE HUMIDITIES

APPENDIX D

SURVIVAL OF OTOBIUS MEGNINI LARVAE UNDER DIFFERENT RELATIVE HUMIDITIES

Otobius megnini lab. rearing is affected by some abiotic conditions such as temperature, photoperiod and relative humidity. To study the effect of different relative humidities on O. megnini larvae, larvae less than one month old were counted and distributed in vials (5 larvae/vial); a total of 15 vials were used. The lids of the vials were cut on the top and covered with a piece of cloth in order to permit gaseous exchange between inside and outside of the vial. The vials were put inside small humidity chambers (3 vials/humidity chamber). Each humidity chamber was a fish bowl in which relative humidity was maintained using salt solutions according to the method of Winston and Bates (1960). Four specific humidity values were maintained i.e., 0, 40, 76, and 100%. Salts used in each were P_2O_5, $MgCl_2$, Nacl, $K_2Cr_2O_7$, respectively. The humidity chambers were held in a chamber using 12D:12LE photoperiod at 26.7 °C. Observations, counting the number of larvae alive in each vial under a stereomicroscope, were made each 48 h during a period of 386 hours. Special care was taken to avoid loss of humidity by covering each humidity chamber with parafilm paper held tightly with a rubber band. The vial was closed during observations to avoid change in relative humidity.

Table 6. Percent survival of Otobius megnini under different relative humidities over time.

	R. H. (%)			
Time	0	40	76	100
0 hr	100	100	100	100
48 h	86.7	86.7	93.3	100
96 h	73.3	73.3	86.7	100
144 h	46.7	73.3	80.0	100
192 h	13.3	66.7	53.3	93.3
240 h	0.0	40.0	53.3	93.3
288 h	0.0	13.3	46.7	86.7
336 h	0.0	13.3	6.7	86.7
384 h	0.0	6.7	0.0	73.3

My observations show that larvae survive longer at high humidity (100%) than at lower humidities.

APPENDIX E

SURVIVAL AND DEVELOPMENT OF FIELD COLLECTED NYMPHAL *OTOBIUS MEGNINI* IN THE LABORATORY

APPENDIX E

SURVIVAL AND DEVELOPMENT OF FIELD COLLECTED NYMPHAL OTOBIUS MEGNINI IN THE LABORATORY

During the research period 1983-1986, Otobius megnini engorged nymphs were collected in two ways in order to maintain the colony: a) in the field, from the ear canal or cattle; or b) from experimental infestations of cattle, sheep or rabbits maintained in the Medical Entomology Laboratory. The objective of this work was to report the characteristics of the nymphs and adults obtained from field samples. These samples were collected at Spiro, Ok during the fall 1984 and at Perkins, Ok during the fall 1985.

Two samples of O. megnini nymphs were obtained from 10 Hereford cows at Spiro, Oklahoma. The first sample (145 nymphs) was collected in September and the second sample (226 nymphs) in November. The nymphs were taken directly from the ears of the 10 cows that were the control units for an experiment conducted to test the efficiency of three insecticide ear tags.

The nymphs were transported to the Medical Entomology Lab. at Stillwater (O.S.U. campus) in small containers. In the lab., the nymphs were cleaned, counted and separated into engorged and unengorged groups based on color characteristics and sizes. All engorged nymphs were weighed with a Fisher electronic balance (Model 200), placed in a humidity chamber at a temperature of 20°C, with 75% relative humidity

and a photoperiod of 24 dark hours. In the humidity chamber the nymphs molted and then the adults were counted, sexed and weighed. the parameters observed were: a) nymphal weight, b) molting time, c) percent molting, d) female weight, e) male weight, f) sex ratio, and g) sample weight distribution (Tables 7-9). The difference in weight between males and females was statistically compared using Student's t tests.

It was observed that male weight variation was less than females. Mean male weight was lower than female.

Table 7. Characteristics of Otobius megnini nymphs obtained from cattle in the fall 1984 at Spiro, Oklahoma.

	Sample	
	I	II
Sample size	146	226
A. Nymphs (engorged)		
a) number	91	99
b) weight (mg)	65.1 ± 27.7	84.0 ± 36.5
c) molting		
1) period (days)	18.6 ± 7.3	7.7 ± 2.1
2) range (days)	(11.3 – 25.8)	(5.7 – 9.8)
d) mortality	15.4%	16.2%

Table 8. Characteristics of Otobius megnini adults collected from cattle as nymphs in the fall 1984 at Spiro, Oklahoma.

	Sample	
	I	II
a) Sample size	78	83
b) Females		
1) number	27	40
2) weight (mg)	69.3 ± 27.5	94.7 ± 40.7
c) Males		
1) number	51	43
2) weight (mg)	41.9 ± 11.2	49.5 ± 10.5
d) Sex ratio	1.0 : 1.89	1.00 : 1.08

Table 9. Weight distribution for Otobius megnini obtained from cattle in the fall 1984 at Spiro, Oklahoma.

Wt Range mg	Nymphs		Female		Male	
	Sample					
	I	II	I	II	I	II
0-50	22	11	6	6	31	14
50-100	57	57	14	15	20	29
100-150	12	23	7	14	0	0
150-200	0	8	0	5	0	0

refer to Tables 7 and 8 for sample means.

It is important to note that the same cows were heavily infested with nymphs two months after all nymphs had been removed from the ears.

This indicates the presence of an active population of O. megnini larvae in the pasture during (9/12/84-11/8/84) at Spiro, Oklahoma.

Engorged O. megnini nymphs were collected from ears of cattle sold at a local auction near Perkins, Ok. Nymphs were transported to the lab. and located in a humidity chamber under constant conditions (85% R.H., 21°C, 24 h DD). The day after collection the nymphs were cleaned and weighed in a Mettler electronic balance (Model AC 100). Nymphs molted in the humidity chamber and adults were used to maintain the colony. The parameters observed were a) nymphal weight, b) molting time, c) percent mortality, d) female weight, e) male weight, f) sex ratio and g) weight distribution. Four samples, a total of 165 engorged nymphs, were collected; but all the parameters were determined only in the first two samples from 9/24/85 and 10/1/85 (Table 10-12).

Table 10. Characteristics of Otobius megnini nymphs obtained from cattle in the fall 1985, at Perkins, Oklahoma.

	Sample	
	I	II
Sample size	25	28
Nymphs (engorged)	22	12
weight (mg)	45.6 ± 39.8	70.8 ± 57.1
molting period (days)	10.2 ± 2.5	11.3 ± 1.3
mortality (%)	12	57.14

The background of cattle in a local auction is unknown. Variation on breed, sex, age, weight, and geographic origin makes a study with cows of this type of situation very difficult; the data collected from Spiro are more useful.

Table 11. Characteristics of adult Otobius megnini obtained from nymphs collected on cattle in the fall 1985 at Perkins, Oklahoma.

	Sample	
	I	II
a) Sample size	22	12
b) Females		
1) number	8	5
2) weight (mg)	71.6 ± 46.8	95.5 ± 65.8
c) Males		
1) number	14	7
2) weight (mg)	30.3 ± 21.8	38.4 ± 20.6
d) Sex ratio	1.14:1.00	1.43:1.00

As in the Spiro sample, from Table 6 it was clear that male weight distribution was greater than female. Mean male weight was lower than female mean weight. Table 13 shows nymphal weight distribution, for samples 3 (11/20/85) and sample 4 (11/27/85).

Table 12. Weight distribution for Otobius megnini obtained from cattle in 1985 at Perkins, Oklahoma.

Wt. Range (mg)	Nymph		Female		Male	
	Sample					
	I	II	I	II	I	II
0–50	17	23	3	2	12	5
50–100	6	3	3	0	2	2
100–150	2	1	2	2	0	0
150–200	0	0	0	1	0	0
200–250	0	1	0	0	0	0

Table 13. Weight distribution for Otobius megnini nymphs, obtained from cattle in 1985, at Perkins, OK (Samples 3 adn 4).

Wt. Range (mg)	Sample	
	III	IV
0–50	57	30
50–100	10	6
100–150	8	2
Mean ± S.D.	43.8 ± 37.4	34.7 ± 30.3

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