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Effect of surface area to weight ratio of egg masses on the hatchability of *Boophilus microplus* eggs

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Abstract

Background & objectives: On global basis, ticks transmit a number of pathogens than any other arthropod vector, and are among the most important vectors of diseases affecting humans, livestock and companion animals. Control of the vector has been focused on integrated management involving strategic use of insecticides, use of vaccines, use of herbal acaricides and breed resistance. It has been established that tick vaccine is working on limiting the egg laying potentiality and subsequent hatchability of the ticks fed on immunized animals. To generate entomological data following immunization of animals against ticks an experiment was conducted to establish the role of water content in egg masses for successful hatching into larvae.

Methods: Different size and shape of egg masses of *Boophilus microplus*, Izatnagar isolate were obtained by manipulating the egg laying process. The weight of the egg masses was measured, keeping their integrity and surface areas of respective egg masses were calculated with the aid of computer software. Larvae hatched from the respective egg masses were counted individually.

Results: It is clear that, with an increase in the exposed surface area of the cylindrical egg mass per unit weight, there is a reduction in the number of larvae hatched out. Also, the spherical egg masses significantly (0.026 at 95% confidence level) yielded more larvae per unit weight in comparison to the cylindrical egg masses.

Interpretation & conclusion: It has been established that the larval count yielded from an egg mass is more or less dependent on the surface area : weight ratio of the respective egg masses rather than on either the surface area alone or weight of the egg mass alone.

Key words *Boophilus microplus* – egg mass – hatchability – surface area

Introduction

On global basis, ticks are among the most important vectors of diseases affecting livestock, humans and companion animals[1]. It has been estimated that the global cost of controlling ticks and tick-borne diseases (TTBDs) in cattle is between US$ 13.9 and 18.7 billion annually[2]. In India, the cost of containing ticks and tick-borne diseases in animals has been estimated in the tune of US$ 498.7 million per annum[3]. At present, TTBDs control is mainly focused on widespread use of various acaricides like organophosphates, carbamates, pyrethroids, BHC/cyclodines, amidines, macrocyclic lactones and benzoylphenylureas leading to various problems such as resistance, residues, environmental pollution and high cost. These factors reinforce the need for alternative approaches to control tick infestations.

The integrated pest/vector management (IPM) has been identified as the future sustainable option for tick control[4,5]. As an important components of IPM, cattle tick vaccine developed by Australian and Cuban scientists has been proved effective, and has been adopted in the tick control programme in the respective countries. Since the vaccine has been advocated as a population control method, the effect of vaccine on the production of egg masses by the individual tick fed on immunized animals has been considered as one of the important criteria[6–8]. In the laboratory we are working on development of tick vaccine suitable to Indian conditions in which viability of eggs is considered one of the most important criterion for evaluation of vaccine efficacy. It is a well-established fact that water loss from the egg masses is directly linked with the viability of eggs[9]. In the present experiment we found that surface area to weight ratio of egg masses of laboratory reared *Boophilus microplus* Izatnagar isolates which were fed on healthy non-immunized calves, influences the hatchability of egg masses. Moreover, in this paper we described a computer-based technique to measure the surface area of egg masses laid by the ticks. The details of observations are reported here.

Material & Methods

Host animals: Three cross-bred male calves of 4–5 months of age and free from *Babesia bigemina* infection were kept in the tick proof animals shed of the Division of Parasitology. The animals were fed with calf starter ration and water *ad libitum*. The blood films of these animals were examined regularly to check infection.

Rearing of ticks: The laboratory maintained *Babesia* spp infection-free homogenous colony of *Boophilus microplus* that was kept inside sterile glass tubes of diameter 25 mm and height 55 mm to lay eggs. The tubes were kept in desiccators containing 10% KOH to maintain 85–90% humidity. The desiccators containing the tubes were kept in an incubator setting the temperature at 28°C.

The *B. microplus* ticks are maintained for the last several years in standard rearing conditions at the Entomology Laboratory of the Division of Parasitology for conducting various experiments. The adults dropped from the *Babesia bigemina* infection-free cross-bred calves were collected, cleaned, weighed and kept in the desiccators. The egg masses laid by the individual tick were allowed to hatch and 7–10 days old unfed larvae of *B. microplus* were released on ear pinna of *Babesia* spp infection-free cross-bred calves by ear bag method[8]. The ear bags were checked daily. After 18–20 days of feeding the engorged adults were dropped in the ear bags and were collected. The weight of the individual tick was taken and was grouped accordingly. Each tick was kept inside sterile glass tubes of diameter 25 mm and height 55 mm and kept in the desiccators as mentioned above. The tubes were checked daily to note the date of initiation of egg laying.

Once the egg laying is started, few of the laying ticks were moved forward a little gently with the help of a soft brush, to facilitate the laying of different clumps of egg masses varying in size and shape. Once the laying was completed, egg masses were carefully collected without disturbing their integrity and weighed using an electronic balance (Mettler Toledo B204-S). Each mass was then incubated at 28°C with 85–90% relative humidity (RH).

Calculation of the surface area: To calculate the surface area of egg masses, digital photographs from two to three angles were taken using Kodak easyshare DX 6490 zoom, 4.0 megapixel, 10x zoom camera. The photographs were viewed with the programme Adobe Photoshop 7.0, on a computer. The diameter of each of the bottles was measured using the ruler provided in it and was compared with diameter in millimeters, using a scale manually in the corresponding bottle. The fractional relation hence obtained was used to deduce the approximate diameter of the spherical and cylindrical masses as well as the length in case of the latter. In each case the measurements were taken at three different positions or levels and the mean was used to minimize any manual errors. Within 13 to 16 days of egg laying, the eggs hatched to larvae which were counted. Diameter and length of egg masses were calculated using the following formulae:

Diameter of egg mass (mm) $D = D'd/d'$
Length of egg mass (mm) $L = D' l/d'$

Where; D' = Manually measured diameter of bottle (mm); d' = Units corresponding to the diameter of bottle in photograph; d = Units corresponding to diameter of egg mass in photograph; l = Units corresponding to the length of egg mass (cylindrical) in the photograph.

Surface area of sphere = $2/3\pi r^2$ ($r = D/2$)
Surface area of cylinder = $\pi r^2 L$

Counting of larvae: A large petri dish was filled two-third with water and was kept on a white background. To stop the mobility of the larvae, tubes containing larvae were kept at 4°C for 15 min. Few of the freshly hatched larvae were taken at a time from the glass tube using zero number brush and spread over the surface of water in a petri dish. The larvae were allowed to spread over the water surface by their active movement. All the larvae were counted at a stage in which individual active larvae were discernible. The number of larvae hatched from 13 selected egg masses were counted individually and tabulated. The weight per egg mass, number of larvae per egg mass (1/mg), surface area of the egg mass (mm^2) and surface area-weight ratio (mm^2/mg) were deduced, tabulated and plotted.

Statistical analysis: The data were analysed by paired '*t*'-test[10].

Declaration: The experiments have been conducted in accordance with the approved guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

Results

There were 13 egg masses of which four were distinctly spherical, seven were cylindrical, one was manually spread and one bottle contained a single egg. From the tabulated data (Table 1) it is clear that, with an increase in the exposed surface area of the

Table 1. Showing different parameters of the egg masses

Egg mass shape	Egg mass weight (mg)	Radius (mm)	Length (mm)	Surface area (mm^2)	Larvae/egg mass (1/mg)	Surface area: weight (mm^2/mg)
Sphere	12.8	1.538		3.22	21.48	0.25
Sphere	14.4	1.136		2.70	22.70	0.18
Sphere	28.2	2.380		11.86	19.40	0.42
Cylinder	6.7	0.968	8.065	23.19	13.13	3.46
Cylinder	20	2.080	3.125	42.46	13.05	2.12
Cylinder	29.9	2.498	2.664	52.19	14.44	1.75
Cylinder	72.8	1.728	9.60	90.01	18.17	1.25
Cylinder	76	2.28	11.20	176.46	14.54	2.32
Cylinder	87.1	2.04	11.22	146.62	16	1.68
Cylinder	94.8	2.15	9.615	139.56	16.16	1.47
Sphere	3.2	1.38		3.98	0	1.24
Single egg	–	–	–	–	0	–
Spread	45.6	–	–	–	0	–

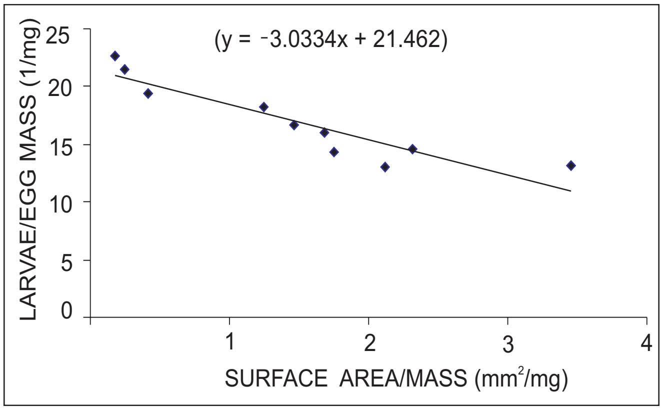

Fig. 1: Larvae/egg mass to surface area mass ratio plot

cylindrical egg mass per unit weight, there is a reduction in the number of larvae hatched out. Also, the spherical egg masses significantly (0.026 at 95% confidence level) yielded more larvae per unit weight in comparison to the cylindrical egg masses. It can be deduced from the generated data (Fig. 1), the larval count yielded from an egg mass is more or less dependent on the surface area : weight ratio of the respective egg masses rather than on either the surface area alone or weight of the egg mass alone.

It was observed that single egg never hatched when incubated in the same conditions. Likewise, the egg mass that was spread on to the wall of the bottle (that resulted in the subsequent loss of integrity of the egg mass) also failed to hatch. It was noticed that the egg mass of 3.2 mg did not hatch to produce viable larvae and they proceeded till the pre-hatching stage only and then ceased to develop.

Discussion

The spherical egg masses yielded more viable larvae probably owing to their lower exposed surface area as compared to egg masses of cylindrical shape, having more surface area which yielded comparatively less larvae per unit egg weight. The longest developmental period occurs in the egg stage. This fact, together with the small size of egg, makes this stage most susceptible to desiccation. Wax coating of the eggs by the Gene's gland during laying is a significant contributor to the maintenance of integrity of the egg mass, as it provides condition for the retention of the shape of the heap when laid. This is very relevant when the hatchability is considered. Wax coating tends to prevent the water loss from the eggs otherwise eggs may desiccate and kill the embryo within the egg, hence proper wax coating is mandatory for proper hatching of eggs and maintenance of the fecundity of the ticks[11]. More surface area of the egg mass tends to provide more exposure of more number of eggs to the environment, than those in the egg mass with a lesser surface area, where more number of eggs would be held within the interior. This tends to facilitate more water loss from the egg masses with more surface area and hence a lesser hatchability. Wax coating on the individual egg surface though tends to minimize the desiccation but in fact this can only incompletely cover each egg[11] which indicates the relevance of the surface area factor in the egg mass hatchability. Considering the number of eggs laid, and the size of each egg laid, any slight variation in the surface area affects the number of eggs exposed to the environment that can lead to water loss and hence a fall in percent hatchability.

The study undertaken here tends to point towards one of the key entomological parameters used for evaluation of effect of vaccination on challenged ticks based on the difference in egg masses[7,12–15] in the ticks fed on control and in immunized animals rather than correlating it with their shape as well. If the control as well as the experimental groups were constituted by significantly variable shapes and sizes, they cannot be compared within an acceptable standard deviation from the normal since the surface area exposed in each case can determine the larval production rather than the mass alone. This calls in for application of techniques that would yield uniform shape to the egg masses laid within a comparable range. Ticks tend to produce cylindrical egg masses, when they are provided with enough space to move. In field situation, the engorged dropped ticks tend to lay eggs in cracks and in small crevices only where they are least compelled to move by external interventions, or various environmental factors which aid in keeping up the eggs as a mass, yielding minimal exposed surface area per unit mass of the egg.

For generating experimental data a slight modification in the shape of the rearing bottle with minimum base area, preferably with a conical or hemispherical base may facilitate the egg masses to be formed with a minimum surface area is exposed and thus can easily be compared in between the ticks dropped from the control and the immunized group of animals with minimum standard deviations under laboratory conditions.

This study hence adds to the significance of waxing that not only coats individual eggs to prevent moisture loss, but also aids in holding the eggs to form a clump, which reduces the exposed surface area and hence aids in better hatchability. This makes clumping of eggs to yield only a minimal surface area to be exposed outside, more significant. The results of the study further suggested the wax producing organ of the tick as a possible candidate for drug target[16,17] or vaccine target that can reduce the fecundity and hence check the proliferation of ticks to a great extent.

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