REVIEW ARTICLE

GUIDELINES FOR THE EVALUATION OF IXODICIDES AGAINST THE CATTLE TICK *BOOPHILUS MICROPLUS* (CANESTRINI, 1887) (ACARI: IXODIDAE).

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SUMMARY: Engorged females and unfed larvae are the two instars of *Boophilus microplus* currently used for the *in vitro* evaluation of ixodicides in cattle. After formulated as a solution or an emulsion, experimental compounds are injected under the ventral cuticle of tick adult females. Alternatively a drop of the formulation is placed on the ventral surface of the integument. The most common test, however, is the immersion of engorged females in a liquid formulation. Unfed larvae are also submitted to immersion tests or exposed to a layer of the compound in impregnated envelopes. Laboratory tests are merely indicative of efficacy in the process of the evaluation of an ixodicide, and need to be completed by *in vivo* experiments. Notwithstanding, *in vitro* trials are the best choice for the diagnosis of resistance. Among the *in vivo* tests, the two methods currently used to determine the effective doses of an ixodicide are: a) the stall test, in which tick females naturally dropping from housed hosts are collected, counted and incubated for reproduction studies; b) the periodic counting of tick females measuring 4.5 to 8.0 mm on one side of the host. Susceptible cattle, carrying an induced infestation by all parasitic stages of *B. microplus* is used in these tests. The dosage determinations obtained in a stall test are very accurate and reliable. The results may not demonstrate the effect of environmental factors on topical, non-systemic ixodicides, if animals are maintained under shelter, protected from the sun and rain. The prophylactic effect of an ixodicide can be measured using the stall test. Tick-free cattle are housed, treated with the experimental compound and periodically challenged with unfed larvae of *B. microplus*. When developing a new ixodicide, final confirmation trials are made under normal field conditions, in experimental farms, for 6-8 months. The physical and chemical stability of cattle dips or spray formulation is studied under the pressure of common pollutants. Progressive loss of the active ingredient in the formulation caused by the passage of cattle should be determined by chemical assays. The required reinforcements to maintain the effective concentration should be recommended after these trials.

KEY WORDS: Cattle ticks, *Boophilus microplus*, Ixodicides, Ixodidae, Antitick Compounds, Ectoparasites, Acaricides, Acari, Guidelines.

INTRODUCTION

Trials for the evaluation of ixodicides may have two main objectives:

a. Discovery or development of a product for tick control or tick eradication purposes, or composing the registration dossier.

b. Diagnosis or measurement of the degree of resistance of a tick strain.

Although there are different methods of conducting this type of trial, or variable criteria in interpreting some of the results, there is a worldwide trend to improve and standardize the relevant methodology. In this review article, we have tried to describe only those methods that have been tested by time and experience, becoming accepted by most of the research organizations involved, as well as by registration authorities.

IN VITRO TRIALS

Laboratory trials are used as an indication of efficacy in preliminary screening programs. Some researchers working with chlorinated hydrocarbons, cyclodienes and organophosphate acaricides (GRILLO TORRADO *et alii*, 1971; DRUMMOND *et alii*, 1973) established theoretical indices for the efficacy or potency of those compounds, based on *in vitro* tests. An index would be useful to make comparisons or to predict the field efficacy of a compound under test in the laboratory.

Such criteria is not shared by other investigators (PALMER, 1976), mainly after the appearance of new chemical structures showing different mode of action.

We should keep in mind that laboratory trials are merely indicative; they must always be complemented and confirmed by *in vivo* experiments.

Nevertheless, laboratory trials are the best choice for the diagnosis or measurement of the degree of resistance of a tick strain. The 50% Effective Concentration (EC_{50}) and 99% Effective Concentration (EC_{99}) for immersion of engorged females or the Lethal Dose 50% (LD_{50}) and Lethal Dose 99% (LD_{99}) for killing unfed larvae should be determined and compared with the same parameters of a susceptible strain. Resistant factors are calculated by dividing the LD_{50} obtained for larvae of the unknown strain by the equivalent figure determined for a reference strain.

In a specific technique to evaluate drugs from the formamidine group, young adults, males or females, just hatching from moulting nymphs, are used to infest mice. Those drugs induce the quick detachment of ticks from their hosts (STONE & KNOWLES, 1973). However, for general purposes, only fully engorged females and / or unfed larvae are used in current *in vitro* tests.

TRIALS WITH ENGORGED FEMALES

Fully engorged tick females may be picked up, directly from the host, by pulling them upwards. This breaks the cement that keeps the rostrum attached to the host skin, causing no harm to the ticks. Otherwise, when cattle are housed in pens with slatted floors, ticks may be recovered from a screen filtering the floor washings. Care should be taken to not wash the floor with a water pressure above 130-150 lb/p.s.i. (9,14 - 10,5 kg/cm^2) which could damage the ticks. It is good practice to wash them in distilled water and to wait 24 hours before conducting the trial, since damaged specimens die, and those showing an abnormal aspect are discarded from the test. After engorged females are exposed to an ixodicide they are not classified as being alive or dead in the following hours, or within a short time, since they may show immobility for long periods making this classification very difficult. The ixodicide effect is judged by oviposition. Under favourable environmental conditions, oviposition begins after the second day of detachment. It attains about 50% of the total volume between the 4th and the 5th day, reaching the end on the 9th - 14th day. There is a linear correlationship between the size and weight of tick females and their oviposition capacity (BENNET, 1974). Once the oviposition is finished, the tick female dies within 2-15 days. Some variation may occur in those periods, depending upon the environmental conditions.

Topical application/ Injection: Engorged females are fixed by the dorsal surface on a double faced adhesive tape, glued to a card or a Petri dish during the operation. A drop of the formulation under test is placed over the ventral integument, or injected under the abdominal surface by means of a micro-syringe (KITAOKA & YAJIMA, 1961; KITAOKA & MORII, 1963). The glue or the immobilization does not affect the oviposition (GREEN *et alii*, 1979). Using tick females of similar size and weight, it is possible to determine with accuracy the effective *in vitro* dose levels of the ixodicide. The method is very reliable for the determination of the 50% Effective Dose (ED_{50}) or the 99% Effective Dose (ED_{99}) for adult ticks, but when running screening programs, in routine tests, the method is considered slow and laborious. In addition, many experimental formulations, particularly powder suspensions cannot be used in a micro-syringe.

Immersion tests: The immersion of engorged tick females in ixodicide preparations is the most currently used *in vitro* test. Commercial formulations are normally used for resistance diagnosis in field work. When experiments are conducted with new drugs, however, the compounds should be formulated before use. The most commonly used solvent is acetone; it does not interfere with the oviposition of *B. microplus* when used up to 40% in the formulation. A standard formulation for the purpose is 25 parts of the ixodicide, 10 parts of octoxynol (Triton X-100) and 65 parts of xylene (DRUMMOND *et alii*, 1973). The amount of ixodicide can be changed to obtain the target concentration and the other components in the formula are adjusted accordingly. When commercial formulations are used, it is necessary to differentiate the effect of the ixodicide from the action of solvents and emulsifiers. Control groups should be immersed in these components that sometimes affect the results (BEADLES *et alii*, 1973). The same may occur with insecticides (BRATTSTEN & WILKINSON, 1977). A very important factor on the *in vitro* results of an ixodicide is the period of immersion. When running screening programs, the current standard time is usually 2-5 minutes. Some compounds such as benzene hexachloride or chlordane need a longer immersion time of up to 30 minutes (ARNOLD, 1949). Among the organophosphates, the immersion time also affects the results, as observed with ethion (PALMER, *op. cit.*; CARVALHO, 1974). Therefore, the most effective or

at least the minimum effective immersion time must be determined for each ixodicide particularly when conducting tests for the diagnosis of resistance. The arbitrary use of a very short time when comparing the effect of different compounds in the same test may generate misleading results. Groups of 10-20 engorged females of similar size and with a normal appearance are weighed and placed in a ~100 ml beaker. The formulation is poured in the container to a level sufficient to cover the ticks. A stopwatch is activated, and after the expiration of the time of immersion, the liquid is discarded through a metal sieve.The retained ticks are then placed on a pad of absorbent paper surrounded by a fence (segments of plastic tubes, 4 inches , 1 inch height) and allowed to dry for 15-20 minutes. They are then placed in vials, Petri dishes, test tubes, or glued on cards with adhesive tape, as previously described in the paragraph referring to topical application. To observe oviposition and hatching, ticks are incubated for two weeks under 27-29°C and >75% relative humidity (RH). This limit of RH is critical for larval hatchability (SHAW, 1965). Water trays may be used to generate humidity in common incubators, but there is a risk of undesirable water condensation inside tick vials. Immersed in water, tick females may stop egg-laying. Excessively high RH (99-100%) encourages the growth of mould and the best method is to use an incubator with environmental control. Alternatively, vials corked with cotton plugs, wetted once a day with 3-5ml of distilled water (SHAW,. *op. cit.*) can be used in small trials.

After two weeks, the egg masses are weighed, and the results of treated groups are compared with those of controls.

Inhibition of egg-laying may be expressed by the following formula:

$$\% \text{ Inhib} = 100 - \frac{\text{Wgt of Eggs of the Treated Gr. x Wgt Fem. of Control Gr.}}{\text{Wgt of Fem.of the Treated Gr. x Wgt of Eggs of Control Gr.}} \times 100$$

Key: Inhib = inhibition; Wgt= weight; Gr= group; Fem: female

A possible complication in tick immersion tests is the infection by *Cedecea lapagei* (Enterobacteriacea) of the female genital tract (BRUM, *et alii,* 1989). Egg-laying is impaired, pus is seen in the genital aperture, some ticks may show a black color and the results of tests become unreliable. Previous desinfection of the incubator and glassware with ethyl alcohol 90°G.L., may be helpful to solve the problem.

When assessing larval hatchability, egg masses are placed in transparent wide-mouth vials closed with fine-mesh gauze tissue. Vials are kept in the incubator under the temperature and RH previously recommended, for an incubation period of 22 to 26 days, counting from the beginning of oviposition. Percent hatchability can be estimated only a week or more after hatching, when larvae leave the eggs to gather in clusters at the top of the vial. The assessment of larval hatchability is subjective and is made by the comparison of: *a.* number of unhatched eggs; *b.* egg shells; *c.* larval clusters. This estimation may show different values when made by different technicians. A more accurate determination that can be carried out in a short time -10 days- is the spectrophotometer method developed by LONDT, (1975).

LARVAL TESTS

Unfed larvae of *B.microplus* are delicate organisms, very sensitive to handling and to some environmental factors. For this reason, larval tests require clean laboratory materials, free of contamination by toxicants, similar adequate environmental conditions, and experienced technicians.

Time: It is essential to determine the necessary period of contact of the larvae with each ixodicide, as well as the best moment for the assessment of mortality. As an example, SHAW (*op. cit.*) observed that while the ideal time to read the results of some chlorinated hydrocarbons and organophosphates was 17 hours, carbophenothion required 72 hours. The same author showed that the LD_{50} of dioxathion for larvae of *Boophilus decoloratus* was 21 ppm after an immersion time of 1 minute; it decreased to only 2 ppm when the time of immersion increased to 10 minutes. Chlordimeform required 144 hours of contact with larvae to produce consistent results (KNOWLES *et alii.*, 1973).

Formulation: As already mentioned in the section dealing with engorged females, results may show a great variation depending upon the solvent used. SHAW (*op.cit.)* working with a strain of *B. decoloratus* showed that the LD_{50} of dioxathion diluted in xylene was 1.5 ppm. This value increased to 3.5 ppm when the solvent was changed to heavy aromatic naphtha. The method of STONE & HAYDOCK (1962) is recommended to make equivalent comparisons on the effect of different compounds. In this method the ixodicides are formulated with the same ingredients. Larvae are exposed to a layer of the ixodicide in impregnated paper packets, after the evaporation of solvents.

Age: A very important point in larval tests is to use organisms of uniform age, since tick larvae show a different susceptibility to the ixodicide depending on age. Considering that egg-laying takes about two weeks, if the whole egg mass is incubated for larval production, the last larvae that hatch from the same batch, will be 15 days older than the first ones. It is therefore recommended to discard eggs laid after the 7th day to reduce that lag time. Larvae younger than 7 days or older than 4 weeks should not be used in larval tests.

METHODS FOR TESTING LARVAE

Two methods are used for larval tests:

1. *Immersion test* (SHAW *op.cit.*): Larvae, 14 to 21 days old, are removed from their holding vial by means of a small paint brush. About 300 larvae are collected and evenly distributed

on a 11-cm Whatman No. 1 filter paper lying in a 15-cm Petri dish. The formulated ixodicide is mixed well and 10 ml withdrawn with a pipette. Some 3 ml of the formulation is pipetted on to the dish beneath the filter paper, which is temporarily lifted and the remaining 4 ml poured over the larvae on the filter paper. Another 11-cm filter paper is placed over the larvae and the remaining 3 ml poured over it. The filter paper sandwich containing the tick larvae is thus saturated with 10 ml of formulation. The standard immersion period for organophosphates is 10 minutes, during which it is possible to test other concentrations in the range selected at intervals of 1.5 minutes. When the immersion period is over, the sandwich is removed from the Petri dish and placed in a double thickness of 24-cm Whatman No. 1 filter paper, opened and moved to dry areas of the filter paper after the initial absorption. After a period, the length of which will depend on the temperature of the formulation and the room, both of which should be constant, the larvae begin to walk. A sample of 70-100 larvae is then carefully removed from the filter paper by means of another paint brush and placed in the apex of a 15-cm. Whatman No. 1 filter paper, folded and sealed. The sealed filter papers are stored in a rack and kept in the incubator at 80% RH and 27C. for 17 - 18 hours. The assessment of the results is made by cutting off the sealed end of the filter paper, opening, and counting the dead and live larvae. An electronic instrument may be used for the purpose (BONIN & HILGNER, 1974). Each concentration of the ixodicide should be evaluated in two or three groups of 70-100 larvae each. Results should be corrected by the Abbott's formula (ABBOTT, 1925), as follows:

$$\% \text{ Mortality} = \frac{\% \text{ Mortality of treated} - \% \text{ Mortality of Controls}}{100 - \% \text{ Mortality of Controls}} \times 100$$

At least five concentrations should be evaluated. The corrected percentage mortalities are plotted on probit mortality / logarithm concentration paper. The 50% Lethal Concentration (LC_{50}) is estimated from a line drawn by eye to fit the points. Data can also be statistical analysed by several other methods.

2. *Packet technique. Confinement of larvae to a treated surface* (STONE & HAYDOCK op. cit.): In this method the ixodicide is diluted at successive concentrations in a mixture of:

Olive oil*	50 ml
Chloroform	100 ml

*Risella oil, according to the original paper.

One ml of the solution is withdrawn with a pipette and poured over a Whatman filter paper No 1 (11cm diameter) in a spiral movement, to lay an even oily film on the surface. The papers are allowed to dry for 3-4 days. Using a small paint brush, samples of 70-100 larvae are removed from the rim of the vial where they have hatched, and transferred to the center of the impregnated paper. The paper is then folded and sealed to make an envelope. A minimum of two / three replicates are made per concentration. Envelopes are maintained in the incubator during the recommended period up to the assessment of results. Dead and live larvae are counted and results corrected and calculated as previously described.

IN VIVO TRIALS

Evaluation of Efficacy: The *stall test* and the *counting of half-engorged females 4.5 - 8.0 mm in lenght, on one side of the host body* are the two methods that have gained general acceptance for this purpose. In both methods, groups of five or six susceptible calves, should be used, preferably 6 months to 1 year old, not previously subjected to large tick infestations.

Calves are experimentally infested with 2500 - 3000 larvae, two to three weeks old, on alternate days, for three to four weeks, when all parasitic stages of *B. microplus* will be present.

STALL TEST

This test was firstly developed in Australia by LEGG & FORAN (1929) and LEGG & SHANAHAN (1954) and further refined by ROULSTON & WILSON (1965).

Calves are housed in individual pens with slatted floors. Engorged tick females naturally detaching from the hosts fall down through the slats on to the paved floor below. They are recovered once or twice a day from a filter screen, when the floor is washed with water under moderate pressure (100 - 120 lbs. p.s.i. or 7.0 - 8. 44 kg. cm2). Calves should be housed about a week before the day of treatment (day 0) to allow them to adapt to feed and to the new environment. Tick counts are made for three days before treatment to compose treatment groups. When the experimental infestation is discontinued after day 0, tick counts are made at every day or every other day, until no more ticks are collected from the control group. If the persistent effect of treatments is required to be determined, experimental infestations may be continued up to days 7-14 after treatment, tick counts being extended accordingly. The possible distressed condition of the control group by the end of the trial, may make it preferable to conduct a specific independent experiment to determine the prophylactic effect of the ixodicide.

In addition to determining the percent efficacy of each treatment based on the number and weight of tick females dropping from each group, the effect of an ixodicide on the reproductive capacity of ticks should also be measured. From each daily collection, a sample of up 10 ticks if available, is counted, weighed and incubated at 27 C and 75% RH. Egg masses are weighed after two weeks and kept in the incubator for up to 42 days, counted from the first day of incubation. Larval hatchability is then estimated and the Index of

Reproduction is calculated as described by DRUMMOND *et alii*, (1969) as follows:

$$IR = \frac{\text{No. Females Collected}}{\text{No. Cattle}} \times \frac{\text{g eggs}}{\text{No. Females saved}} \times \frac{20{,}000 \text{ larvae}}{1 \text{ g eggs}} \times \% \text{ hatch}$$

or otherwise by a simplified formula suggested by CRAMER *et alii*. (1988b):

$$IR = \frac{\text{Total Weight (g) Females}}{\text{Collected per animal}} \times \frac{\text{Egg masses from Oviposition (g)}}{\text{Total Weight (g) of Females Incubated}} \times \frac{\% \text{ Hatchability}}{100}$$

Independently from the variable or parameter used in the calculation (number or total weight of females or IR), the percent efficacy of the compound may be expressed as:

$$[(C-T) / C] \times 100$$

where:

C = value of the control group.
T = value of the treated group.

In the original method of calculation proposed by ROULSTON *et alii*., (1968), results are expressed as the daily percentage of tick survival by the following formula:

$$\frac{ad}{bc} \times 100$$

Where:

a is the mean number of ticks collected from the control (s) group (s) in the three tick countings before treatment,

b is the mean number of ticks collected from the control (s) group (s) on a particular day after treatment of the other groups,

c is the number of ticks collected from a treated group during the three tick countings done before treatment,

d is the number of ticks collected from the treated group on a particular day after treatment.

The factor *a* is a constant for all calculations. The factor *c* is a constant for the calculation of each group. At the beginning, a constant factor for each group is obtained by dividing *a* by *c*. After it, the factor *d/b* is obtained for each day, and the result is multiplied by the constant of the group.

The stall test is very accurate and reliable for dosage determinations.

If the animals are protected from sun rays, topical non-systemic products subjected to degradation by UV light, such as some organophosphates or pyrethroids, may show better results in sheltered animals than those obtained in the field. Building stalls or pens covered with roofs running on wheels that are moved out for some hours, solve the problem of exposing cattle to sun or rain, simulating a natural environment.

COUNTING OF HALF-ENGORGED TICK FEMALES 4.5 - 8.0 MM IN LENGHT, ON ONE SIDE OF THE HOST.

The assessment of the efficacy of ixodicides in the field would be most accurate when based on counts of total tick population, but this is impracticable. The most easily counted tick stage is the engorged female, but fully engorged females have a negative phototropism. Tick infested cattle usually show a large number of specimens at dawn; these detach soon in the early morning. Counts of engorged females are therefore not comparable because they depend upon the time of operation, and this is nearly impossible to synchronize.

WHARTON & UTECH (1970) showed that half-engorged females of *B. microplus*, measuring 4.5 - 8.0 mm in length, engorge overnight and detach from the host in the early hours of the following day. *They do not detach on the day in which they are counted.* This finding makes the countings of ticks of that size very reliable, to measure the degree of tick infestation *any time of the day*. The distribution of tick infestation is similar on both sides of the body and to save labor, only one side of the body may be counted with reliable and practical results (WHARTON *et alii*., 1970). To assist in distinguishing females tick 4.5 - 8.0 mm in length, a metal gauge, with circular holes of that diameter, is used to measure any tick of doubtful size. The cattle are examined in a squeeze crush that holds them firmly and allow all areas to be searched carefully.

As in the stall test, treatment groups of about five or six susceptible calves, experimentally infested by all stages of *B. microplus*, are composed by the consideration of three pre-treatment counts. Animals are kept in a paddock under normal environmental conditions and should receive some supplemental food to compensate for the distress of tick parasitism. Post-treatment counts are made at every day, or every other day, up to three to five weeks. Samples of up to ten engorged females, when available, may be collected at each counting operation from different group treatments, to study the reproductive capacity of ticks, as described previously.

It is a good practice to repeat counts of the same animal at random by two or three different technicians, results being known to and recorded by, a trial coordinator, only. This eliminates mistakes due to operator fatigue that may occur especially when dealing with large numbers of cattle.

Evaluation of Prophylactic Effect

CRAMER *et alii*., 1988a developed a method that evaluated, with great accuracy, the persistent effect of an experimental compound against larval infestations of *B. microplus*. Eight tick-free Holstein calves were allocated, by restricted randomisation of bodyweight, to two groups and housed in individual pens with slatted floors. One group was treated

with the compound, the other group was kept as untreated control. After treatment, each calf was experimentally infected with about 5000 *B. microplus* unfed larvae on days 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21. Ticks dropping through the slatted floor of each individual pen were collected on days 21, 24, 26, 28, 31, 33, 35, 38, 40 and 42. After collection, female ticks were counted and weighed. The mean weights of ticks collected from the two groups were calculated for each collection day. The number of ticks/day was transformed to the natural logarithm of $(n+1)$ to stabilise variances between treatments. The data for each day were analysed separately using one-way analysis of variance (ANOVA).

Comparison between tick collections from treated cattle and controls expressed the lethal effect of the experimental compound against any of the tick parasitic stages.

Dose confirmation trials.

These trials should be conducted in herds composed by bovine of different dairy and beef cattle categories, in farms from different physiographical regions with records of tick infestation. The ixodicide should be used according to the cattle management conditions of each region.

The product should be used in the definitive commercial formulation, following the proposed label recommendations for dose levels, mode of administration and precautions.

Tests should run for 6 - 8 months, for a full tick season, or for the time required to obtain a satisfactory control.

The degree of tick control should be periodically assessed in a group of 10 - 15 susceptible cattle selected from the herd at the beginning of the experiment. Tick counts of engorged females 4.5 - 8.0 mm in length, over one side of the body host will be used to judge results of treatments. Countings should be done before each treatment and a week later.

When treatments are made by immersion dippings, samples of dipwash should be collected:

1. After filling the dipping tank and stirring up the mixture.
2. Just after dipping the first 10-15 cattle.
3. After the dip level drops by 500 or 1000 liters keeping records of the type of cattle dipped (calves, yearlings, adults).
4. Each day, at the end or at the beginning of the dipping operation.
5. After the dip remains unused for some time such as a period of two or three weeks between dippings.

Dipwash samples should be assayed by using an accurate and well-proven method of analysis such as Spectrophotometry, Gas-Liquid-Chromatography (GLC) or High-Performance Liquid-Chromatography (HPLC). Based on chemical assays, the progressive loss (stripping) of active ingredient that usually occurs after the passage of cattle should be determined and the required compensation (reinforcements) may be established and can be recommended on the label. The possible degradation of the active ingredient under unusual conditions, such as high alkaline pH, or pollutants may also be detected and prevented by corrective measures.

A similar procedure is used for samples collected from cattle spray races.

FINAL COMMENTS

Each method has advantages, as well as some deficiencies. An ideal evaluation of a new ixodicide should be conducted by using all the available methods mentioned in this paper, including the confirmation trials in the field. Because of the inexorable building up of tick resistant strains after some time, the further use of an antitick compound should be monitored in the field by *in vitro* trials to detect the appearance of that problem.

SUMÁRIO

Fêmeas ingurgitadas, assim como larvas não alimentadas, são os dois ínstares de *Boophilus microplus* utilizados em provas *in vitro*, na avaliação de carrapaticidas para bovinos. Após formulados como solução ou emulsão, as drogas candidatas são injetadas sob a pele do ventre da teleógina com micro-seringa. Alternativamente pode-se fazer aplicação tópica sobre o tegumento ventral. O teste mais comum é o da imersão de teleóginas numa formulação líquida do produto. Larvas também são submetidas à provas de imersão, ou expostas dentro de envelopes impregnados com o ixodicida. As provas de laboratório são meramente indicativas da eficácia de um produto, devendo ser complementadas por testes *in vivo*. Não obstante, elas são o método mais prático e seguro para o diagnóstico de resistência. Dentre as provas *in vivo*, os dois métodos mais utilizados para determinar a dose eficaz de um carrapaticida são: a) o teste de estábulo, no qual as teleóginas que se desprendem naturalmente do hospedeiro são contadas e incubadas para estudo de sua capacidade reprodutiva. b) a contagem de fêmeas semi-ingurgitadas medindo 4,5 a 8,0 mm, de um lado do corpo de animais mantidos a campo. Nesses testes utilizam-se bovinos suscetíveis, preferencialmente com infestações induzidas, abrangendo todos os estágios parasitários do *B. microplus*. O teste de estábulo é de grande precisão, porém os seus resultados não refletem os possíveis efeitos ambientais sobre carrapaticidas de contato, não sistêmicos, se os animais foram mantidos sempre abrigados, protegidos do sol e da chuva. O efeito profiláctico de um produto pode ser determinado no teste de estábulo. Animais livres de carrapatos são tratados com o produto experimental e a seguir infestados periodicamente com larvas de *B. microplus*. Os testes de confirmação final de dosagem são feitos em fazendas sob

condições normais de uso, durante um período de 6 a 8 meses, ou pelo tempo que for necessário para satisfazer o objetivo do teste. Nos produtos utilizados sob a forma de banho ou em bretes de aspersão, analisa-se periodicamente amostras do líquido do banheiro ou do tanque do brete, para determinar a perda progressiva de ingrediente ativo da formulação e recomendar os reforços necessários.

PALAVRAS-CHAVE: carrapatos, *Boophilus microplus*, ixodicidas, Ixodidae, carrapaticidas, ectoparasitos, Acari, acaricidas, metodologia.

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