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Succinctus

Formula to evaluate efficacy of vaccines and systemic substances against three-host ticks

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ABSTRACT

The control of ticks with vaccines is of global interest. Experimental vaccines incorporate new technologies as soon as they are available. Historically, the main vaccine studies have focused on the one-host cattle tick *Rhipicephalus microplus*, and efficacy evaluations have been standardised for this tick species. On the other hand, evaluations of vaccine candidates for three-host ticks are being done somewhat arbitrarily and thus comparisons within the current literature on the efficacy of vaccines, as well as other methods of control, are difficult. We herein provide a formula for the evaluation of efficacy of a vaccine designed against three-host ticks that incorporates the whole life cycle of the tick.

The calculation of tick vaccine efficacy is used to report the percentage of immunoprotection of a product against the parasite. This calculation has been applied around the world overwhelmingly for products designed against the cattle tick, *Rhipicephalus microplus* (and *Rhipicephalus australis*). In the first report of vaccine efficacy against the cattle tick, Wong and Opdebeeck (1989) used a formula containing only three parameters: total number of detached adults, oviposition of adults and egg mass weight. Eight years later, Canales et al. (1997) calculated for the first time the current standard test for vaccine efficacy against ticks. In fact, this formula was used to test the efficacy of the commercial vaccine Gavac™ (Heber Biotec S.A., Havana, Cuba), developed to control the *R. microplus* and *Rhipicephalus annulatus* populations (Fragoso et al., 1998). This formula calculates the efficacy (E%) based on three variables: number of detached engorged adults (CRT), egg mass weight (CRO) and fertility of eggs (CRF) as follows (Canales et al., 1997; Andreotti, 2007; Miller et al., 2012):

$$E\% = 100 * [1 - (CRT * CRO * CRF)].$$

At the same time, control of the three-host tick species, such as *Rhipicephalus sanguineus*, *Amblyomma variegatum* and *Ixodes scapularis*, is also on the research agenda but a standard formula has not yet been established. In the last few years it has been proposed to validate vaccine efficacy by correlating antibody titers in individual animals with some of the tick parameters, which we included in the formula discussed below (Szabó and Bechara, 1997; Almazán et al., 2003, 2005; Trimnell et al., 2005; Pérez-Pérez et al., 2010; Anisuzzaman et al., 2012). Almazán et al. (2005) calculated the efficacy of an antigen against the three-host tick *I. scapularis*, based only on its effect against larvae. Trimnell et al. (2005) tested a cement antigen as a vaccine in guinea pigs against *R. sanguineus* and in hamsters and rabbits against *Ixodes ricinus*, however, in all cases the final efficacy was not calculated and only some life cycle parameters were compared using ANOVA. Pérez-Pérez et al. (2010) inoculated dogs with Bm86 antigen (Hebercani, Heber Biotec) to evaluate its efficacy against *R. sanguineus*; nevertheless they only compared some parameters with means comparison using a Student's *t*-test. Similar analysis has been done previously when *R. sanguineus* gut extract was tested as a vaccine in guinea pigs against this tick (Szabó and Bechara, 1997). The work of Rodríguez-Mallon et al. (2012) was the only known report on vaccine efficacy against a three-host tick which considered the cumulative effect of the product against larvae, nymphs and adult

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stages. These authors, however, summarised the effects on recovery and viability of engorged larvae in only one parameter, disregarded the number of engorged nymphs that molt to adults, and used twice the number of detached engorged females in their formula. Thus, the vaccine efficacy was over-estimated. The final formula reported was:

$$E(\%) = 100 * [1 - (VL * RN * RA * VA * OA * FE)]$$

where

VL is the effect of each immunogen on larval viability.
RN is the effect of each immunogen on nymph yield.
RA is the effect of each immunogen on adult yield.
VA is the effect of each immunogen on adult viability.
OA is the effect of each immunogen on oviposition of females.
FE is the effect of each immunogen on fertility of eggs.

Here we propose an alternative formula of vaccine efficacy for three-host ticks, which cumulatively calculates the values of each instar, avoiding an over-estimated result, and calculates the ability of each tick instar to complete its blood meal as well as to molt to the next stage. The ability of adult females to lay eggs and larvae hatchability were also considered, resulting in a final percentage that includes values of larvae, nymphs and adults/weight of mass of eggs of three-host ticks. Thus, we propose to separate the larvae parameter into two and add one parameter for nymphs in relation to the formula cited above. For larvae, we considered separately the effect on recovery, which counts the total number of engorged larvae from the vaccinated group in relation to the control group. For nymphs, we included their viability, in other words, the total engorged nymphs that are able to molt to adults from the vaccinated group compared with the control group.

The formula cited by Rodríguez-Mallon et al. (2012) includes four parameters for adults, but two of those cause an over-estimation of the final value since they calculate values for the same condition. The VA variable calculates the viability of an engorged female to lay eggs by counting how many females within the recovered total have started oviposition. On the other hand, the OA variable shows the same when the average mass weight of eggs per female recovered is calculated. All eggs came from females that had already started oviposition, so when the total mass of eggs was divided by the total number of recovered females, the same parameter that had previously been calculated is included, which was the number of engorged females that had started oviposition. We propose including only three parameters for adults in the formula, one being the parameter that calculates both the effects of the reduction in females able to lay eggs and the average weight of eggs laid by each engorged female. Each value was determined by the ratio between the average values of the vaccinated group and the control group. All cross-linked parameters are multiplied, providing the final percentage of efficacy. The final value is subtracted from one and multiplied by 100, to obtain the overall reduction, as a percentage, caused by the vaccine in tick yield throughout the whole of the tick life cycle. Thus, we can use the effects on both the host and environment to determine the effect of the vaccine on the parasite burden.

The final formula is:

$$E(\%) = 100 * [1 - (RL * VL * RN * VN * RA * OA * FE)]$$

where

E is the vaccine efficacy percentage.
RL is the effect on recovery of engorged larvae. This variable is calculated by the ratio between engorged larvae recovered from the vaccinated (rlv) group in relation to the control group (rlc). RL = (rlv/rlc).
VL is the effect on the viability of engorged larvae. This variable is calculated based on the ratio of the total number of engorged larvae recovered that molted to nymphs from the vaccinated (vlv) group compared with the control group (vlc). VL = (vlv/vlc).
RN is the effect on recovery of engorged nymphs. This variable is calculated as the ratio between engorged nymphs recovered from the vaccinated (rnv) group in relation to the control group (rnc). RN = (rnv/rnc).
VN is the effect on the viability of engorged nymphs. This variable is calculated based on the ratio of the total number of engorged nymphs recovered that molted to adults from the vaccinated (vnv) group compared with the control group (vnc). VN = (vnv/vnc).
RA is the effect on recovery of engorged adults. This variable is calculated as the ratio between engorged adults recovered from the vaccinated (rav) group compared with the control group (rac). RA = (rav/rac).
OA is the effect on oviposition of adult females. This variable is calculated based on the average weight of egg mass laid by engorged adult females recovered from the vaccinated (oav) group compared with the control group (oac). OA = (oav/oac).
FE is the effect on egg fertility. This variable is calculated based on the ratio between the hatchability, as a percentage, of larvae from eggs laid by engorged adults recovered from the vaccinated group (fev) compared with the control group (fec). FE = (fev/fec).

Including all proposed parameters in the new formula, and not only those that are statistically significantly different between treated and control groups, may dilute antagonistic effects of a vaccine or a drug on specific parameters of ticks. However, for those who will use a vaccine against ticks, the described efficacy should include the overall effect on the parasite. Also, additive effects on various parameters throughout the tick life cycle should be considered. In this regard, it is possible to suppose that the sum of small, non-significant negative effects of several of the parameters will result in a final, significant negative effect on the efficacy for tick control. Last but not least, apart from the proposed formula, it is always important to evaluate the effect of a vaccine on specific parameters. Such an approach will be important to better understand the mode of action of the product.

Although it was developed to calculate efficacy under laboratory conditions, it is also possible to use this formula in the field, however, the data for all tick stages would likely be more time-consuming to collect than under laboratory conditions because some developmental stages might not feed on hosts at the same time. Using *Amblyomma cajennense* as an example where the life cycle usually takes 1 year to complete (Guglielmone et al., 1990), the treatment of hosts should be planned to provide protection for at least 1 year and tick collection should aimed to recover all tick stages at the end of the trial, providing the researcher with all of the data that the formula requires. Another scenario that could improve the accuracy of the formula is to use the same ticks from larvae until oviposition of eggs, under laboratory conditions. This way, the real cumulative effects will be preserved, because each tick stage would load the vaccine/substance effect from the previous stage.

In conclusion, it is possible to use this formula under any conditions as the required parameters can be collected and measured. Therefore, we suggest this formula as useful for vaccine evaluation or substance efficacy in three-host ticks.

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