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A Novel Method for Determining Efficacy of Acaricides under Field Conditions Using the Relative Risk of Tick Infestation (RRTI)

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Abstract

In Uganda and several other countries, regulatory frameworks mandate that new acaricides undergo field trials prior to registration and licensing. We reviewed the various methods recommended for determining the field efficacy of new acaricides by reputable organizations and methods proposed by scholars. The methods were found to have some shortcomings affecting the quality of the field trial results. These included failure to consider the use of a comparable control product in the control group and failure to meet some principles of epidemiology, good clinical practice and animal welfare. To address these shortcomings and the lack of published literature concerning acaricide field trials, we propose an epidemiologically plausible and comprehensive novel method for determining the efficacy of acaricides under field conditions using the relative risk of tick infestation (RRTI) with the following formula: RRTI = $\frac{at(dc+ac)}{ac(dt+at)}$. We also determined the desired outcome for acaricide application, the variable for measurement of the outcome, designed a data capture form for trial data collection, developed formulae for determining efficacy, and provided guidance for interpretation of results as well as decision-making. The method is compliant with the principles of epidemiology, good clinical research practice, and animal welfare and addresses key realities in field settings, thus generating accurate, reliable and credible results. Researchers and regulatory bodies may consider embracing this method to promote the fair and plausible evaluation of new acaricides under field conditions.

Keywords

Acaricides, Acaricide Field Trials**,** Efficacy, Method, Relative Risk of Tick Infestation, Ticks, Tick Infestation, Tick Detachment

1. Introduction

Acaricides are commonly used to control ticks on livestock [1]-[4]. It is good practice to subject new acaricide formulations to both laboratory and field-based trials to assess and determine their *in vitro* and *in vivo* acaricidal efficacy, respectively. In Uganda, there has been a regulatory requirement to conduct field trials for newly introduced acaricide molecules before acaricide registration and licensing. The National Drug Authority (NDA) of Uganda developed guidelines for conducting field trials of ectoparasiticides (acaricides) [5]. The guidelines address a number of issues, including documentation, protocol development, approval processes, regulatory fees, reporting, monitoring of trials and efficacy determination. Countries such as Zimbabwe and economic blocks such as the European Union have also developed comparable guidelines for conducting ectoparasiticide field trials [6] [7]. In this study, we reviewed the various methods recommended by various regulatory bodies, scholars [8] and international organizations [7] [9] for the determination of the efficacy of acaricides under field trial conditions. We assessed the methods for their strengths and weaknesses and identified several key gaps. The gaps included failure to conform to some principles of randomized controlled trials such as product efficacy determination using longitudinal data and use of a comparable product in the control arm. The methods assumed use of a placebo within the control group instead of a comparable product, failure to state the decision criteria for the registration of new products and failure to provide comprehensive criteria for the interpretation of results. The previously recommended methods do not consider the issue of tick acaricide resistance or how to distinguish it from poorly performing acaricides. In other words, we could not attribute the poor efficacy results to the inherent poor performance of the acaricide or the general challenge of tick acaricide resistance. These gaps could have led to unfair judgment of the efficacy of new acaricides, whose efficacy was determined following previous methods and formulae.

Therefore, the purpose of our work was to develop and propose a comprehensive method for determining the efficacy of acaricides under field trial conditions. The verifiable desired outcome for acaricide application on animals, the appropriate variable to measure the outcome and a data capture form for collecting the appropriate data to measure the desired outcome are also proposed. Furthermore, the necessary formulae for calculating efficacy were developed. Finally, we provide guidance to regulatory bodies regarding possible decisions to make after receiving reports of acaricide field trials where this method is adopted.

2. Materials and Methods

We searched and reviewed all the accessible relevant literature on methods and formulae that were proposed for the determination of the efficacy of acaricides under field trial conditions by scholars and reputable organizations. Only published documents (articles, guidelines and reports) which described methods and or formulae for determining efficacy of acaricides against ectoparasites or ticks without restricting the geographical coverage were included in the review. We searched for both published research articles, international and national guidelines for conducting acaricide/ectoparasiticide field trials in all regions where ticks are endemic. The methods which qualified for review included the following:

2.1. Methods Recommended by Uganda's National Drug Authority

We reviewed the recommended method/formula described in the guidelines for demonstrating the efficacy of ectoparasiticides during ectoparasiticide trials in Uganda by Uganda's National Drug Authority (NDA) [5]. The guidelines state that the percentage efficacy for each species of ectoparasite could be determined by comparing the treated group and the control group via the following formula:

$$C - T/C \times 100 = \%\ efficacy$$

where: *C = the mean of the control group.*

T = mean of the treated group.

The above method has the following weaknesses: the NDA formula does not consider the use of a current standard practice (comparable control product) or a reasonable alternative. The formula assumes the use of a placebo in the control group, which is not only impractical in field settings but also unethical, as it violates animal welfare. It does not clearly show the desired outcome or the best data to collect to measure the outcome. The guidelines do not state which variable mean is being considered for the determination of efficacy. The best time post acaricide application to measure efficacy is not specified, including failure to account for various realities in field settings, such as difficulties in verifying tick mortality after acaricide exposure. Therefore, the formula fell short of some key principles of efficacy determination during RCT and good clinical research practices (GCPs) in field trials [10]-[12]. Importantly, when a comparable control product is used, the efficacy of the new product decreases to zero.

2.2. Methods Used by Some Scholars

In a study conducted in Ethiopia titled "*In vitro* and *in vivo* acaricidal efficacy of amitraz and diazinon against some tick species infesting *Camelus dromedarius* around Jigjiga, Eastern Ethiopia", the authors applied the following method/formula for determining the efficacy of acaricides under field conditions [8].

$$AE = \left[B - T/B\right]/B$$

where:

AE is the antiparasitic efficacy,

B is the mean number of surviving ticks in the control,

T represents the mean number of surviving ticks in the treatment group.

The same challenges that were noted with the NDA's recommended method were also observed in the method proposed by Feyera *et al*. (2015). The same method assumes comparable levels of tick infestation in the control and treatment groups. This situation is unrealistic in field settings, as tick infestations differ from one farm to another despite being located in the same agro-ecological zone.

2.3. Methods Recommended by Food and Agriculture Organization of the United Nations

The Food and Agriculture Organization of the United Nations (FAO) recommended formula for determining the efficacy of acaricides is as follows [9]:

$$Corrected\ \%\ mortality = \frac{\left(\%\ test\ mortality - \%\ control\ mortality\right)}{\left(100 - \%\ Control\ mortality\right)} \times 100$$

The FAO guidelines suggest that when mortality in the non-treated packet is less than 5%, the control product and investigational product-induced mortality values would be stated without correction. However, if the mortality in the untreated packets was between 5% and 10%, the above formula was applied. The above formula is well applicable to laboratory settings, as it grossly failed to account for field realities. It also went ahead to assume that the control group would be treated with a placebo and yet is not ethical in the field setting. If the above formula is used to estimate efficacy in situations where the comparable control and investigational products are equally efficacious or equally nonefficacious, the formula would collapse, as it would yield zero values. The use of mortality as an indicator of acaricide efficacy in field settings is also quite misleading, as it cannot easily be verified. In addition, not all acaricides act by causing immediate mortality but rather can weaken ticks through mechanisms such as paralysis, leading to detachment and later death. Since we cannot easily confirm whether the detached ticks died in field settings, the use of mortality as an indicator of acaricide efficacy becomes impractical.

2.4. Methods Recommended by the European Medicines Agency (EMA)

According to the European Medicines Agency (EMA), Abbott's formula for calculating the acaricidal or repellent efficacy (%) is recommended. Abbott's formula is as follows [7]:

$$Acaricidal\ efficacy\ (\%) = \frac{\left(mC - mT\right)}{mC} \times 100$$

where:

Control group (mC): Mean number of live ticks (attached, free) on the host animals (Control group).

(mT): Mean number of live ticks (attached, free) on the host animals (treatment group).

This particular method faced the same shortcomings noted with earlier methods [5] [8] [9], as it was based on the same principle. This method also falls short of guidance in decision-making after the results are obtained.

2.5. Methods Recommended by the Medicines Control Authority of Zimbabwe

According to the guidelines of the Medicines Control Authority of Zimbabwe, the acaricide efficacy under field trial conditions was determined via the following formula [6]:

$$\%\ Efficacy = \frac{(Mc - Mt)\times 100}{Mc}(Abbott's\ formula)$$

where:

Control group (*Mc*): Mean number of live parasites (ticks) on the untreated host animals;

Treatment group (*Mt*): Mean number of live parasites (ticks) on the treated host animals.

The guidelines provided acceptance criteria that required the new product to exhibit an efficacy of more than 90%. However, this method was similar to what the European Medicines Agency recommended and therefore faced the same shortcomings discussed earlier.

2.6. Methods Recommended by the World Association for Advancement of Veterinary Parasitology (W.A.A.V.P)

The World Association for Advancement of Veterinary Parasitology (W.A.A.V.P) also recommended the use of Abbott's formula for determining the efficacy of ectoparasiticides in dogs and cats [13] [14]. Some international (World Organization for Animal Health), regional (such as the African Union, East African Community, etc.) and national regulatory agencies lack guidelines or remained silent on prescribing appropriate methods for determining the efficacy of new acaricides under field conditions, which has left a knowledge and method standardization gap [15]-[18]. Overall, literature shows that Abbots formula has been widely adopted by several regulatory agencies, international organizations and scholars for determining the efficacy of acaricides. However, Abbots formula is only applicable to the laboratory settings and its use under field settings faces a number of limitations as well articulated above.

2.7. Methodological Gaps and Other Cross-Cutting Issues

Generally, all the formulae discussed above are not well applicable for analyzing longitudinal data but rather seem more applicable to data generated by cross-sectional studies and laboratory-based *in vitro* experiments. Apart from the formula-specific weaknesses, we observed gaps in both the literature and regulations regarding the following aspects of acaricide field trials. For example, failure to define a measurable desired outcome for field acaricide application (what do we want to

achieve whenever we apply acaricides?). Secondly, failure to define a verifiable indicator variable to measure the outcome as well as lack of a comprehensive data capture form. Another important issue was the lack of a plausible formula for acaricide efficacy determination under field trials to guarantee credible results. Other gaps identified included; failure to provide an epidemiologically plausible guide to data collection, data management, analysis, result interpretation and decision-making rules for the regulators on whether to pass or fail the new product. The shortcomings of the methods and formulae proposed by international organizations, national regulators and some scholars coupled with the gross insufficiency of published literature about acaricide field trials provided sufficient justification for the development of a comprehensive method for determining the efficacy of acaricides under field trial conditions.

2.8. Addressing the Identified Methodological Gaps and Cross-Cutting Issues

To address the several identified gaps in methodology and literature, we provided a clear desired outcome for acaricide application, a clear variable to accurately measure the desired outcome, and we further provided a data capture form for collecting the data used to measure the outcome. With respect to the determination of acaricide efficacy, we developed a novel formula, provided guidance for the interpretation of results and further provided decision-making rules for competent authorities responsible for the registration and licensing of new acaricides on the basis of the results of this method. We also tested and validated the method via the use of hypothetical computer-generated data for a five-cycle hypothetical acaricide field trial.

3. Results

This section is divided into two parts. In the first part, we report the step-by-step process of how the novel method was developed. This includes defining the desired outcome for acaricide application, variables to measure the outcome, data capture form for collecting important trial data, deriving the formula for efficacy determination and guiding the interpretation of results. The second part reports a hypothetical field trial that was implemented using computer-generated data for the validation of the novel method. In this section, we demonstrate the step-by-step process of data management, analysis and result interpretation. We also provide guidance to regulatory authorities regarding decision options for the registration and licensing of new acaricide products.

3.1. Part One: Deriving the Novel Method for Determining the Efficacy of Acaricides under Field Trials

3.1.1. Defining the Desired Outcome for Acaricide Application and Variables to Measure the Outcome

The method starts by defining the desired outcome for acaricide application in animals. In this case, we inquired about the measurable and verifiable goal of ap-

plying acaricides on animals in the field setting. To answer the above question, several more questions have been raised. For example:

I. Is it to see that the acaricide can kill ticks?

II. Is it to free animals of ticks?

III. Is it to free animals of tick-borne diseases (*TBDs*)*?*

IV. Is it to free animals of nuisance flies?

V. Is it to kill flies?

An affirmative answer to the first question would be a good outcome, although it is very difficult to determine the number of dead ticks, let alone to verify mortality as an outcome under field settings. The affirmative answer to the third question provided another potential desired outcome (animals free of TBDs). However, this is an indirect outcome and could take a longer time to measure significant changes than measuring direct outcomes on the basis of the effect of the acaricide on ticks. For the fourth and fifth questions, seeing animals free of nuisance flies may be desirable but may not be the main reason for acaricide application since not all acaricides have an effect on nuisance flies, such as amidines. Additionally, since the chemical is an acaricide, the desired outcome should be directed primarily toward ticks, not flies, unless such label claims are made. The effect on flies could be an added advantage of the acaricide product. The mortality of ectoparasites due to acaricide application is difficult to verify under field conditions. Therefore, affirmative answers to the first, third, fourth and fifth questions above could not provide a good desired outcome that could easily be determined and verified. On the basis of our assessments, experiences and professional judgment, the desired outcome of acaricide application is to free animals of ticks or reduce tick infestations on animals. In other words, the acaricide should be able to reduce the risk of tick infestation in the animals where it is applied. Under field conditions, it did not matter whether the ticks died after detachment, as long as the animals were free of ticks and the acaricide application preceded the tick detachment (temporality of events). This outcome could easily be determined and verified using daily counts of attached ticks and estimation of counts of detached ticks. Therefore, the daily number of ticks attached to the animals was the best indicator of whether the animals were free of ticks or not.

3.1.2. Designing the Data Capture Form for Enumeration of Attached Ticks

To facilitate the collection of accurate, complete and detailed data for the trial, we designed a Data Capture Form (DCF) for the collection of data on attached tick counts per farm per day. Individual animal half-body counts for attached ticks should be performed by counting and categorizing the ticks counted per specified body region according to the number of adults, nymphae and, where possible, larvae (requiring the use of a magnifying glass). The total number of attached ticks per animal (half body) should be determined as the sum of all ticks (all genera) regardless of the developmental stage counted in all the body regions on one of the sides (right or left side) of the animal. The total number of attached ticks on

animals on a particular farm per day of a particular cycle is the sum of the half-body tick counts of individual animals. Data should be collected on days 0 (acaricide application day) and days 1, 2, 3, 4, 5, etc., post acaricide application until the next acaricide application day (day 0), which starts another cycle of acaricide application. The duration of data collection post treatment in each acaricide application cycle will depend on the product application intervals recommended by the manufacturer. We considered a 7-day application interval, as it is the recommended application interval for the majority of acaricides belonging to the classes of synthetic pyrethroids, organophosphates and amidines. Daily counts of attached ticks should be conducted on at least 10 randomly selected animals on each farm using DCF (Table 1). One DCF should be used to collect data from one farm per day.

3.1.3. DCF for Enumeration of Attached Ticks

Acaricide code: …… Farm code: ……Trial site: …….

Sub county……Parish…… Village……

Acaricide Application interval: …… Application method: Spraying/dipping

Day post acaricide application: ……. (0, 1, 2, 3, 4, 5, 6)

Date: …… Time for Tick counts: …….Weather (Sunny/rainy/cloudy: ….

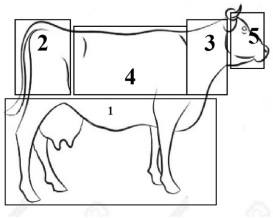

Table 1. DCF for enumeration of attached ticks.

S/N	Ear tag No	Half body tick counts from different body regions															Total (HB)	Tick Genera	Initials
		1			2			3			4			5					
		A	N	L	A	N	L	A	N	L	A	N	L	A	N	L			
1																	T1		
2																	T2		
3																	T3		
n^{th}																	Tn		
Total																	X		

The diagram of a cow used above was adopted from the data capture forms developed for the implementation of the parasite mapping project in Uganda. Keywords: A = Adult, N = Nymph, L = Larvae, HB = Half body, n^{th} = last animal from which ticks were counted, T1 to T3 = total half-body counts of attached ticks per animal on a particular trial farm on a particular day of the cycle and X = total counts of attached ticks for a particular farm on a particular day of the cycle (X = T1 + T2 + T3 + ……. Tn).

3.1.4. Half-Body Regional Demarcations

1: includes the lower fore (starting from the elbow joint) and lower hind limbs (starting from the knee joint), the tail switch, half udder/half scrotum and the lower abdomen (the upper boundary: runs from the elbow joint to connect to the

knee joint of the same side, the lower boundary: the lower midline stretching from the udder/scrotum to the brisket/anterior end of sternum). 2: includes the upper part of the tail, perineum, gluteal area and thigh. 3: Neck and dewlap. 4: Thoracic area and the para lumber fossa (the upper midline forms the upper boundary, whereas the upper line for region 1 forms the lower boundary of region 4). 5: Includes the ear, head, horn and half of the muzzle.

3.1.5. Deriving the Formula for Determining Acaricide Efficacy in Field Trials

The formula is built from the data collected from the DCF we developed above, and this constitutes the total number of attached ticks for the various days of the cycle. These data should be collected from the field. In this study, we propose that for randomized controlled trials, the efficacy of the trial acaricide should be compared with that of the current best practice, a comparable product or a reasonable alternative. The control product should be a registered product (standard of practice) by the competent regulatory authority of a given country. Ideally, both the trial and control products should belong to the same class of acaricide and therefore have similar mechanism of action as well as similar method and application interval. However, in some situations the control product can belong to a different class and can have a different mechanism of action to that of the trial product and would serve as a reasonable alternative. Situations that could warrant use of reasonable alternative control products may include trial of novel new products belonging to a new class with a new mechanism of action.

In the development of the formula, we assumed that two farms located within the same region were randomly allocated to the trial (investigational product) and control products. The field trial duration was assumed to cover five weeks. We also assumed that both the trial and control products had the similar method and application interval of seven days. These products were also assumed to have the similar mode of action and dilution rate. Therefore, we considered one farm with a trial product and another farm with the control product. Similar processes, including routine data collection, were expected to occur at both farms. To obtain credible data, counts of attached ticks were supposed to occur daily throughout the entire five-week period on both farms. Tick counts were conducted from at least ten randomly selected animals on each of the days of the cycle. For a period of 5 weeks, a total of 35 DCFs would be collected from each of the farms. The total counts of attached ticks for a particular farm on each of the days of the cycle (X) were used to populate **Table 2** and **Table 3**, which summarize the total counts of attached ticks for each of the days within the five acaricide application cycles. Importantly, if two or more farms are allocated to the trial or control product per region, the total daily counts of attached ticks per farm should be added together to generate the total for the trial farms or the control farms in a particular region. For comparison purposes, for every step and aspect of the data management, we present data from the trial farm alongside data from the control farm.

Table 2. Summary of counts for attached ticks for a complete trial period (7-day acaricide application cycle) for the trial product.

Cycle	Total counts of attached ticks per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	X_1	X_2	X_3	X_4	X_5	X_6	X_7
2	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
3	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	X_{20}	X_{21}
4	X_{22}	X_{23}	X_{24}	X_{25}	X_{26}	X_{27}	X_{28}
5	X_{29}	X_{30}	X_{31}	X_{32}	X_{33}	X_{34}	X_{35}

Key: $X_{1\text{-}35}$ = Total counts for attached ticks for the trial farm per day for a complete trial period.

Table 3. Summary of counts for attached ticks for a complete trial period (7-day acaricide application cycle) for the control product.

Cycle	Total counts of attached ticks per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
2	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	Y_{14}
3	Y_{15}	Y_{16}	Y_{17}	Y_{18}	Y_{19}	Y_{20}	Y_{21}
4	Y_{22}	Y_{23}	Y_{24}	Y_{25}	Y_{26}	Y_{27}	Y_{28}
5	Y_{29}	Y_{30}	Y_{31}	Y_{32}	Y_{33}	Y_{34}	Y_{35}

Key: $Y_{1\text{-}35}$ = Total number of attached ticks on the control farm per day for the entire trial period.

3.1.6. Determining the Counts of Detached Ticks Post Acaricide Application

The next task was to calculate the total number of detached ticks on each day of the cycle following the guidance provided here. The total number of detached ticks per farm per day per cycle was determined for all the acaricide application cycles that made up the trial period. The total number of detached ticks on a particular day of the cycle (starting with day 1) was determined by comparing the total number of attached ticks on the previous day and the total number of attached ticks on that particular day. If the total number of attached ticks on a particular day was less than the total number of attached ticks on the previous day, the total number of detached ticks was determined by subtracting the counts of that particular day from the counts of the previous day (for example $X_1 - X_2$). If the total number of attached ticks on a particular day was greater than the total number of attached ticks on the previous day, the total number of detached ticks on that particular day was assumed to be zero (Table 4 and Table 5).

Table 4. Summary of counts for detached ticks for a complete trial period (7-day acaricide application cycle) for the trial product.

Cycle	Total counts of detached ticks per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	X_1	X_{d2}	X_{d3}	X_{d4}	X_{d5}	X_{d6}	X_{d7}
2	X_8	X_{d9}	X_{d10}	X_{d11}	X_{d12}	X_{d13}	X_{d14}
3	X_{15}	X_{d16}	X_{d17}	X_{d18}	X_{d19}	X_{d20}	X_{d21}
4	X_{22}	X_{d23}	X_{d24}	X_{d25}	X_{d26}	X_{d27}	X_{d28}
5	X_{29}	X_{d30}	X_{d31}	X_{d32}	X_{d33}	X_{d34}	X_{d35}

Keywords: $X_{1, 8, 15, 22 \& 29}$ = Total counts for attached ticks for the trial farm on day 0 (baseline) for each cycle. X_{d2} - X_{d35} = Total counts for detached ticks for the trial farm per day post acaricide application (excluding day 0) for a complete trial period.

Table 5. Summary of counts for detached ticks for a complete trial period (7-day acaricide application cycle) for the control product.

Cycle	Total counts of detached ticks per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	Y_1	Y_{d2}	Y_{d3}	Y_{d4}	Y_{d5}	Y_{d6}	Y_{d7}
2	Y_8	Y_{d9}	Y_{d10}	Y_{d11}	Y_{d12}	Y_{d13}	Y_{d14}
3	Y_{15}	Y_{d16}	Y_{d17}	Y_{d18}	Y_{d19}	Y_{d20}	Y_{d21}
4	Y_{22}	Y_{d23}	Y_{d24}	Y_{d25}	Y_{d26}	Y_{d27}	Y_{d28}
5	Y_{29}	Y_{d30}	Y_{d31}	Y_{d32}	Y_{d33}	Y_{d34}	Y_{d35}

Keywords: $Y_{1, 8, 15, 22 \& 29}$ = Total counts for attached ticks for the control farm on day 0 (baseline) for each cycle. Keywords: Y_{d2} - Y_{d35} = Total counts for detached ticks for the control farm per day post acaricide application (excluding day 0) for a complete trial period.

3.1.7. Determining Tick Attachment and Detachment Rates Per Day of the Cycle

This was followed by standardizing each of the data in the tables above to a standard population of 1000, 10,000 or 100,000 tick populations, depending on the highest value of the counts of attached ticks. The baseline (day 0) counts were not standardized since they served as the denominator (baseline). The denominator for attached and detached ticks for a particular product in a particular cycle was the same. For example, $X_2/X_1 \times 1000$ (Attached) or $X_{d2}/X_1 \times 1000$ (detached). Depending on the level of tick infestation, the standardization could be out of 100 (if the highest daily total count is less than 100 ticks), 1000 (if the highest daily total count is greater than 100 ticks but less than 1000 ticks) or 10,000 (if the highest daily total count is greater than 1000 ticks but less than 10,000 ticks) tick populations [11] [12]. Standardized tables were generated as shown below (**Tables 6-9**). The mean tick infestation and detachment rates were calculated for each day of the cycle post acaricide application, as shown in the tables below.

Table 6. Tick infestation/attachment rate for the trial product for a complete trial period.

Cycles	Tick infestation rate per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	X_1	q_{a1}	r_{a1}	s_{a1}	t_{a1}	u_{a1}	v_{a1}
2	X_8	q_{a2}	r_{a2}	s_{a2}	t_{a2}	u_{a2}	v_{a2}
3	X_{15}	q_{a3}	r_{a3}	s_{a3}	t_{a3}	u_{a3}	v_{a3}
4	X_{22}	q_{a4}	r_{a4}	s_{a4}	t_{a4}	u_{a4}	v_{a4}
5	X_{29}	q_{a5}	r_{a5}	s_{a5}	t_{a5}	u_{a5}	v_{a5}
	Mean	**a_{t1}**	**a_{t2}**	**a_{t3}**	**a_{t4}**	**a_{t5}**	**a_{t6}**

Key: $X_{1, 8, 15, 22, 29}$ = total counts for attached ticks for the trial farm on day 0 (baseline) for each cycle. $q_{a1\text{-}5}$ = Tick attachment rate for the trial farm on day 1 postacaricide application for five cycles. $r_{a1\text{-}5}$ = Tick attachment rate for the trial farm on day 2 post acaricide application for five cycles. $s_{a1\text{-}5}$ = Tick attachment rate for the trial farm on day 3 post acaricide application for five cycles. $t_{a1\text{-}5}$ = Tick attachment rate for the trial farm on day 4 post acaricide application for five cycles. $u_{a1\text{-}5}$ = Tick attachment rate for the trial farm on day 5 post acaricide application for five cycles. $v_{a1\text{-}5}$ = Tick attachment rate for the trial farm on day 6 post acaricide application for five cycles. $a_{t1\text{-}6}$ = Mean tick attachment rate for the trial farm on days 1 to 6 post acaricide application for a complete trial period.

Table 7. Tick infestation/attachment rates of the control product for the entire trial period.

Cycles	Tick infestation/attachment rate per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	Y_1	qc_{a1}	rc_{a1}	sc_{a1}	tc_{a1}	uc_{a1}	vc_{a1}
2	Y_8	qc_{a2}	rc_{a2}	sc_{a2}	tc_{a2}	uc_{a2}	vc_{a2}
3	Y_{15}	qc_{a3}	rc_{a3}	sc_{a3}	tc_{a3}	uc_{a3}	vc_{a3}
4	Y_{22}	qc_{a4}	rc_{a4}	sc_{a4}	tc_{a4}	uc_{a4}	vc_{a4}
5	Y_{29}	qc_{a5}	rc_{a5}	sc_{a5}	tc_{a5}	uc_{a5}	vc_{a5}
	Mean	**a_{c1}**	**a_{c2}**	**a_{c3}**	**a_{c4}**	**a_{c5}**	**a_{c6}**

Key: $Y_{1, 8, 15, 22, 29}$ = Total number for attached ticks for the control farm on day 0 (baseline) for each cycle. $q_{c1\text{--}5}$ = Tick attachment rate for the control farm on day 1 postacaricide application for five cycles. $r_{c1\text{-}5}$ = Tick attachment rate for the control farm on day 2 post acaricide application for five cycles. $s_{c1\text{-}5}$ = Tick attachment rate for the control farm on day 3 postacaricide application for five cycles. $t_{c1\text{-}5}$ = Tick attachment rate for the control farm on day 4 post acaricide application for five cycles. $u_{c1\text{--}5}$ = Tick attachment rate for the control farm on day 5 post acaricide application for five cycles. $v_{c1\text{-}5}$ = Tick attachment rate for the control farm on day 6 post acaricide application for five cycles. $a_{c1\text{-}6}$ = Mean tick attachment rate for the control farm on days 1 to 6 post acaricide application for a complete trial period.

Table 8. Tick detachment rate for the trial product for a complete trial period.

Cycle	Tick detachment rates per day						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	X_1	q_{d1}	r_{d1}	s_{d1}	t_{d1}	u_{d1}	v_{d1}
2	X_8	q_{d2}	r_{d2}	s_{d2}	t_{d2}	u_{d2}	v_{d2}

Continued

3	X_{15}	q_{d3}	r_{d3}	s_{d3}	t_{d3}	u_{d3}	v_{d3}
4	X_{22}	q_{d4}	r_{d4}	s_{d4}	t_{d4}	u_{d4}	v_{d4}
5	X_{29}	q_{d5}	r_{d5}	s_{d5}	t_{d5}	u_{d5}	v_{d5}
	Mean	**d_{t1}**	**d_{t2}**	**d_{t3}**	**d_{t4}**	**d_{t5}**	**d_{t6}**

Key: $X_{1, 8, 15, 22, 29}$ = total counts for attached ticks for the trial farm on day 0 (baseline) for each cycle. $q_{d1\text{-}5}$ = Tick detachment rate for the trial farm on day 1 postacaricide application for five cycles. $r_{d1\text{-}5}$ = Tick detachment rate for the trial farm on day 2 postacaricide application for five cycles. $s_{d1\text{-}5}$ = Tick detachment rate for the trial farm on day 3 postacaricide application for five cycles. $t_{d1\text{-}5}$ = Tick detachment rate for the trial farm on day 4 postacaricide application for five cycles. $u_{d1\text{-}5}$ = Tick detachment rate for the trial farm on day 5 postacaricide application for five cycles. $v_{d1\text{-}5}$ = Tick detachment rate for the trial farm on day 6 postacaricide application for five cycles. $d_{t1\text{-}6}$ = Mean tick detachment rate for the trial farm on days 1 to 6 post acaricide application for a complete trial period.

Table 9. Tick detachment rate for the control product for a complete trial period.

Cycle	Tick detachment rate per day						
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
	Saturday	**Sunday**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
1	Y_1	qc_{d1}	rc_{d1}	sc_{d1}	tc_{d1}	uc_{d1}	vc_{d1}
2	Y_8	qc_{d2}	rc_{d2}	sc_{d2}	tc_{d2}	uc_{d2}	vc_{d2}
3	Y_{15}	qc_{d3}	rc_{d3}	sc_{d3}	tc_{d3}	uc_{d3}	vc_{d3}
4	Y_{22}	qc_{d4}	rc_{d4}	sc_{d4}	tc_{d4}	uc_{d4}	vc_{d4}
5	Y_{29}	qc_{d5}	rc_{d5}	sc_{d5}	tc_{d5}	uc_{d5}	vc_{d5}
	Mean	**d_{c1}**	**d_{c2}**	**d_{c3}**	**d_{c4}**	**d_{c5}**	**d_{c6}**

Key: $Y_{1, 8, 15, 22, 29}$ = Total number for attached ticks for the control farm on day 0 (baseline) for each cycle. $qc_{d1\text{-}5}$ = Tick detachment rate for the control farm on day 1 post acaricide application for five cycles. $rc_{d1\text{-}5}$ = Tick detachment rate for the control farm on day 2 post acaricide application for five cycles. $sc_{d1\text{-}5}$ = Tick detachment rate for the control farm on day 3 post acaricide application for five cycles. $tc_{d1\text{-}5}$ = Tick detachment rate for the control farm on day 4 post acaricide application for five cycles. $uc_{d1\text{-}5}$ = Tick detachment rate for the control farm on day 5 post acaricide application for five cycles. $vc_{d1\text{-}5}$ = Tick detachment rate for the control farm on day 6 post acaricide application for five cycles. $d_{c1\text{-}6}$ = Mean tick detachment rate for the control farm on days 1 to 6 post acaricide application for the entire trial period.

3.1.8. Deriving the Formula for Calculating the Relative Risk of Tick Infestation for Determining the Efficacy of New Acaricide (Trial) Products

The efficacy of the trial product can be determined for each day of the cycle post acaricide application by determining the relative risk of tick infestation (RRTI). This method compares the risk of tick infestation (risk of tick attachment) among the animals on farms exposed to the trial product and the risk of tick infestation among the animals on farms exposed to the control product. Therefore, we started by determining the risk of tick infestation on a particular day post acaricide application among herds/farms exposed to the trial product and control product.

Using the data from the above tables, we constructed a 2 × 2 table for each of the days in the cycle after acaricide application to derive the formula for determining the efficacy, as shown below (Table 10).

Table 10. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on a particular day (day 1) of the cycle after acaricide application.

	Average tick detachment rates	Average tick attachment rates	Total
Trial Product	d_{t1}	a_{t1}	$d_{t1} + a_{t1}$
Control Product	d_{c1}	a_{c1}	$d_{c1} + a_{c1}$
Total	$\mathbf{d_{t1} + d_{c1}}$	$\mathbf{a_{t1} + a_{c1}}$	$\mathbf{d_{t1} + d_{c1} + a_{t1} + a_{c1}}$

where; $\mathbf{a_{t1}}$ = average tick infestation rates on animals on the farm (s) exposed to the trial product on day 1 or any specific day of the cycle post-trial acaricide application for the entire trial period. $\mathbf{d_{t1}}$ = average tick detachment rates on animals in the farm(s) exposed to the trial product on day 1 or any specific day of the cycle post-trial acaricide application for the entire trial period. $\mathbf{a_{c1}}$ = average tick infestation rates on animals on the farm (s) exposed to the control product on day 1 or any specific day of the cycle after control acaricide application for the entire trial period. $\mathbf{d_{c1}}$ = average tick detachment rates on animals on the farm (s) exposed to the control product on day 1 or any specific day of the cycle after control acaricide application for the entire trial period. $\mathbf{d_{t1} + a_{t1}}$ = Total tick infestation on animals on the farm (s) exposed to the trial product on day 1 or any specific day post acaricide application. $\mathbf{d_{c1} + a_{c1}}$ = Total tick infestation on animals in the farm (s) exposed to the control product on day 1 or any specific day post acaricide application. $\mathbf{d_{t1} + d_{c1}}$ = Total tick detachment rate among animals in the farm(s) exposed to the trial and control products on day 1 or any specific day post acaricide application. $\mathbf{a_{t1} + a_{c1}}$ = Total tick attachment rate among animals in the farm(s) exposed to the trial and control products on day 1 or any specific day post acaricide application.

From the above table, the risk of tick infestation among the animals on the farm(s) exposed to the trial product on day 1 of the cycle (R_{t1}) or any other day of the cycle (R_{t2}, R_{t3}, R_{c4}, R_{t5}, …. R_{tn}) is determined using the following formula:

$$\mathbf{R_{t1} = a_{t1}/(d_{t1} + a_{t1})} \quad \text{(Equation 1)}$$

Similarly, the risk of tick infestation among the animals on the farm(s) exposed to the control product on day 1 of the cycle (R_{c1}) or any other day of the cycle (R_{c2}, R_{c3}, R_{c4}, R_{c5}, …. R_{cn}) is determined using the following formula:

$$\mathbf{R_{c1} = a_{c1}/(d_{c1} + a_{c1})} \quad \text{(Equation 2)}$$

By dividing equation (1) by equation (2), we derive a formula for determining the relative risk of tick infestation ($RRTI_1$), which is used to determine and compare the efficacy of the new acaricide with that of the control on day 1 of the cycle, or any other day of the cycle ($RRTI_2$, $RRTI_3$, $RRTI_4$, $RRTI_5$ …… $RRTI_n$).

$$\mathbf{RRTI_1 = R_{t1}/R_{c1}} \quad \text{(Equation 3)}$$

Therefore, $\mathbf{RRTI_1 = a_{t1}/(d_{t1} + a_{t1}) \div a_{c1}/(d_{c1} + a_{c1})}$

$$\mathbf{RRTI_1 = a_{t1}/(d_{t1} + a_{t1}) \times (d_{c1} + a_{c1})/a_{c1}}$$

$$\mathbf{RRTI_1 = a_{t1}(d_{c1} + a_{c1}) \div a_{c1}\,(d_{t1} + a_{t1})}$$

$$\mathbf{RRTI_1 = \frac{at1(dc1+ac1)}{ac1(dt1+at1)}} \quad \textbf{(Formula)}$$

To further investigate the RRTI results, the relative tick infestation risk reduc-

tion (RTIRR) and absolute tick infestation risk difference (ATIRD) can be determined [11] for each day of the cycle after acaricide application for the entire trial period using the formulae below.

Relative tick infestation risk reduction $\mathbf{(RTIRR) = 1 - (a_{t1}\,(d_{c1} + a_{c1})/a_{c1}\,(d_{t1} + a_{t1}))}$

Therefore, $\mathbf{RTIRR_1 = 1 - RRTI_1}$.

Absolute tick infestation risk difference $\mathbf{(ATIRD) = (a_t/d_t + a_t) - (a_c/d_c + a_c)}$

Therefore, $\mathbf{ATIRD_1 = R_{t1} - R_{c1}}$.

3.1.9. Interpretation of RRTI Results

1. If RRTI = 1: The trial product is as effective/efficacious or ineffective as the control product

2. If RRTI < 1: The efficacy of the trial product is superior to that of the control product

3. If the RRTI > 1: The efficacy of the trial product is inferior to that of the control product

4. If RRTI = 0 or very close to 0 (<0.5), the trial and control products are equally highly effective or efficacious.

5. The results of the relative tick infestation risk reduction (RTIRR) are interpreted as the combined protective efficacy of the trial and control products, and the larger the difference is, the better.

6. The results of the absolute tick infestation risk difference (ATIRD) are interpreted as the reduction in tick infestation risk attributable to the trial product. In this case, a negative difference indicates a superior trial product, whereas a positive result indicates an inferior trial product compared with the control product. A zero difference indicates comparable performance of the trial and control products.

3.1.10. Guidance to Decision-Making Authorities

In the process of deciding whether to pass or fail a new product, the regulator needs to consider the following before making any decision (**Table 11**):

1. The RRTI values for each of the days in the cycle after acaricide application.

2. In the cases where the RRTI values suggest that the trial product was inferior, consider the individual Rt and Rc values used to calculate the RRTI for each day of the cycle post acaricide application.

3. Assess the comparability of the trial and control product.

4. Consider the larval packet test (LPT) results for tick populations collected from the respective study sites. This helps to corroborate the field findings with the laboratory findings. The LPT results reveal the actual performance of both products when other environmental factors are eliminated. Therefore, LPT results can be used to differentiate between tick acaricide resistance and an ineffective product. In situations of tick acaricide resistance, the efficacy of both the trial and control products against tick larvae (obtained from engorged ticks collected from the respective trial farms) that have never been exposed to acaricides is expected to be poor. In the absence of tick acaricide resistance among the trial and control farms, the efficacy of both the trial and control products against tick larvae would

be good or excellent. Finally, susceptible colonies of ticks should be exposed to both the trial and control products to assess their performance. An efficacious trial product is expected to cause 100% mortality among susceptible tick colonies. This should be the case for the control product. Failure to cause significant mortality among susceptible colonies is an indication that the trial product has a limited acaricidal effect.

Table 11. Guide to decision makers.

RRTI value	Interpretation	Possible decision
0	Trial product is very effective.	Perfect Pass
>0 <1	Trial product is superior	Pass
1	Trial product is as effective or ineffective as the control product	Pass or Fail depending on Rt and Rc values plus LPT results. If the Rt and Rc values are closer to zero than to 1 (<0.5), pass the trial product. If the Rt and Rc values are closer to 1 than zero (0.5 and above), both the trial and control product are equally ineffective and therefore the trial product could fail.
> 1	Trial product is inferior	Pass or fail depending on Rt and Rc values. If both Rt and Rc values are below 0.5, pass the trial product. If both Rt and Rc values are equal or above 0.5, consider failing the trial product. If the Rt values are equal or above 0.5 while the Rc values are below 0.5, consider failing the trial product. Last, consider the type of control used and LPT results.

3.1.11. Summary of the Key Steps of the Novel Method

The diagram below provides the key steps to take while using this method for determining the efficacy of acaricides under field trials. It summarizes the long processes described above for the benefit of the readers/users of the method (**Figure 1**). Both the trial (treatment) and control arms are well showed in the diagram including showing the stage at which data from the two arms converges during the data management and analysis phase.

3.2. Part Two: Validation of the Novel Method

To test and validate this method, we used computer-generated data (www.random.org) to mimic data collected from a hypothetical field acaricide trial to apply the method to generate, interpret and use the results to make a regulatory decision of whether to pass or fail the hypothetical trial acaricide product. In this hypothetical field trial, we compared the efficacy of an investigational acaricide product (trial product) and a comparable control product (control product). The control product was supposed to be a registered product (standard of practice) by the competent regulatory authority of a given country. We assumed that both the trial and control products belonged to the same class of acaricide and therefore had the similar mechanism of action. However, the control product can belong to a different class and thus a have a different mechanism of action to that of the trial product and would still serve as a reasonable alternative. We also assumed that both the trial and the control products were tested within the same geographical

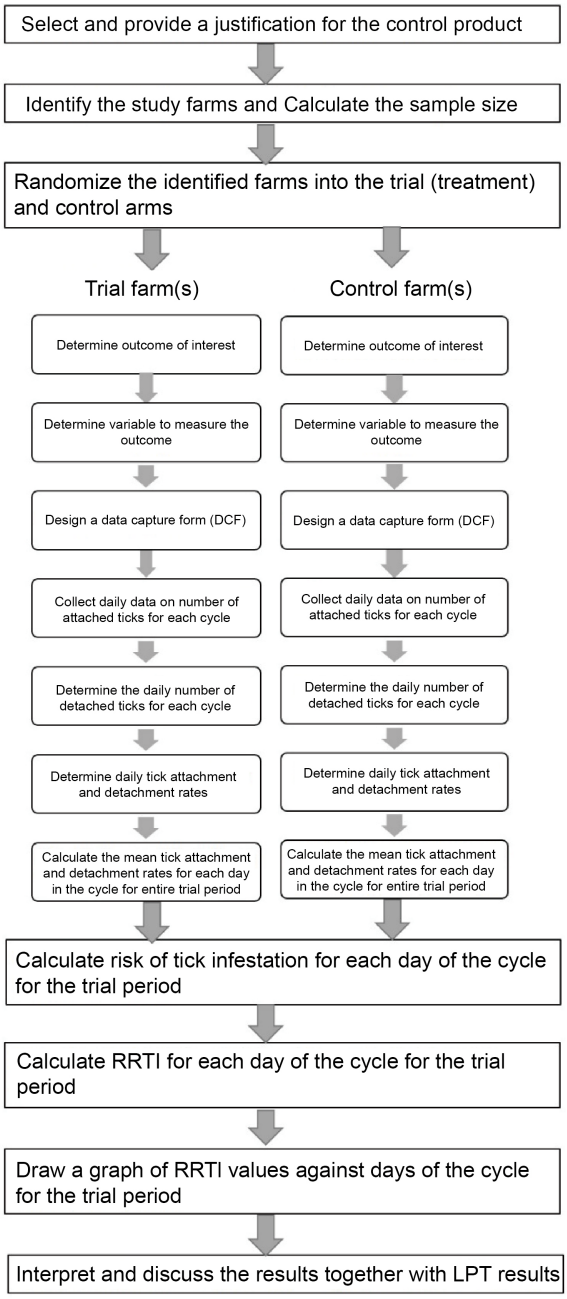

Figure 1. Summary of key steps to be followed while using the novel method for determining efficacy of acaricides under field trials.

location. The two products were randomly allocated to the two farms. The farm that received the trial product became the trial farm, whereas the farm that received the control product became the control farm. The products were assumed to have similar application intervals of seven days, although products of different application intervals can also be used. Ideally, control products with similar application intervals to that of the trial product are preferred. The method of application was spraying via a bucket pump, which is the most common method in sub-Saharan countries [19]. The day for spraying was assumed to be Saturday

(Day 0) every week. We further assumed that the entire trial lasted for a period of five weeks (5 cycles). The duration of a field trial is not standard and varies from one regulatory authority to another; however, it may also be influenced by the available resources, seasons and other product-related factors, such as the mechanism of action and application intervals. We assumed that half-body tick counts were performed daily on 10 randomly selected cattle from both farms and that the data were recorded via the DCF proposed in this paper. The total half-body tick counts per day per farm were computed to generate the data in the tables below for the trial and control products. This is a summary of the primary data captured from the field (Table 12 and Table 13). Notably, the data follow field observations, where tick infestation tends to decrease drastically immediately after the day of acaricide application (day 0), especially when an effective acaricide is applied. This is followed by low or very low infestation for some days in the middle of the cycle, and tick infestation tends to increase toward the end of the cycle, with day 0 expected to have the highest tick infestation per cycle. The tables below contain values obtained by summing the half-body tick counts of 10 randomly selected animals on each day of the cycle per farm. These data are obtained from the daily DCF for tick enumeration.

Table 12. Total daily half body counts for attached ticks for 5 cycles at the trial farm.

Cycles	Total daily half body counts for attached ticks						
	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	600	200	96	22	15	60	124
2	220	50	0	0	28	58	118
3	204	42	8	0	12	32	88
4	144	12	0	0	33	65	99
5	176	34	7	0	13	40	102

Table 13. Total daily half-body counts for attached ticks for 5 cycles at the control farm.

Cycles	Total daily half body counts for attached ticks						
	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	164	50	10	5	17	53	92
2	135	10	12	9	22	79	87
3	149	27	30	21	35	81	101
4	151	18	11	0	0	13	27
5	60	2	0	0	19	24	45

3.2.1. Determining the Counts of Detached Ticks Post Acaricide Application

With the above data, we determined the total number of detached ticks per day of the cycle post acaricide application for the trial and control farms following the guidance provided above. The data are presented below (Table 14 and Table 15).

Table 14. Total daily half-body counts for detached ticks for 5 cycles at the trial farm.

Cycles	Total daily half body counts for detached ticks						
	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	600	400	104	74	7	0	0
2	220	170	50	0	0	0	0
3	204	162	34	8	0	0	0
4	144	132	12	0	0	0	0
5	176	142	27	7	0	0	0

Table 15. Total daily half-body counts for detached ticks for 5 cycles on the control farm.

Cycles	Total daily half body counts for detached ticks						
	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	164	114	40	5	0	0	0
2	135	125	12	3	0	0	0
3	149	122	30	9	0	0	0
4	151	133	7	11	0	0	0
5	60	58	2	0	0	0	0

3.2.2. Determining Tick Attachment and Detachment Rates Per Day of the Cycle

After determining the total daily half-body counts for both attached and detached ticks for all cycles, the next step was to standardize the total daily half-body counts for both attached and detached ticks to generate the tick attachment rates and tick detachment rates per 1000 tick population per day, respectively. The following formula was used:

$X_2/X_1 \times 1000$ tick population (Attached) or $X_{d2}/X_1 \times 1000$ tick population (detached). where X_2 = total half-body count for attached ticks on day 2, X_1 = total half-body count for attached ticks on day 0 (baseline), X_{d2} = total half-body count for detached ticks on day 2 and X_1 = total half-body count for attached ticks on day 0 (baseline). The choice of multiplying with 1000 was informed by the highest value for the total daily counts for both attached and detached ticks, which was less than 1000 tick populations. In other words, if we had a value greater than 1000 in any of the tables above, we would have chosen to use a population of 10,000

ticks. Below are the daily tick attachment and tick detachment rates for both the trial and control farms (Tables 16-19) derived from the above tables.

Table 16. Tick attachment rates per day for 5 cycles for the trial farm.

	Tick attachment rates per day						
	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Cycles	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	600	333.3	160	36.7	25	100	206.7
2	220	227.3	0	0	127.3	263.6	536.4
3	204	205.8	39.2	0	58.8	156.8	431.4
4	144	83.3	0	0	229.2	451.4	687.5
5	176	193.2	39.8	0	73.8	227.3	579.5
Mean	**269**	**209**	**48**	**7**	**103**	**240**	**488**

Table 17. Tick attachment rates per day for 5 cycles for the control farm.

	Tick attachment rates per day						
Cycles	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	164	304.9	61	30.5	103.6	323.2	561
2	135	74.1	88.9	66.7	163	585.2	644.4
3	149	181.2	201.3	141	234.9	543.6	677.8
4	151	119.2	72.8	0	0	86.1	178.8
5	60	33.3	0	0	316.7	400	750
Mean	**132**	**143**	**85**	**48**	**164**	**388**	**562**

Table 18. Tick detachment rates per day for 5 cycles for the trial farm.

	Tick detachment rates per day						
Cycles	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	600	667	173	123	12	0	0
2	220	773	227	0	0	0	0
3	204	794.1	166.7	39.2	0	0	0
4	144	916.6	83.3	0	0	0	0
5	176	806.8	153.4	39.8	0	0	0
Mean	**269**	**792**	**161**	**40**	**2**	**0**	**0**

Table 19. Tick detachment rates per day for 5 cycles for the control farm.

	Tick detachment rates per day						
	Day 0 (Baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Cycles	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	164	695.1	244	30.5	0	0	0
2	135	926	89	22.2	0	0	0
3	149	818.8	201.3	60.4	0	0	0
4	151	880.8	46.3	72.8	0	0	0
5	60	966.7	33.3	0	0	0	0
Mean	**132**	**857**	**123**	**37**	**0**	**0**	**0**

3.2.3. Determining the RRTI Per Day of the Cycle

The mean tick attachment and detachment rates per day for the entire trial period were calculated, as shown in the last rows of the above tables. The mean tick attachment and detachment rates per day post acaricide application for the entire trial period for both the trial farms and the control farms were used to create a 2 × 2 table. The mean tick attachment and detachment rates for the experimental/treatment and control farms were compared each day post acaricide application via 2 × 2 Tables 20-25 below. From the 2 × 2 table, we calculated the risk of tick infestation among herds exposed to the trial (R_t) and the control (R_c) products. These values can be calculated manually or by use of statistical software such as STATA and R software to generate R_t, R_c, RRTI, confidence intervals, p-values and risk differences.

Table 20. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on day 1 of the cycle post acaricide application.

		Mean tick detachment rates	Mean tick attachment rates	Total tick infestation
Day 1	Trial product	792	209	1001
	Control product	858	143	1001
	Total	**1,650**	**352**	**2002**

R_{t1} = 209/1001 = 0.209; R_{c1} = 143/1001 = 0.143; $RRTI_1 = R_{t1}/R_{c1}$ = 0.209/0.143; $RRTI_1$ = 1.46.

Table 21. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on day 2 of the cycle post acaricide application.

		Mean tick detachment rates	Mean tick attachment rates	Total tick infestation
Day 2	Trial product	161	48	209
	Control product	123	85	208
	Total	**284**	**133**	**417**

R_{t2} = 48/209 = 0.23; R_{c2} = 85/208 = 0.41; $RRTI_2 = R_{t2}/R_{c2}$ = 0.23/0.41; $RRTI_2$ = 0.56.

Table 22. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on day 3 of the cycle post acaricide application.

		Mean tick detachment rates	Mean tick attachment rates	Total tick infestation
Day 3	Trial product	40	7	47
	Control product	37	47	84
	Total	**77**	**54**	**132**

R_{t3} = 7/47 = 0.149; R_{c3} = 47/84 = 0.560; $RRTI_3 = R_{t3}/R_{c3}$ = 0.149/0.560; $RRTI_3$ = 0.27.

Table 23. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on day 4 of the cycle post acaricide application.

		Mean tick detachment rates	Mean tick attachment rates	Total tick infestation
Day 4	Trial product	2	103	105
	Control product	0	164	164
	Total	**2**	**267**	**269**

R_{t4} = 103/105 = 0.98; R_{c4} = 164/164 = 1; $RRTI_4 = R_{t4}/R_{c4}$ = 0.98/1; $RRTI_4$ = 0.98.

Table 24. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on day 5 of the cycle post acaricide application.

		Mean tick detachment rates	Mean tick attachment rates	Total tick infestation
Day 5	Trial product	0	240	240
	Control product	0	388	388
	Total	**0**	**628**	**628**

R_{t5} = 240/240 = 1; R_{c5} = 388/388 = 1; $RRTI_5 = R_{t5}/R_{c5}$ = 1/1; $RRTI_5$ = 1.

Table 25. A 2 × 2 table for the calculation of the efficacy of a trial acaricide product with a comparable control product on day 6 of the cycle post acaricide application.

		Mean tick detachment rates	Mean tick attachment rates	Total tick infestation
Day 6	Trial product	0	488	488
	Control product	0	562	562
	Total	**0**	**1,050**	**1050**

R_{t6} = 488/488 = 1; R_{c6} = 562/562 = 1; $RRTI_6 = R_{t6}/R_{c6}$ = 1/1; $RRTI_6$ = 1.

3.2.4. Interpretation of RRTI Results

On the first day post-application, the trial product was less effective than the control product, with RRTI values greater than 1. However, on days 2, 3 and 4 post acaricide application, the efficacy of the trial product was superior to that of the control product, although the level of superiority decreased as the number of days

after acaricide application increased. On the 5th and 6th days of the cycle, the trial product was as ineffective as the control product, as evidenced by RRTI values (RRTI = 1) as well as high (close to 1) R_t and R_c values (Table 26 and Figure 2).

Table 26. RRTI values for entire acaricide application cycle.

Day of the cycle	RRTI Value (CI)	P-value	Risk difference (CI)
Day 1	1.46 (1.206 - 1.771)	0.0001	0.066 (0.032 - 0.099)
Day 2	0.56 (0.421 - 0.749)	0.0001	−0.179 (−0.268 - −0.089)
Day 3	0.27 (0.151 - 0.470)	0.0000	−0.410 (−0.587 - −0.234)
Day 4	0.98 (0.960 - 1.002)	0.1515	−0.019 (−0.040 - 0.002)
Day 5	1.0		0.0
Day 6	1.0		0.0

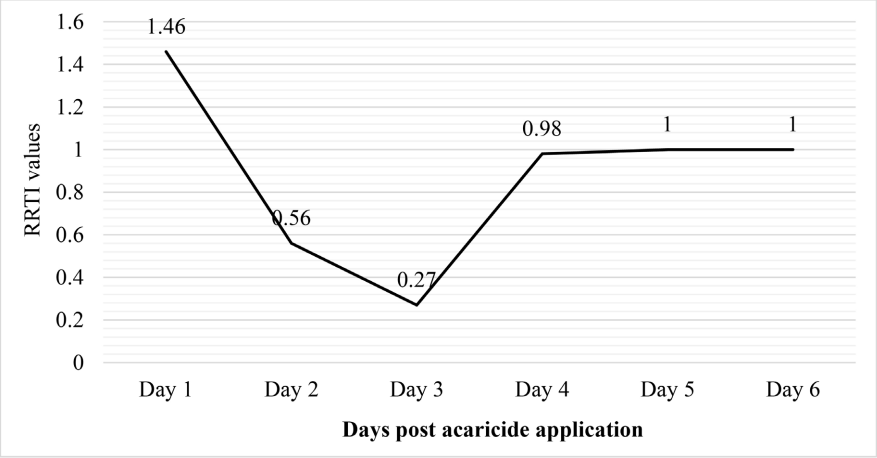

Figure 2. Trends of RRTI over the acaricide application cycle.

Epidemiologically, the above results could also be interpreted as follows: on the first day post acaricide application, the risk of tick infestation among animals on farms exposed to the trial product was 1.46 times that among animals on farms exposed to the control product. The association was statistically significant as evidenced by the confidence interval (1.206 - 1.771) which does not include the null and a corresponding p-value of less than 0.05 (Table 26). The risk of tick infestation among animals on farms exposed to the trial product was 0.56, 0.27 and 0.98 times that among the animals on farms exposed to the control product on the 2nd, 3rd and 4th days post acaricide application, respectively. The association was statistically significant on days 2 and 3 but the association on the 4th day post acaricide application was not statistically significant as evidenced by the confidence intervals and corresponding p-values (Table 26). On the 5th and 6th days post acaricide application, the risk of tick infestation among the animals on farms exposed to the trial product was similar to that among the animals on farms exposed to the control product. In other words, both the trial and control products were equally in-

effective on days 5 and 6 post acaricide application.

The line graph above (**Figure 2**) shows how the relative risk of tick infestation changes over the course of the acaricide application cycle, and this information could be used to recommend the appropriate acaricide application interval and determine the residual period (protective period) of the new product. The residual period can be determined on the day when the RRTI values start to approximate 1.

Overall, the trial product demonstrated superior efficacy compared to the control product, indicating its potential for effective tick control. The trial product was able to reduce the risk of tick infestation in cattle, and its effect was comparable to that of the control product. Therefore, the trial product would qualify for a pass and be recommended for possible registration and licensing by the relevant authorities.

4. Discussion

In this section, we provide a detailed discussion of this novel method, with a focus on the important assumptions to consider when the method is used. In addition, we discuss in detail the strengths, implications and weaknesses of the method. The method makes a number of assumptions, which include the following: all visible attached ticks can be counted on a daily basis after acaricide application for the entire cycle. This is expected to increase the accuracy of the calculated number of detached ticks per day and to accurately determine the protective period/residual period of the acaricide. Tick counting is expected to take place while the animals are well restrained within the confines of a standard cattle crush while strictly observing animal welfare. The other assumption is that the number of 3 and 2 host ticks that detached for reasons other than the effect of the acaricide is negligible during the cycle so that all the observed detachment is attributed to the effect of the acaricide. We also assume that random animals are selected to count visible attached ticks daily and that the animals are well identified with ear tags or other methods of identification. Additionally, to increase the accuracy of the counts, we assume the selected herds to participate in the field trial have cattle breeds with short fur to increase the visibility of the attached ticks, hence easing the tick counting process. The other important assumption is that the trial and control products should share some common attributes. Furthermore, the trial and control products have the similar or related active ingredients and similar or related mechanism of action (the control products is a good comparator of the trial product or a reasonable alternative). However, some new acaricide products, especially novel molecules may not have good comparator control products. In this case, the investigators could justify the selection of a reasonable alternative control product that must share one or more attributes with the trial product. Finally, we assumed that randomization occurred at the farm level rather than at the animal level. This meant that selected farms were randomly allocated to receive the trial and control products.

This method provides a fair and credible evaluation of the efficacy of a new

acaricide, as its efficacy is compared with that of already registered and licensed comparable control products or at least a reasonable alternative. For that reason, this method is well applicable in situations of tick acaricide resistance, as both products would be equally challenged. This would be evidenced by high values of risk of tick infestation (R_t and R_c values close to 1) and low LPT results (percentage mortality of less than 90%). In non-inferiority trial designs, the objective is to investigate whether a new acaricide is at least not inferior to the existing best comparable acaricide [11]. Therefore, the purpose of the field trial is to demonstrate that the new acaricide has a comparable effect on the risk of tick infestation to that of the comparable control product or that its performance is superior or inferior to that of the comparable control product. However, inferior efficacy may not be desirable for a new acaricide product. However, inferior performance may be expected in circumstances where a reasonable alternative control product is used and in situations of highly varying degrees of tick acaricide resistance among the farms participating in the field trial. The method provides comprehensive step-by-step guidance from data collection, data analysis, result interpretation and decision-making, which was not given attention in the earlier proposed methods and formulae [5]-[9] [13] [14]. In addition, the method considers all the longitudinal data collected for the entire trial period and presents results using the appropriate measures of association (relative risk) which addresses the gaps identified in the previously recommended methods. Using this method, the efficacy of the trial product can be determined for each day of the cycle post acaricide application and therefore it makes it easy to determine the residual period of the new acaricide product. The method is not affected by varying tick infestations on the trial and control farms and is thus applicable in situations of low and high tick infestations. The other important strength of this method is its ability to generate results that are easy to interpret for both the investigator and the regulator with a standard interpretation and decision-making rules. The method complies with the principles of randomized controlled trials (use of comparable control product in the control group and use of appropriate measures of association for result presentation), good clinical research practices [10], ethical standards and animal welfare. Using this method, the investigator can present the risk of tick infestation and RRTI in a graphical format to show trends as well as in a tabular format to include RRTI values, the confidence intervals and p-values over the days post acaricide application. The graphs can be used to determine the protective period/residual period of the new acaricide. The adoption of this method is expected to improve on the data quality of acaricide field trials and also standardize the manner in which these field trials are conducted across researchers and countries for ease of reproducibility and repeatability of results. This invention is also expected to support the review of the international, regional and national guidelines for the conduct of acaricide field trials to address the gaps we identified and also standardize the practice across researchers and countries.

However, the method requires at least two trained field assistants to work to-

gether during data collection on each of the farms and requires daily data collection, which may require slightly more resources than previously proposed methods. To use this method, researchers and regulators require prior training to be able to generate credible results and interpret the results correctly. Good data management skills are needed to handle and analyze longitudinal data for the entire trial period.

5. Conclusion

We present a robust method for evaluating acaricide efficacy in field trials, addressing critical limitations in existing approaches. This method offers comprehensive guidance, from defining the intended outcomes of acaricide application to providing a standardized data capture framework. Furthermore, it supports researchers through the entire lifecycle of field trial data management from collection and analysis to interpretation while also aiding decision-makers, particularly regulators overseeing the registration and licensing of new acaricide products. The method's underlying assumptions, strengths, and limitations are clearly articulated to ensure transparency. Future research should focus on applying this method to other ectoparasiticides and validating its efficacy across diverse field conditions. Additional validation using empirical field trial data is recommended, though this will necessitate dedicated resources. We advise competent regulatory authorities for veterinary products to integrate this method into national guidelines for Good Clinical Practice (GCP) field trials on ectoparasiticides, thereby addressing gaps in previously proposed formulae. International bodies such as the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (WOAH), which shape guidelines for tick and tick-borne disease control, may also wish to incorporate this approach into their global standards. Finally, academic institutions are encouraged to include this method in curricula for veterinary medicine, epidemiology, public health, and related disciplines to foster evidence-based practices in the field.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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