Egypt. J. Vet. Sci. **Vol. 51,** No. 2, pp. 153-161 (2020)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

*In vitro*Analysis of the Efficacy of Selected Commercial Acaricides on The Cattle Tick *Rhipicephalus* (*Boophilus*) *annulatus* (Acari: Ixodidae)

Foluke A. Akande[1], Ahmed O. Garba[1] and Olubukola T. Adenubi[2]
[1]*Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture. Abeokuta, Ogun State. Nigeria.*
[2]*Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Federal University of Agriculture. Abeokuta, Ogun State. Nigeria.*

TICKS are important to the Nigerian livestock industry because of their ability to cause huge economic loss directly or indirectly. Chemical acaricides have played important roles in control of tick infestation for many years but development of acaricide resistance as being reported worldwide is a huge problem facing the livestock industry and the veterinarians. Thus, this study evaluated the efficacy of available commercial acaricides on *Rhipicephalus (Boophilus) annulatus.* Using Adult Immersion Test (AIT): the LC_{50}, inhibition of oviposition, control and resistance rates, gradation of tarsal reflexes were determined for ticks treated with different acaricides at five different concentrations the discriminating dose (DD) (the manufacturer's recommended dose) and four other different concentrations of two below and two above the DD with the aid of prohibit regression. The association between weight and number of eggs laid was analyzed by linear correlation. Macrocyclic lactone(66.7%), Phenylpyrazole (62.5%), Cypermethrin (62.5%), Formamidine(33.3%) and Phenylpyrazole(13% egg production), Macrocyclic lactone (18% egg production), Cypermethrin(20% egg production), Formamidine(49% egg production) have the highest to lowest percentage control and oviposition inhibition values respectively. The LC_{50} of (Formamidine), (Phenylpyrazole), (Synthetic pyrethroids), (Macrocyclic lactone) against adult *R. (B.) annulatus* were 100, 8000, 2300 and 6000ppm respectively.The demonstration of acaricide resistancedevelopmentas demonstrated by the ticks in laying of eggs after dipping in acaricideis a call to the need for discovery and use of alternative tick control methods with good therapeutic dose and the need for continuous evaluation of available acaricide.

Keywords: Acaricides, *Rhipicephalus (Boophilus) annulatus*, Adult Immersion Test, Tarsal Reflex, Discriminating Dose.

Introduction

Ticks are obligate ectoparasites, which feed on a variety of vertebrate hosts worldwide [1]. They belong to the phylum Arthropoda, class Arachnida, order Acari, suborder Ixodida. There are over 800 tick species classified intothree families - 650 species in the family Ixodidae (hard ticks), 150 species belong to the family Argasidae (soft ticks) and one species to the family Nuttaliellidae [2]. Ticks are the most important ectoparasites of livestock because of their heavy rate of infestation that can cause significant damage to hides and skin as well as their ability to transmit diseases (bacteria, protozoa, rickettsia and viruses) to their host [3,4]. The huge impact of ticks and tick borne diseases on the individual and national economics warrants application of appropriate tick control strategies [5] .

Rhipicephalus (*Boophilus*) *annulatus* is a typical one host tick. The hosts include cattle, sheep, goats and wild ungulatesbut cattle are probably the only maintenance host for this tick).

Corresponding auther: Foluke A. Akande, E-mail: dayoakande2006@gmail.com, Tel. +23408035008607.
(*Received* 27/12/2019, *accepted* 13/02/2020)
DOI. 10.21608/ejvs.2020.21560.1144

The period of infestation of cattle is approximately three weeks and the common feeding sites are dewlap, legs, belly and neck. This species of tick causes bovine babesiosis and anaplasmosis by transmitting *Babesia bigemina*, *Babesia bovis* and *Anaplasmamarginale* to cattle [6,7].

Acaricides are chemicals, mostly neurotoxins, which act either systemically or by direct contact with ticks following external application [8]. Three main chemical groupings of common acaricides are the organochlorines (e.g. benzene hexachloride), organophosphates (e.g. coumaphos, diazinon, dichlorvos) and synthetic pyrethroids (e.g. permethrin, deltamethrin, cypermethrin). Other groups which are also used include the carbamates (e.g. butocarb, carbaryl, carbanolate), formamidines (e.g. amitraz), phenypyrazoles (e.g. fipronil), macrocyclic lactones (e.g. ivermectin) and triazines [9] with the recently introduced isooxazoline groups.In many situations, animal health personneland livestock farmers complain of failure of acaricides to kill ticks and toxicity associated with their usage. Inappropriate acaricide use with incorrect concentrations probably contributes to the development of resistance, which leads to tick-control program failures [10]. This widespread multidrug resistance has been reported against nearly all commercially available acaricides [11-15]. Although such has been observed in Nigeria, there is yet to be an empirical report on the level of acaricide resistance in ticks.

In view of the resistance report over the years, the study aimed to check for the efficacy of the commercially available acaricides in the study area to determine if they are still potent or ticks have developed resistance to them.

Materials and Methods

The study area

Ogun State (Gateway to Nigeria) is one of the states in Southwest Nigeria with coordinates 7° 00′N 3°34 59.99′E [16]. The State has twenty local government areas with Abẹ́òkúta being the capital and the largest city [17]. Ogun State covers an estimated land area of about 16 409 km^2 which represents approximately 1.7% of the total land area of Nigeria [17]. Livestock farming is one of the major occupations of the inhabitants of the State [18].

Acaricides

Four acaricide preparations belonging to different chemical classes (formamidine, phenypyrazole, macrocyclic lactone and synthetic pyrethroid {cypermethrin}) obtained from Agrovet shops in the study area were used. The brand names of the acaricides were coded for anonymity and to avoid any misinterpretation as promotion or demotion of such products based on theresult.These preparations prepared according to manufacturer's instruction (discriminatory dose) with two doses each below and above the discriminatory doses were diluted with distilled water into five different concentrations namely 150, 200, 250, 300 and 350ppm for ACARICIDE A (formamidine), 6000, 8000, 10000, 12000 and 14000ppm for ACARICIDE B (phenylpyrazole), 30000, 40000, 50000, 60000 and 70000ppm for ACARICIDE C (cypermethrin) and 3000, 4000, 5000, 6000 and 7000ppm for ACARICIDE D (macrocyclic lactone). The series of concentrations for each acaricide had one concentration at the manufacturer recommended dose (DD)of 250, 10000, 50000 and 5000ppm,respectively, while two other concentrations were two concentrations prepared above and below the DD. Solution E (Distilled water) and Solution F (Normal saline) and were used as the positive control and negative control respectively.

Collection and transportation of ticks

Engorged female ticks were hand-picked from herds ofcattle in the study area. All the cattle from which the ticks were obtained had no history of acaricide usage in the last thirty days before they were harvested. The ticks were transported tothe Parasitology Laboratory, College of Veterinary Medicine, Federal University of Agriculture Abeokuta (FUNAAB) in labeled plastic containers with perforated lids for aeration. All the tick samples collected from an area werepooled together, washed in tap water and dried on an absorbent paper. The ticks were thereafter kept in a tick rearing chamber and maintained at 27-28°C and 80-95% relative humidity. Of the total ticks harvested, 492 engorged adult female ticks were used for this study after they were sorted and identified to species level.

Morphological identification

The identification of *Rhipicephalus* (*B.*) *annulatus*wasdone based on morphological characteristics by using a stereoscope (80-fold magnificationDigital USB Microscope, Unimake®) and a light microscope (100-200 fold magnification, Leica®) as described by Walker et al. [19].

Adult Immersion Test (AIT)

The ticks were assigned randomly tosix groupsof six ticks per group, the average weight of each group was taken and recorded. The six engorged female ticks per group were immersed in 10ml [9,20]of respective concentrations of acaricidal preparations at room temperature for two min in a 25ml beaker under gentle agitation to ensure uniformity. Six ticks per group were also immersed in normal saline and distilled water. Each treatment group was replicated four times. After dipping, the acaricide solution was sieved off the ticks and theticks wereplaced on a clean paper towel. The ticks were thereafter kept into an individual tick rearing chamber and maintained at 27-28°C and 80-95% relative humidity for oviposition. The ticks were monitored daily for oviposition and mortalities, and the eggs laid by individual tickswerecounted [9,21].

Estimation of acaricide efficacy

The percentage resistance was calculated as:

$$\textbf{Percentage resistance}\ (\%) = \frac{\boldsymbol{Nt}}{\boldsymbol{Nw}} \times \mathbf{100}$$

Where,*Nt*= number of treated ticks laying eggs; *Nw*= number of untreated ticks laying eggs [9].

The percentage control was calculated as:

$$Percentage\ control = 100 - Control\%$$

Biological parameters were monitored for upto 14 days to allow egg laying and the following parameters wererecorded:

(a) Mortality=engorged females that oviposit are considered as live and females that do not oviposit or which oviposit small amount of black eggs (non-viable) were considered as dead.

(b) Mass of eggs on day 14 post Adult Immersion Test

(c) Reproductive index (RI)

$$= \frac{Mass\ of\ eggs\ (mg)}{Weight\ of\ engorged\ female\ (mg)}$$

(d) % Inhibition of oviposition (IO)

$$= \frac{RI\ control - RI\ treated}{RI\ control} \times 100$$

Tarsal reflex scoring

The tarsal response of the treated ticks to light and touch were observed, graded on a scale of 0-5 and recorded. The touch and light stimuli were delivered using a probe and light source (Digital USB Microscope, Unimake®) respectively. This was done immediately after removing the ticks from the test solutions. Grade 0 stand for no movement or response, 1 stand for movement of one or two limbs slowly, 2 stand for movement of one or two limbs at a fast rate, 3 stand for movement of more than two limbs, 4 stand for movement of greater than two but less than 8 limbs at a slow rate while 5 stand is used for movement of all the limbs after dipping in acaricides.

Data analysis

Data was recorded in Microsoft excel and mean mortality and standard error determined. Dose-response data were analyzed by Probit method [22]. The 50% lethal concentration (LC_{50}) was determined by applying regression analysis to the Probit transformed data of mortality using SPSS version 25.Relationship between the weights of the ticksand the number of eggs produced post-treatment was determined using the value of co-efficient of correlation (r).

Results

Tick identification

The ticks collected were identified as *R. (B.) annulatus*based on the presence of 4+4 column hypostomal teeth, the internal margin of the first palp article was long and slightly concave, coxae 1spurs were present and distinct while coxae 2 and 3 were absent.

Mean weight of ticks

A group of six ticks weighed between 0.88 and 1.63. The mean weight of ticks immersed in acaricides and the standard error of the mean for each acaricidal group is as presented in Table 1.

Tarsal reflex score

None of the ticks got drowned in the acaricide or control solutions used in this study (Table 2). Both ticks in treatment and control groups displayed visible response after immersion, 75 ticks were recorded to move 1-2 limbs slowly while 88 ticks moved the same number of limbs at a faster rate after dipping. Majority of the ticks (147) moved more than 2 limbs at a slower pace while 59 ticks moved all the limbs after immersion.

TABLE 1. Weight of replicate group treated with acaricides.

	Weight (grams)
Mean of Replicate group	1.23±0.01
Number of Replicate group	80

TABLE 2. Tarsal reflex scoring.

Response	Score	A	B	C	D	Positive Control	Negative Control	Total
No Response	0	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Moving 1-2 Limb Slowly	1	9	12	27	27	Nil	Nil	75
Moving 1-2 Limbs At A Fast Rate	2	24	28	22	14	Nil	Nil	88
Moving More Than 2 Limbs But Slow	3	33	35	43	31	2	3	147
Moving >2 But <8 Limbs But Fast	4	32	35	18	35	1	2	123
Moving All Limbs	5	22	10	10	13	3	1	59

Percentage resistance and control

The percentage resistance and control for each concentration of ACARICIDE A, ACARICIDE B, ACARICIDE C, ACARICIDE D and control solutions were obtained. Percentage resistance and control for the acaricides used ranged from 33.3-100% and 0-66.7% respectively (Table 3)

The pre-oviposition period for the ticks was three days while the oviposition period differs among the ticks.Among the treatment group, Acaricide A treated ticks laid the highest number of eggs while Acaricide B laid the lowest number of eggs, the oviposition period stays between 3-16 days post treatment (Table 4).

TABLE 3a. Percentage control and resistance of acaricides.

Acaricides	Concentration (ppm)	Percentage Resistance (%)	Percentage Control (%)
FORMAMIDINE	150	95.8	4.2
	200	66.7	33.3
	250	95.8	4.2
	300	100	0
	350	83.3	16.7
PHENYLPYRAZOLE	6000	37.5	62.5
	8000	37.5	62.5
	10000	58.3	41.7
	12000	54.2	45.8
	14000	45.8	54.2
CYPERMETHRIN	30000	75	25
	40000	54.2	45.8
	50000	37.5	62.5
	60000	41.7	58.3
	70000	37.5	62.5
MACROCYCLIC LACTONE	3000	33.3	66.7
	4000	70.8	29.2
	5000	41.7	58.3
	6000	62.5	37.5
	7000	70.8	29.2

TABLE 3b. Means of percentage control and resistance.

Acaricides	%Control ±SE	% Resistance±SE
FORMAMIDINE	11.68±6	88.32±6
PHENYLPYRAZOLE	53.34±4.25	46.66±4.25
CYPERMETHRIN	50.82±7.14	49.18±7.14
MACROLYTIC LACTONE	44.16±7.75	55.82±7.75

TABLE 4. Mean number of eggs and oviposition for each acaricide concentration.

Acaricide	Conc. 1 (Eggs)	Conc. 2 (Eggs)	Conc. 3 (Eggs)	Conc. 4 (Eggs)	Conc. 5 (Eggs)	Total Egg	Range Of Oviposition Period (Days)
A	738	437	1264	1133	1243	4815	3-16
B	345	178	338	259	192	1312	3-13
C	685	343	311	322	310	1971	3-9
D	206	178	328	394	513	1619	3-11
E	--	---	---	---	---	5439	3-13
F	--	--	---	---	---	---	3-13

Table 5 shows LC_{50} for each acaricide and the number of tick dead and alive 7 days post treatment. Acaricide A,B,C and D had 100,800, 23000 and 6000ppm respectively.

TABLE 5. Number of acaricide treated ticks that were alive after 7 days.

Acaricide	Concentration (ppm)	Tick Alive	Tick Death	Lc_{50}(ppm)
A	150	15	9	100
	200	14	10	
	250	23	1	
	300	21	3	
	350	19	5	
B	6000	14	10	8000
	8000	12	12	
	10000	11	13	
	12000	7	17	
	14000	4	20	
C	30000	10	14	23000
	40000	10	14	
	50000	6	18	
	60000	6	18	
	70000	6	18	
D	3000	1	23	6000
	4000	10	14	
	5000	12	12	
	6000	9	15	
	7000	13	11	

The coefficient of correlation between the weight and the number of eggs laid by ticks treated in ACARICIDE A, B and D shows positive relationship which is not significant at ≤0.05 P-value as shown below Table6.

TABLE 6. Co-efficient of corelation for weights and number of eggs laid by ticks treated tick .

Acaricides	Co-efficient of Correlation(r)	p-Value
A	0.88	0.04*
B	0.53	0.35
C	0.33	0.58
D	0.59	0.29

Stastitically Significant

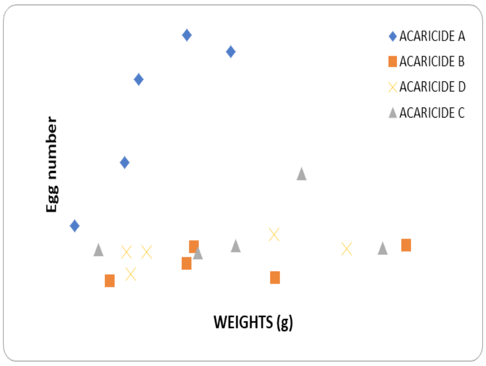

Fig. 1. Corelation relationship between weights and number of eggs laid by ticks treated .

Discussion

Ticks transmit diverse pathogens (including viruses, bacteria and protozoa) from host to host during feeding and are the most important vectors of pathogens affecting cattle worldwide [23,24]. Tick-borne diseases such as babesiosis, anaplasmosis, theileriosis and cowdriosisgenerally affects the blood and lymphatic systems. In such situations of disease outbreaks, chemical acaricides with different modes of action are generally used [25]. However, the widespread and indiscriminate use of these acaricides, especially at doses lower than the recommended dose, has led to the development of acaricidal resistance. Resistance to more than one chemical class of acaricide is an alarming phenomenon considering that there are no new drugs with a novel mode of action available in the market to control multidrug resistant ticks. This study is in line with farmers' claims of lost efficacy to commercial acaricides in this environment. In the present study, four acaricides viz. ACARICIDE A (formamidine), ACARICIDE B (phenylpyrazole), ACARICIDE C (Cypermethrin) and ACARICIDE D (macrocyclic lactone) were evaluated for their efficacy using AIT and it can be deduced that ticks have developed resistance to them.

Tarsal reflex scoring of ticks used in this study was between 0-5. The use of tarsal reflex in this study helped to detect any immediate effect (if any) of the acaricides. Of the 492 ticks that were used, no tick was graded 0 while 15% (75/492), 18% (88/492),30% (147/492),25% (123/492) and 12% (59/492) ticks were graded 1,2,3,4 and 5 respectively. Factors responsible for variation in response to stimuli may include health status, size, constituents of solution and state of sensory apparatus. Check for tarsal reflex is a visual observation which has been used in experiments by various authors including [26, 27]. Tick body surfaces and appendages possess sensory apparatus or organs making them sensitive to touch stimuli [28,29]. The presence of eyesin *R. (B) annulatus*also makes them sensitive to light stimuli [30]. In many animals, intersegmental reflexes are important for postural and movement control[27].A detailed understanding of such a reflex response could be included in the design of orthoses or functional electrical stimulation treatments.

Formamidines (ACARICIDE A) are widely used in veterinary medicine for the control of external parasites such as ticks, mites and lice on cattle, dogs, goats, pigs and sheep [31]. Its mode of action probably involves interaction with octopamine receptors in the nervous system of the ticks causing an increase in nervous activity [32]. At 200ppm concentration, ACARICIDE A (Formamidine) elicited 33.3% control, better than the DD of 250ppm which produced 4.2% control. 300ppm experienced 100% resistance while ticks treated in 200ppm laid the smallest number of eggs. The LC_{50} value of ACARICIDE A (Formamidine) was 100ppm (0.01%), a value that is lower than the DD of 250ppm (0.025%).

*Rhipicephalus. (B) annulatus*showed high level of resistance to ACARICIDE A which is in line with many authors [33,34], who reported low *invitro* efficacy levels of the formamidines in the southern region of Rio Grande do Sul (Brazil) using the AIT and larvae packet tests. The low percentage control displayed by all the concentrations of the formamidines indicate that this drug is poor in controlling *R.* (*B.*) *annulatus*

The phenylpyrazoles are broad-spectrum insect control agents that act to block the gamma-aminobutyric acid (GABA) regulated chloride channel in the insect nervous system [35]In this present study, ACARICIDE B (Phenylpyrazole) displayed dose-dependent inhibition of oviposition of *R.*(*B.*)*annulatus*. Both 6000ppm and 8000ppm of ACARICIDE B displayed higher percentage control (62.5%) than any other concentration, which supports the report of Shyma, et al. [20] on the existence of low-grade resistance of *Rhipicephalus* species to fipronil, with the DD of 10000ppm having 41.7% control. The LC_{50}value for fipronil against *R.* (*B.*) *annulatus*was determined as 12.78 ppm by Amithamol, et al. [36]. The LC_{50} estimated was 0.2% lower than the DD. Having a lower LC_{50} may promote the use of lower but effective concentration which may minimize animal toxicity and environmental pollution. This will also save cost as farmer will have to pay for lesser quantity to achieve the same result.

Pyrethroids act on sodium and chloride channels of nerve cells of ectoparasites thereby delaying closing of the sodium ion channels, leading to multiple nerve impulses [37]. The DD of 50000ppm and 70000ppm ACARICIDE C (Synthetic pyrethroid) showed the highest percentage control (62.5%). The estimated $LC_{50}$was 2.30% while the DD was 5%. This is higher than 138.5ppm, the LC_{50} of cypermethrin against *R.* (*B.*)*microplus* [38] and 184ppm, this suggests moderate control at the DD.

In arthropods, the avermectins blocks nerve signals by interfering with the glutamate-gated chloride (GluCl) channel receptors [39]. ACARICIDE D (Macrocyclic lactone), at 3000ppm, a concentration lower than the DD of 5000ppm elicited 66% control while the latter controlled 58.3%. The estimated $LC_{50}$was 0.6%, which is higher than the DD (0.5%).

None of the five concentrations of ACARICIDE A, ACARICIDE B, ACARICIDE C and ACARICIDE D showed 100% tick control.

Complete inhibition of oviposition (100%) was not observed, even at concentrations higher than the DD. Ticks treated with ACARICIDE B laid the lowest number of eggs before death. The number of eggs laid by ACARICIDE C treated ticks indicate great inhibition of oviposition on *R.* (*B.*) *annulatus* which increases with concentration. This agrees with [40]who reported areduction in thepercentage of egg-laying in *Dermacentorreticulatus* females treated with Cypermethrin. There was increase in the number of eggs laid by ACARICIDE D-treated ticks withincrease in acaricide concentration. ACARICIDE A had low oviposition inhibition, with the ticks treated in it producing the highest number of eggs. Laying of eggs after dipping in acaricide indicate the presence of resistance from the ticks to the acaricide [9].

The pre-oviposition period of three days is in line with [41]. There was no inhibition of oviposition as most of the treated ticks laid through out before death. No significant change in laying pattern between Solution E and F treated ticks was observed and, in most cases,mortality was not seen until 11th day post treatment. Start of mortalities 11th day post treatment suggests a possibility that if the ticks are left in control solution more than two minutes used in AIT, death and better oviposition inhibition can be achieved [42].

The correlation regression between the weights of ticks (groups) and the number of eggs laid by acaricide (B,C,D) treated ticks suggest no relationship between the two variables according to the obtained co-efficient of correlation (r), which can be explained as a result of average percentage control and inhibition of oviposition that was observed in the drugs. There is a relationship between the weights of ticks and the number of eggs laid by ticks treated with ACARICIDE A, which is in line with Davey, et al. [41] due to low inhibition of oviposition and percentage control.

Both the oviposition period and the number of eggs obtained from the control group are the same.

This is an indication that little or no inhibition of oviposition when compared to the control group. Solution E and F show no significant oviposition or acaricidal property.

With the level of resistance recorded in this study, it can be deduced that the available acaricides in our markets are no longer effective in tick control. Discovery of better acaricides possibly biological which are cheap and environmentally friendly is needed. Possibility of alternating acaricide use should be considered to reduce the risk of resistance development. Continuous monitoring of the efficacies of available acaricides is also needed to determine the possibility of substandard product provision. Proper combination of these commercial acaricides may give better result than individual use. The result obtained on this current study also confirms the presence of commercial acaricide resistance *Rhipicephalus (B.) annulatus* and this is the first report in Nigeria.

With these findings the need for alternative acaricides is apparent, more so with diverse reports on increase tick population in the country and invasion of newer tick species.

Acknowlegment: we want to appreciate the cattle owners who agreed to us sampling their animals from which the ticks were gathered

Funding statement: No fund or grant was gotten for this research work.

Conflict of interest: No conflict of interest exits for any of the author

Ethical consideration:

References

1. Parola, P. and Raoult, D. Tick and Tick-borne Bacterial Disease in Humans. An Emerging Infectious Threat. *Clinical Infection Disease*, **32**, 897-928 (2010).

2. Mekonnen, S. Acaricide Resistance Profiles in Single- and Multi- host ticks in Commercial and Communal Farming Areas of Eastern Cape and North -Western Provinces of South Africa". *In a Masters Dissertation submitted to the Department of Veterinary Tropical diseases*, Faculty of Veterinary science, University of Pretoria. 139(2002).

3. Hend, H.A.M. Abdullaha, Amal, El-Mollab, Fayez, A. Salibb, Alaa, A. Ghazya, Nesreen, A.T. Allam, and Sobhy, Abdel-Shafy. Preliminary detection of Rickettsiae using PCR targeting *OmpA* gene among dogs and horses in Cairo, Egypt**.** *Egyptian Journal of Veterinary Science*s, **50** (special issue), 1- 8(2019).

4. Abdullah, D., ALanazi, Swaid Abdullah, Chris Helps, Richard Wall, Robert Puschendorf, Samir, A. AL Harbi, **Sobhy** Abdel-Shafy and Raafat M. Shapaan. Tick-borne pathogens in ticks and blood samples collected from camels of Riyadh Province, Saudi Arabia. *International Journal of Zoological Research*, **14**(1), 30-36(2018).

5. Bansal, G.C., Bovine Theileriosis in India: An Overview. *Proceedings of the National Academy of Sciences*. India, **75**, 134-143(2005).

6. Mona, S. Mahmoud, Nadia, T. Abu El-Ezz, Sobhy Abdel-Shafy, Somia, A. Nassar, Amera,H. El Namaky, Wagdy, K.B., Khalil and Carlos E. Suarez. Serological and molecular diagnosis of equine Babesiosis in distinct animal populations of Egypt. *Parasites and Vectors*, **9**,260-267(2016). Published online 2016 May 4. doi: 10.1186/s13071-

7. Mostafa, I., Hassan, Hanan, S. M., Gabr, Sobhy Abdel-Shafy, Kotb M. Hammad and Mostafa, M. Mokhtar Molecular detection of *Borrelia* sp. in *Ornithodoros savignyi*and *Rhipicephalus annulatus* by *FlaB* gene and *Babesiabigemina* in *R. annulatus* by *18S rRNA* gene. *J. Egypt. Soc. Parasitol.*, **47** (2),403 – 414(2017).

8. Nanyingi, M.O., Research and validation of the ethnoveterinary remedies for ectoparasites and endoparasites of livestock in Kenya. Ph.D. *thesis.*, University of Nairobi,Nairobi, Kenya(2008).

9. Food and Agricultural OrganizationAcaricide Resistance: Diagnosis, Management and Prevention. Animal Production and Health Division, Agriculture Department Food and Agriculture Organization of the United Nations, Rome, Italy.

10. Bianchi, M.W., Barre, N. and Messad, S., Factors Related to Cattle Infestation Level and Resistance to Acaricides in *Boophilusmicroplus*Tick Populations in New Caledonia. *Veterinary Parasitology*, **112**, 75–89(2003).

11. Martins, J.R. and Furlong, J., Avermectin Resistance of the Cattle Tick Boophilusmicroplus in Brazil. *Veterinary. Record*, **149**, 64-67(2001).

12. Li, A.Y., Davey, L.R. and George, J.E., Carbaryl resistance in Mexican Strains of the Southern Cattle Tick (Acari: Ixodidae). *Journal of Economic Entomology,* **98**, 552–556(2005).

13. Alonso-Diaz, M.A., Rodriguez-Vivas, R.I., Fragoso-Sanches, H. and Rosario-Cruz, R.,Resistance of the Tick *Boophilusmicroplus* to the Ixodicides. *Archivos de MedicinaVeterinaria*, **38**, 105–113(2006).

14. Castro-Janer, E., Martins, J.R., Mendes, M.C., Namindome, A., Klafke, G.M. and Schumaker, T.T., Diagnoses of Fipronil Resistance in Brazilian Cattle Ticks *Rhipicephalus(Boophilus) microplus* using *in vitro* Larval Bioassays. *VeterinaryParasitology*, **173**, 300–306(2010).

15. Haggag, Y.N., Samaha, H.A., Nossair, M.A. and Habib, H.M., Comparative in Vitro Study on the Efficacy of Some Commercial Insecticides against *Rhipicephalus (Boophilus) annulatus* Ticks. *Alexandria Journal of Veterinary*

16. Omotayo, A.M. A land-use system and the challenge of sustainable ago-pastoral production in southwestern Nigeria. *The International Journal of Sustainable Development and World Ecology***,** **9**, 369-382(2002).

17. Ebuehi, O.M., Campbell, P.C. and Campbell, O.E.P. Attraction and retention of qualified health workers to rural areas in Nigeria: a case study of four LGAs in Ogun State, Nigeria. *Rural Remote Health,* **11**, 1-11(2011).

18. Taiwo, O.T., Sam-Wobo, S.O., Idowu, O.A., Talabi, A.O. and Taiwo, A.M. Comparative assessment of intestinal helminths prevalence in Water, Sanitation and Hygiene (WASH) intervention and non-intervention communities in Abeokuta, Nigeria. *Asian Pacific Journal of Tropical Biomedicine* **7**, 524-532(2017).

19. Walker, A.R., Bouattor, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., and Preston, P.M. Ticks of domestic Animalsin Africa: A Guide to identification of Species (2014).

20. Shyma, K.P., Gupta, J.P, Singh, V. and Patel. K.K.., *In Vitro* Detection of Acaricidal Resistance Status of *Rhipicephalus (Boophilus) microplus*against CommercialPreparation of Deltamethrin, Flumethrin, and Fipronil from North Gujarat, India. Hindawi Publishing Corporation *Journal of Parasitology*. *Journal of Parasitology*. **2015**, article ID 506586 7 pages http://dx.doi.org/10.1155/2015/506586(2015).

21. Drummond, R.O., Ernest, S.E., Trevino, J.L., Gladney, W.J. and Graham, O.H.*Boophilusannulatus*and *Boophilusmicroplus*. Laboratory Tests for Insecticides. *Journal of Eco*Finney, D.J., Probit Analysis-A Statistical Treatment of the Sigmoid Response Curve. *Cambridge University Press, Cambridge*. 1–318(1952).

22. Peter, R.J, Van den Bossche, P., Penzhorn, B.L. and Sharp, B., Tick, Fly, and Mosquito Control-Lessons from the Past, Solutions for the Future. *Veterinary Parasitology*, **132***,*205–215(2005).

23. De la Fuente, J., Estrada-Pena A., Venza J.M., Kocan K.M. and Sonenshine D.E., Overview: Ticks as Vectors of Pathogens that Cause Disease in Humans and Animals. *Frontiers in Biosci*

24. Ravindran, R., Jyothimol, G., Amithamo, K..K.., Sunil A.R., Chandrasekhar L. , Lenka D.R., Amritha, A., Sreelekha, K., Sathish, N., Udayan, D., Krishna, T. P., Juliet, T.M., Kumar, K.G. , Nair, S.N. and Ghosh, S., *In vitro* Efficacy of Amitraz, Coumaphos, Deltamethrin and Lindane Against Engorged Female *Rhipicephalus* (*Boophilus*) *annulatus*and *Haemaphysalisbispinosa*ticks. *Experimental and Applied Acarology,* **75**(2),241-253(2018). doi: 10.1007/s10493-018-0262-y

25. Carvalho, L.B., Furlong, J., Prata, M. C., Reis, E.S., Batista, E.S., Faza, A.P. and Leite, R.C., Evaluation *in vitro* of the Infection Times of Engorged Females of *Rhipicephalus (Boophilus) microplus*by the Entomopathogenic Nematode *Steinernemaglaseri* CCA strain. *Cienta Rural*, **40**, 939-943(2010).

26. Costalago-Meruelo, A., Simpson, D.M., Veres, S.M. and Newland, P.L. Predictive control of intersegmental tarsal movements in an insect. *Journal of Computational Neuroscience.* **43**, 5-15(2017).

27. Wooley, T.A., Some Sense Organs of Ticks as Seen by Scanning Electron Microscopy.

28. Transactions of the American Microscopical Society,**91**,348-363 (1972).

29. Waladde S.M., The Sensory Nervous System of the Adult Cattle Tick BoophilusMicroplus (Canestrini) Ixodidae. Part 1. Light microscopy. *Journal of Australian Entomological. Society*, **15**, 379-387(1976).

30. Walker, A.R., Bouattour A., Camicas J.L. and Estrada-Pena., Ticks of Domestic Animals in the Mediterranean region, A guide to identification of Species, 1st edition. *Bioscence Reports*, Edin

31. Müller, G.H., Amitraz Treatment of Demodicosis. *Journal of American Animal Hospital*. **19**, 435–441(1983).

32. Roder, T., Pharmacology of the Octopamine Receptor from Locust Central Nervous tissue (OAR3). *British Journal of Pharmacology*, **114**, 210–216(1995).

33. Santos, T.R.B., Farias, N.A.R., Cunha, N.A., and Vaz, I.S.J., Uso de acaricide asem *Rhipicephalus (B.) microplus*de duasregioesfisiograficas do Rio Grande do Sul. *Acta Scientiae Vetirinariae*, **36**, 25-30(2008).

34. Santos, T.R.B., Klafke, G.M., Pappen, F.G., Nizoli, L.Q., Biegelmeyer, P. and Farias, N.A.R., Comparison of Three Larval Bioassays to Evaluate Susceptibility of *Rhipicephalus* (*Boophilus*) *microplus*to amitraz. *Revista. Brasileira. De Parasitoogial Veterianaria*, **22**, 495-501 (2013).

35. Taylor, M.A., Recent developments in Ectoparasiticides. *The VeterinaryJournal*, **161**, 253-268(2001).

36. Amithamol, K.K., Sunil, A.R., Kumar, K.G.A., Nair, S.N., Soorya, V.C., Chandrasekhar, L., Juliet, S., Ravindran, R. and Ghosh, S., Effects of Fipronil against *Rhipicephalus (Boophilus) annulatus*. *International Journal of Acarology*, **38**, 731-735(2012).

37. Eells, J.T., Bandettini, P.A., Holman P.A. and Propp J.M., Pyrethroid Insecticide-Induced Alterations in Mammalian Synaptic Membrane Potential. *Journals of Pharmacology and Experimental Theraupetics*, **262**, 1173–1181(1992).

38. Sharma, A.K., Kumar, R., Kumar, S., Nagar, G., Singh, N.K.., Rawat, S.S., Dhakad, M.L., Rawat, A.K., Ray, D.D. and Ghosh, S., Deltamethrin and Cypermethrin Resistance Status of *Rhipicephalus (Boophilus) microplus*Collected from Six agro-Climatic Regions of India. *Veterinary Parasitology,* **188**, 337- 345(2012).

39. Martin, R.J., Robertson, A.P, and Wolstenholme, A.J., Mode of Action of the macrocyclic lactones. *V*ercruysse, J, and Rew R.S (Eds.), *Macrocyclic Lactones in Antiparasitic Therapy*. *Wallingford, United Kingdom: CABI Publishing*, 125-140(2002).

40. Buczek, A., Bartosik, K. and Kuczyński, P.,. Evaluation of the Effect of Various Concentrations of Selected Pyrethroids on the Development of *Dermacentorreticulates* Eggs and Larvae. *Annals of Agricultural and Environmental Medicine,* **20**, 447–451(2013).

41. Davey, R.B., Garza, J.J., Thompson, G.D. and Drummond, O., Oviposition Biology of the Cattle Tick, *BoophilusAnnulatus* (*Acari: Ixodidae*) in the Laboratory. *Journal of Medical Entomology*, **17**, 287-289(1980).

42. Adejinmi, J.O. "Effect of Water Flooding on the Oviposition Capacity of Engorged Adult Females and Hatchability of Eggs of Dog Ticks: Rhipicephalus sanguineus and Haemaphysalisleachila eachi," *Journal of Parasitology Research*, , Article ID 824162, 5 pages(2011). https://doi.org/10.1155/2011/824162(2011).