European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 8, 2020

EVALUATION OF THE EFFECT OF TWO TICK KILLERS IN SPRAY BATHS ON CATTLE IN THE PARISH OF REGULO DE MORA, CANTON SAN MIGUEL, PROVINCE OF BOLIVAR (ECUADOR)

Anthony García Romero, Riveliño Ramón, Favian Bayas-Morejón , Alejandra Barrionuevo
Faculty of Agricultural Sciences, Career in Veterinary Medicine, Universidad Estatal de Bolívar, CP: 020150, Guaranda-Ecuador

Abstract - The present research evaluated the effect of different pharmacological combinations, in spraying baths against ticks in cattle, in order to know the most effective treatment, the prevalence of ticks in the area and which are the most common anatomical regions of location of ticks; besides, they identified the species of ticks according to their taxonomic classification and determined the presence of milk trace drugs in animals. With the use of a statistical model (DBCA) with 60 animals in study and a total of 3 treatments and 20 repetitions, besides an entomological study of the collected animals and liquid chromatography of the milk obtained from the animals in study. It was determined that the treatment with the highest efficacy and external antiparasitic action was the pharmacological combination 2 that corresponded to the T3 treatment presenting the highest efficacy against tick infestation. We could catalogue as endemic the presence of ticks of the genus (Boophilus microplus), since we could establish 100% of prevalence, besides, a genus of ticks (Boophilus microplus) was obtained. The anatomical region of preference of the tick Boophilus microplus was established in the members of the rear train due to the great musculature found in this area, besides another area that had great predilection was the abdominal region due to the great irrigation it presents. By means of a chromatographic study, it was determined that no residues of Amitraz, Fipronil, Dichlorvos and Cipermetrine were found in raw milk from the animals submitted to spray baths in the present investigation.

Keywords: Ticks, Spray baths, Prevalence, Taxonomy, Chromatography.

INTRODUCTION

In Ecuador, the presence of ticks is one of the most important economic problems for cattle production, due to the direct and indirect effects they have on the animals of the herd. They can also transmit diseases to their hosts, since they act as vectors for protozoa such as *Babesia bigemina and Babesia bovis*, which parasitize the animal, affect production and public health, and of which there is insufficient information in the Ecuadorian context. (Alvarado, 2014)

Amidines are a special class of ectoparasitic active substances with contact activity mainly against ticks, mites and lice. (Pereira, 2019). The main active substance in this class is Amitraz, which is still widely used today in livestock farming against external parasites in cattle, sheep, pigs, poultry and dogs. (Miller, 2018). Amidines are antagonists to the octopamine receptors in the brains of parasites: they cause hyperexcitability and then paralysis and death. (Sumano and Ocampo, 2014)

Cypermethrin has rapid action, its effectiveness is enhanced, and its residual effect is increased. It acts on insects by inhibiting motor nerve centres and microsomal enzyme systems. It is absorbed by the skin of the animals only to a limited extent, which makes it safe to apply. (Woodward, 2014)

Fipronil in turn is a Phenylpyrazole antiparasitic which interferes with the passages of chloride ions in the corresponding GABA-regulated channels, thus interfering with the activity of the CNS. It is used to combat ticks, lice and fleas. (Toriz, 2014)

High Performance Liquid chromatography (HPLC) is a technique used to separate the components of a mixture. It consists of a stationary non-polar phase (column) and a mobile phase. (Naushad and Rizwan, 2014). For the detection and identification of antibiotics in meat, kidney and milk the most used detectors are the ultraviolet detector (UV) and the diode array detector (DAD) (Corcia and Nazzari, 2002).

MATERIALS AND METHODS

The present study consists of a descriptive, experimental and investigative part, over a period of three months, in which spray baths were carried out with the above-mentioned drugs to test their effectiveness against ticks

Samples

The following methods were used for the following investigation.

Chromatographic Field Methodology

The chromatographic method for UPLC-MS proposed by She *et al*. It will be coupled for the UPLC-UV with the application of an isocratic gradient. For this study a reverse phase column Acquity BEH C18 UPLCTM was used. (50mm x 2.1 μm; 1.7 μm. Waters). We also worked with an ACQUITY UPLC photodiode array detector (PDA), which allowed simultaneous operation in 2D and 3D in the software Empower™. The team worked with a column temperature of 30°C (±5) and a sample temperature of 10°C (±5). The flow rate of the mobile phase was 0.25 ml/min.

Field Methodology

Periodic baths were carried out according to the established time, using a fumigation pump according to the proposed treatments, after which a count was carried out in each of the anatomical regions previously chosen to obtain the number of ticks per region.

Treatments

Table 1. Treatments.

Treatment	Code	Detail
1	a1b1	Witness
2	a1b2	Bovine + 20 ml of Pharmacological Combination 1 in 12.5 l water every 15 days
3	a1b3	Bovine + 1 ml of pharmacological combination 2 in 1 l of water every 15 days

Experimental Measurements

The variables to be evaluated were:

- Prevalence to determine the most effective antiparasitic.
- Age.
- Race.
- Prevalence for determining the type of tick
- Percentage of efficiency
- Study of drug residues in milk

RESULTS

Prevalence Of Ticks In The Examined Animals

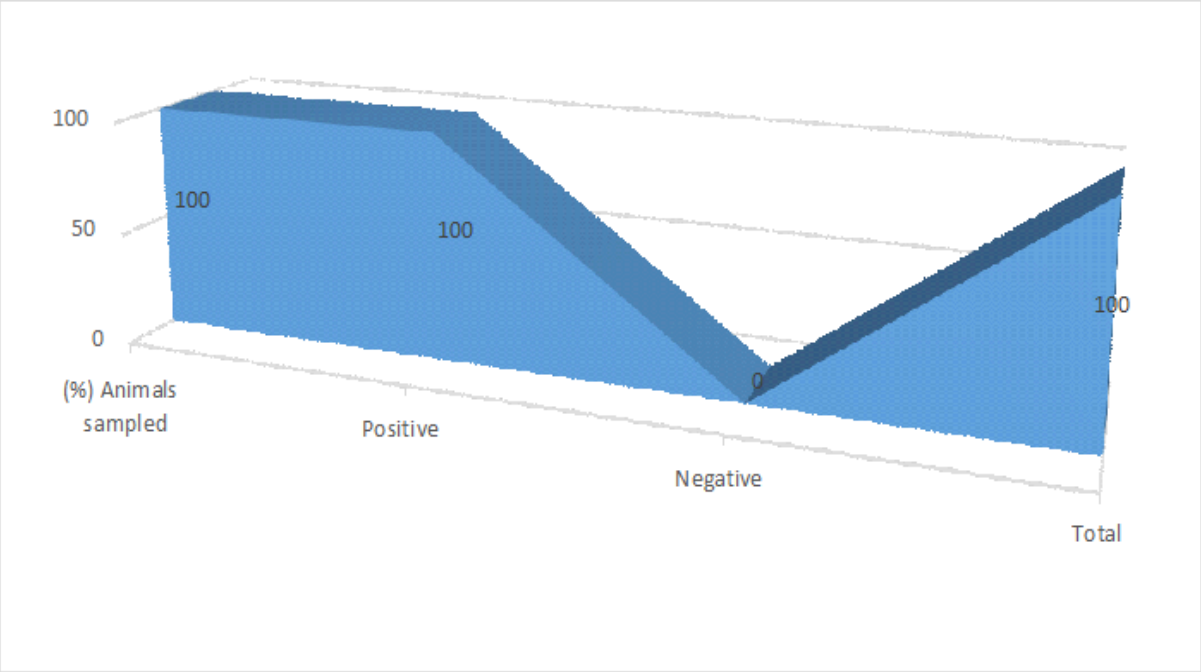
The results obtained in the parish Regulo de Mora belonging to San Miguel canton, in relation to determining the prevalence of ticks in the animals of the sector indicate that, from a total of 60 animals

studied, the following results were obtained: 60 animals were positive representing 100% of prevalence and 0 negative for 0% correspondingly as it is observed in table 2.

Table 2. Prevalence of ticks.

Animals sampled	Positive	%	Negative	%	Total
60	60	100	0	0	100

The results show that the total prevalence found in the parish Regulo de Mora belonging to San Miguel canton can be catalogued as highly infested with ticks. This is due to the fact that 100% of the animals had presence of ticks.



It was determined in the Patate canton that the Holstein breed presented a high level (89%), as opposed to the raza Jersey (80%), we are management and icon control of the ticks areas is better than one presented in the parish, result that no surveillance and that revision of the ticks in the parish is endemic. (Diaz, 2015)

Identification Of Tick Species Affecting Cattle In That Area According To Their Taxonomic Classification

Table 3. Taxonomic study of ticks.

Taxonomic study of ticks							
	Sex					Parasitic stage	
Sample No.	M	F	Family	Género	Species	Adult	Nymph
60	9	51	Ixodidae	Rhioicephalus	Microplus (Boophilus)	51	9

There were obtained a total of 60 specimens of ticks from the proposed treatments, collected in the four data taking carried out; later, the samples were grouped in groups of 5 due to their physical similarity, to be analyzed in a stereomicroscope and through the use of keys.

Determination Of The Most Common Anatomical Location Regions Of Ticks.

In order to determine the greatest concurrence of ticks in the an average of all the data obtained from each tick count the anatomical in which it was found, is presented below:

Location Of Ticks According To Anatomical Regions In The First Count.

Table 4. Results obtained from the first tick count according to the anatomical region

First tick count					
No. of ticks per area.	T1 (Witness)	T2 (Pharmacological combination 1)	T3 (Pharmacological combination 2)	Total	Average
Head	15	17	10	42	10,9
Neck	19	16	15	50	12,9
Chest	8	15	12	35	9,0
Abdomen	24	32	24	80	20,7
Previous Member	11	14	13	38	9,8
Rear Member	51	35	56	142	36,7
Total	128	129	130	387	100,0

For the first count of ticks by region we observed the site of predilection of the tick of the genus *Boophilus* was the posterior limb, in which the great majority of mites of this genus was concentrated; it was followed by the abdominal region that comprised part of the udder and the inguinal zone of the animal. For the front part of the animal there was a smaller quantity of located ticks, in their majority they were located in the neck and head of the animal and scarcely in the thorax and the previous members of the bovine.

Location Of Ticks According To Anatomical Regions In The Second Count.

Table 5. Results obtained from the second tick count according to the anatomical region

Second tick count					
No. of ticks per area.	T1 (Witness)	T2 (Pharmacological combination 1)	T3 (Pharmacological combination 2)	Total	Average
Head	10	11	11	32	9,8
Neck	14	8	14	36	11,0
Chest	9	6	10	25	7,6
Abdomen	30	29	21	80	24,4
Previous Member	15	9	10	34	10,4
Rear Member	52	36	33	121	36,9
Total	130	99	99	328	100,0

According to Table 5 that expresses the results of the second tick count, it is observed that the area of predilection in the location with the greatest number of this parasite is the hind limbs with a total of 121 and an average of 36.9; followed by the abdomen with 80 and 24.4 on average; the values in head, neck and forelimbs are very similar unlike the thorax where it is found less with 25 and an average of 7.6.

Location Of Ticks According To Anatomical Regions In The Third Count.

Table 6. Results obtained from the third tick count according to the anatomical region

Third tick count					
No. of ticks per area.	T1 (Witness)	T2 (Pharmacological combination 1)	T3 (Pharmacological combination 2)	Total	Average
Head	15	8	7	30	10,9
Neck	10	4	5	19	6,9
Chest	11	3	7	21	7,6
Abdomen	20	21	19	60	21,7
Previous Member	11	14	13	38	13,8
Rear Member	48	33	27	108	39,1
Total	115	83	78	276	100,0

Regarding the third tick count per region, we observe that the site of predilection of the tick of the genus *Boophilus* is still the posterior limb, as a constant that is being repeated, followed by the abdominal region as another favorite site for this genus.

Location Of Ticks According To Anatomical Regions In The Fourth Count.

Table 7. Results obtained from the fourth tick count according to the anatomical region

Fourth tick count					
No. of ticks per area.	T1 (Witness)	T2 (Pharmacological combination 1)	T3 (Pharmacological combination 2)	Total	Average
Head	12	9	7	28	10,8
Neck	15	11	9	35	13,5
Chest	16	5	8	29	11,2
Abdomen	21	14	14	49	18,8
Previous Member	15	9	10	34	13,1
Rear Member	37	27	21	85	32,7
Total	116	75	69	260	100,0

For the fourth and last count of ticks by region we observed that the tick of the genus *Boophilus* had a great affinity for the area comprising the hind limbs and the abdominal region. This constant was observed from the first count and identification of the anatomical regions of the animals in which the ticks were found and the great affinity that the ticks had for these areas, while the areas such as the thorax and the head had less affinity.

To Determine The Presence Of Milk Trace Drugs In Animals Exposed To Drugs.

In order to rule out the presence of pharmacological traces in milk from animals exposed to spray baths with two types of pharmacological combinations (treatments), a chromatographic study of the animals under study was carried out, revealing the following results:

Table 8. Liquid chromatography method for the determination of drugs in milk.

Sample data							
Type: Raw Milk			Quantity: 1500 ml		Packaging: Plastic, closed.		
Resultados							
Parámetros	**Métodos**	**A2LA**	**SAE**	**Unidad**	**Resultados**	**LOQ**	**LOD**
*Amide	Journal o International Vol. 88 NO.2.2005 (modified) Instrumental Method GC-MS/MS			Mg/kg	ND	0,01	0,08
*Fipronil	Journal o International Vol. 88 NO.2.2005 (modified) Instrumental Method GC-MS/MS			Mg/kg	ND	0,01	0,08
*Cipermetrine	Journal o International Vol. 88 NO.2.2005 (modified) Instrumental Method			Mg/kg	ND	0,01	0,08

	GC-MS/MS						
*Dichlorvos	Journal o International Vol. 88 NO.2.2005 (modified) Instrumental Method GC-MS/MS			Mg/kg	ND	0,01	0,08

Chromatographic Result For Drug Combination 1 (T2).
The first pharmacological combination used in bovine bath by spray for tick control, presented two pharmacological components, which were Amitraz (Amidine) and Fipronil (Phenylpyrazoles).

From the total of samples examined (60), there was 0% presence of residues of these drugs in the milk analyzed, since the concentrations detected do not show a reading of the doses used for the spray bath that can be secreted by galactophore.

Chromatographic Result For Drug Combination 2 (T3)
For the second pharmacological combination used in the spray bath in bovines for tick control, three pharmacological components were used, which were Cypermethrin (Pyrethroid), Dichlorvos (Phosphorus organ) and Fipronil (Phenylpyrazoles).

In the studied samples (60), there was a 0% presence of residues of these drugs in the raw milk samples, since the presented values do not exceed the reference values (0.01-0.008), so we can conclude that the dose used for the spray baths, does not secrete these components by galactophore way.

CONCLUSIONS

It was determined that the most effective treatment with external antiparasitic action was the pharmacological combination 2 (Cypermethrin, Dichlorvos, Fipronil). In addition to this, we were able to catalogue the presence of ticks of the genus (Boophilus microplus) as endemic, in farms in the parish of Regulo de Mora.

In the farms of the Regulo de Mora parish, corresponding to the San Miguel de Bolivar canton, a genus of ticks (Boophilus microplus) was found, presumably due to the low altitude of the pastures, since the Boophilus microplus tick has an affinity for grasses and low vegetation typical of this parish. The anatomical region of preference of the Boophilus microplus tick was established in the members of the rear train, adding this phenomenon to the muscular development and vascularisation of the anatomical area.

It was determined that no residues of Amitraz, Fipronil, Dichlorvos and Cypermethrin were found in raw milk from the animals subjected to spray baths during this investigation.

BIBLIOGRAPHY

- Alvarado, R. (2014). Evaluación de la efectividad de la Cipermetrina, Deltametrina, fipronil y triclorfon como antiparasitarios externos en cuyes. Tesis. Ing. Zootecnista. ESPOCH. Riobamba. 69-70pp.
- Casas E, Trigueros A, Chávez A, Tang J, Ruiz F. (2019). Tratamiento y control de garrapata Boophilus microplus, a través de la combinación de fluazurón/ fipronil pour on, en bovinos de trópico, Pucallpa, Perú. 44-45 pp.
- Castillo, C; Pinedo, R; Rodríguez, L; Chávez, A. (2016). Evaluación de Tres Formulaciones Comerciales de Aplicación Pour-on Bajo Condiciones de Campo y su Efecto in vitro en el Control de Boophilus microplus (Acari: Ixodidae) en Bovinos de Ceja de Selva. Lima, PE. Revista de Investigación Veterinaria. Vol. 27. P 145-147.
- Corcia A, Nazzari M. (2002) Liquid chromatographicmass spectrometric methods for analyzing antibiotic and antibacterial agents in animal food products. Journal of Chromatography A 974:53-89

- Díaz R. (2014). Mecanismos moleculares y bioquímicos de resistencia a acaricidas en la garrapata común de los bovinos Rhipicephalus microplus. Rev Colomb Cienc Anim 5: 72-81pp.
- Miller, T. (2018). Mechanism of resistance to pyrethroid insecticides. Parasitol Today 4,8-12.
- Naushad, M. Rizwan, N. (2014). Ultra Performance Liquid Chromatography Mass Spectrometry. Evaluation and Applications in Food Analysis. B/W Illustrations. ISBN 9781466591547
- Pereira, R. (2019). The efficiency of avermectins (abamectin, doramectin and vermectin) in the control of Boophilus microplus, in artificially infested bovines kept in field conditions. Buenos Aires. 34-35pp
- Sumano, L y Ocampo, C. (2014). Farmacología Veterinaria. 4ed. MacGraw-Hill Interamericana. México D.F. México, p 481-482
- Toriz, Ch. 2014. Antiparasitarios usados en Medicina Veterinaria y Zootecnia. Actividad de apoyo a la docencia. Piretrinas y Piretroides. Pág. 159.
- Wood IB, Amaral NK, Barden K, Duncan JL, Kassai T, Malone JB, Pankavich JA, et al. I(1995). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.). Second edition of guidelines. New York. 67 pp.
- Woodward, K. 2014. Veterinary Pesticides. En: Marrs TC (Ed.). Mammalian Toxicology of Insecticides. Cambridge, Royal Society of Chemistry Publishing, 348-42