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Sustained Release of Ivermectin in Cattle Blood Measured by High Performance Liquid Chromatography Through Time and Its Efficiency Against the Cattle Tick *Rhipicephalus (Boophilus) Annulatus* (Acari: Ixodidae)

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Abstract: Ticks and tick-borne diseases cause great economic losses to livestock in tropical countries and subtropical regions including Egypt. The current study was carried out in order to determine the suitable time of repeating injection of ivermectin according to its actual concentration in blood that could be sufficient to kill the adult stage of *Rhipicephalus (Boophilus) annulatus* that reappear on cattle after injection and to follow up the changes of liver and kidney enzymes post injection. A total of 25 infested cattle with *R. (B) annulatus* ticks of different ages and sexes were selected randomly then they were injected sub subcutaneously in the neck region with commercial formulation of ivermectin at the rate of 200µg/kg body weight. Treated cattle were inspected daily for observing the disappearance of ticks; moreover, serum samples were collected at days 2, 5, 18 and 28 post injection for determination of the level released ivermectin in blood by high performance liquid chromatography (HPLC). In addition, some biochemical parameters of blood were estimated in collected serum samples post injection. The obtained results revealed minor fluctuation of serum ivermectin level over time with mean 11.25±0.57 ppb until the day 18 post injection, while it could not be detected at the day 28 post injection. According to the obtained results, it was concluded that single ivermectin injection at rate of 200µg/kg body weight could give protection over than 3 weeks post injection and so it is advisable to repeat injection after 21 days not after 15 day as frequently used in veterinary practices, moreover, ivermectin injection caused no any adverse effects on liver and kidney as well as on oxidant/antioxidant homeostasis during the period of its release in the blood. Also, it was found that application of HPLC for determination of micro amount of ivermectin in blood samples was simple, rapid and relatively cheap and efficient method.

Key words: Injectable Acaricides · HPLC · Oxidative Status

INTRODUCTION

Rhipicephalus (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *annulatus* ticks are an endemic pest of cattle in tropical and subtropical regions of the world, causing major economic losses to cattle producers through direct physical effects on the parasitized animal and indirectly through transmission of infectious disease agents such as *Babesia bovis*, *B. bigemina* and *Anaplasma marginale* [1, 2]. In addition to the costs of chemicals, labor, equipment and production losses associated with treatment, the cost of maintaining tick-free zones and boundaries is highly expensive [3]. In Egypt, *R. (B). annulatus* tick infestation is considered a cause of major concern as it is a vector for babesiosis and borreliosis [4].

Chemical acaricides have played an essential role in control of this ticks, but intensive use of acaricide has favored the development of resistant populations [5]. Non-chemical control alternatives include the use of resistant cattle breeds, biological control and vaccines. However, the most widely used method is the application of different chemical classes of acaricides and macro cyclic lactones. Macro cyclic lactones (MLs)

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have emerged as an alternative to mitigate the negative effects of ticks, including tick populations resistant to most acaricides [6]. Mls are endectocides are derived from *Streptomyces avermitilis* (avermectins), *S. cyaneogriseus* (milbemicins) and the genus *Saccharopolyspora* (Spinosyns). Because of their chemistry and mode of action, broad spectrum of activity and efficacy at extremely low dosages, they represent an important class of compounds for control and management of arthropod pests of livestock including ticks [7]. Numbers of analytical methods have been reported for the determination of ivermectin in human and animal biological fluids as well as tissue organ extracts including enzyme-linked immunosorbent assay (ELISA) [8], immunobiosensor [9], Thin layer chromatography (TLC) [10] and high performance liquid chromatographic techniques (HPLC) with ultraviolet [11]. HPLC method is a simple, sensitive, selective, reproducible, accurate and suitable to the determination of micro-amount of ivermectin in plasma and serum on human and animals [12, 13]. The tick larval stage has a minimum period of 5-7 days, during which the larvae become engorged with blood, after which they molt to the nymphal stage which has a minimum period of 9-11 days. So to ensure complete destruction of all stages of ticks on animals, any used chemical acaricides should be repeated at least after 14 days [14].

Therefore, the current study was aimed to determine the time required to repeat injection of ivermectin according to it actual concentration in blood which is sufficient to kill adult stage of ticks that reappear on animal after injection and to follow up the changes of liver and kidney enzymes post injection.

MATERIALS AND METHODS

Study Area: The present study was carried out in Behera Province, Western Egypt.

Animals under Investigation: A total of 25 infested Egyptian cattle by *R. (B). annulatus* ticks of different ages and sexes were selected randomly from individually owned cattle. All animals were injected sub subcutaneously in the neck region with commercial formulation of ivermectin (*Ivomec super*^{R} *Merial Saude Animal Ltda*) at the rate of 200µg /kg body weight. Cattle inspected daily for the disappearance of ticks especially at the days 2,5,18 and 28 post injection.

Collection of Blood Samples: Blood samples were collected aseptically from treated cattle and serum was separated immediately. Collection of serum samples was done at the day 2 post injection to determine the initial level of ivermectin in blood, at the day 5 post injection to determine the maximum level of ivermectin in blood according to manufacturer recommendation and at days 18 and 28 to follow up the level of ivermectin through the time. Each serum sample was divided in to 2 parts; the first was send to Drug Residue Laboratory, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt for determination of micro amount of ivermectin in serum samples by HPLC and the second part was sent to the central Laboratory, Faculty of Veterinary Medicine, Damanhur University for biochemical examination. Every five serum samples of the same ages and sex were pooled together to form one sample, that will be analyzed by HPLC.

Determination of the Level of Ivermectin in Serum Samples: It was done by using HPLC [12]. Ivermectin in the five serum pools was extracted with ethyl acetate after the serum protein was precipitated with 0.05% met phosphoric acid-methanol in the ratio of 7:3 (V/V). Then the sample was centrifuged at 4000 rpm for 5 minutes and the supernatant was evaporated to dryness with rotary vacuum evaporator. The residue was dissolved with 0.20 ml of methanol as the sample solution for HPLC analysis. HPLC column used was a phenomenex C18 (5 microm, 250 mm x 4.6 mm) with a same type of guard column. Mobile phase consisted of methanol and water in the ratio of 90:10 (V/V) and the flow rate was 1.0 ml/min. The detection wavelength was 245 nm. Standard ivermectin solution was adopted from Merial Saude Animal Ltda.

Follow up the Changes of Liver and Kidney Functions Post Injection of Ivermectin: Parameters related to liver, kidney functions as well as related to oxidative status were analyzed by commercial kits following manufacturers' instructions. UNICO 2100 UV-Spectrophotometers, Elx800 Absorbance Micro plate Reader and other laboratory equipment aids were used for biochemical analysis.

Statistical Analysis: All statistical analyses used ANOVAs and were conducted with the SPSS 10.0 (SPSS Inc., Illinois, USA) computer program. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Ticks are hematophagous arthropods and obligatory ectoparasites which are considered significant in both veterinary and medical field by acting as vector of serious pathogens such as *Babesia, Thileria, Rickettsia, Anaplasma* and *Borrelia*. When these obligatory ectoparasites attach to a host for the aim of blood sucking, they induce skin irritation and anemia. Moreover, ticks are responsible for serious economic losses of animal owners particularly in livestock industries [15].

Visual Inspection of Treated Cattle with 200µg /K Body Weight Ivermectin: At the day 2 post injection, ticks began to disappear in all treated cattle and by the day 5 post injection, ticks completely disappeared from animals and they remained free until the third week post injection then ticks began to reappear again in some animals. The observed result agreed with that reported by Miller and Oehler, [16] and Cruz *et al.* [17] who reported that the maximum effect of ivermectin was observed at the first 3 week post injection.

Determination of Ivermectin in Serum Samples by HPLC: Serum pools collected at the day 2 post injection showed an initial ivermectin concentration of 10.981, 10.977, 10.619, 10.910 and 10.712 ppb that was insufficient to destroy all ticks. While at the day 5 post injection, ivermectin concentration appeared to reach a plateau of 11.170, 11.180, 11.507, 11.052 and 11.135 ppb that was sufficient to destroy all ticks and ticks completely disappeared. The detected level of ivermectin in examined samples could be taken as a standard level for ivermectin in blood that sufficient to control ticks as it persisted in blood. By following up serum ivermectin level at the day 18 post injection, it was recorded that ivermectin concentration levels in examined serum pools were 11.104,11. 071, 11.075, 11.022 and 11.015 ppb that indicated minor fluctuation than the level recorded in the day 5. This result indicated that single ivermectin injection at rate of 200µg/kg body weight could give protection for about 3 weeks post injection. On contrary, estimation of ivermectin serum levels at the day 28 post injection showed zero ppb in examined serum pools indicating that ivermectin was completely metabolized and become unable to give protection. The detected serum ivermectin levels at all measurements were compatible with visual inspection of treated cattle that was supported by reappearance of ticks as soon the level of serum ivermectin declined. These results were similar to those reported by Miller *et al.* [18]. Also, these results clarified that the use of HPLC was efficient, simple, rapid and relatively cheap method for determination of micro amount of ivermectin in serum samples that was supported by Pietruk and Jedziniak [19].

Biochemical Analysis: The obtained data revealed that, the changes in hepatic and kidney functions and oxidative status were insignificant. These reflect the safety uses of IVM. Our results disagree with Selvakumar *et al*. [20] and Ashang [21], who reported that, ivermectin injection elevate serum urea and ALT. This discrepancy might be due to changes in species or the dose used.

Table 1: Serum concentration of ivermectin measured by HPLC (ppb)

Serum pools	2nd day post injection	5th day post injection	18th day post injection	28th day post injection
1	10.916	11.180	11.104	Not detected
2	10.610	11.170	11.071	Not detected
3	10.769	11.507	11.135	Not detected
4	10.981	11.180	11.075	Not detected
5	10.977	11.201	11.055	Not detected
Mean ± SE	10.85±0.57b	11.25±+0.57a	11.09±0.51a	Not detected

Value carrying the same letter are not significantly different, Significant difference at level P<0.05

Table 2: Effect of IVM on biochemical parameters related to liver and kidney functions and oxidative states.

Item	Control	5th day post injection	18th day post injection	28 th day post injection
Total protein	7.16±0.41^{ab}	8.12±0.39^{a}	6.81±0.3^{ab}	6.38±0.14^{b}
Albumin	2.22±0.05^{b}	2.06±0.09^{b}	2.39±0.09^{b}	2.97±0.06^{a}
Globulin	4.94±0.45^{ab}	6.07±0.37^{a}	4.42±0.3^{bc}	3.41±0.14^{c}
A/G ratio	0.46±0.05^{bc}	0.36±0.02^{c}	0.58±0.04^{b}	0.89±0.04^{a}
Urea	62.36±1.02^{a}	50.87±2.14^{b}	67.86±1.87^{a}	67.74±2.12^{a}
GPT	36.67±2.33a	35±2.35a	28.25±1.11b	28±2.1^{b}
GSH	65.67±2.91^{a}	68.8±3.04^{a}	62.5±3.77^{a}	60.8±1.69^{a}
MDA	92±1.73^{a}	86.8±2.78^{a}	82.25±4.33^{a}	81.2±3.69^{a}

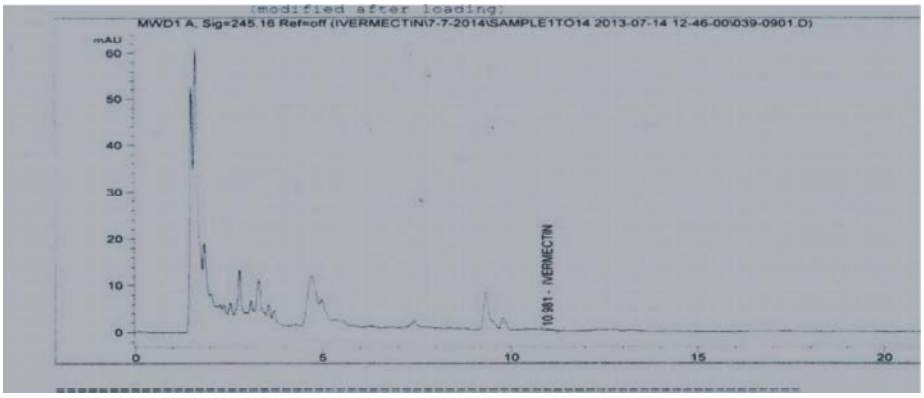

Fig. 1: Concentration of ivermectin in samples collected at the 2nd day post injection (10.981 ppb). *mAU* = milliabsorbance units

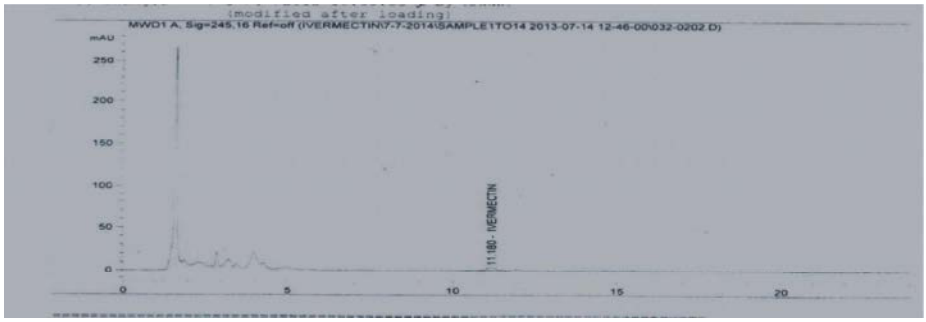

Fig. 2: Concentration of ivermectin in samples collected at the 5th day post injection (11.180 ppb)

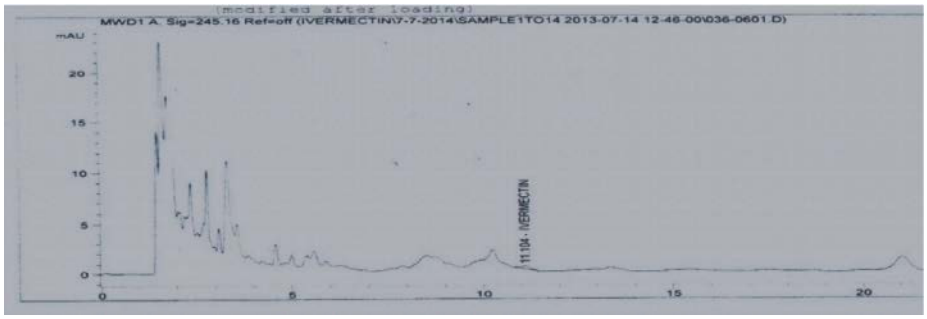

Fig. 3: Concentration of ivermectin in samples collected at the 18th day post injection (11.104 ppb)

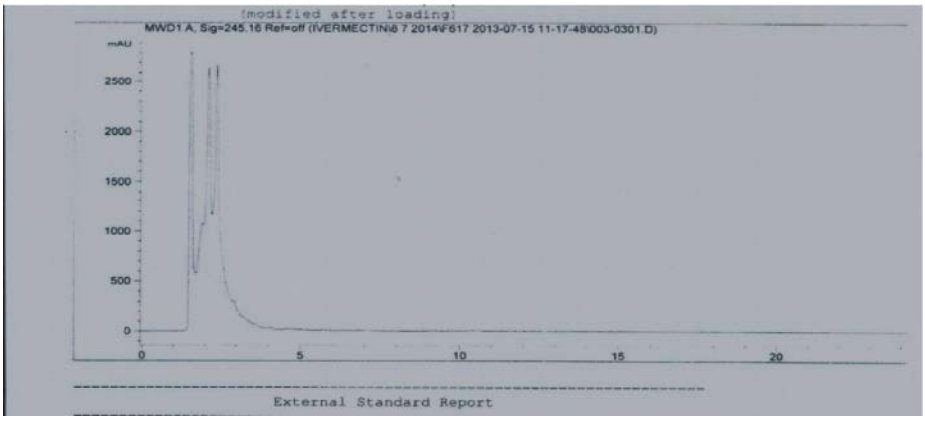

Fig. 4: Concentration of ivermectin in samples collected at the 28th day post injection (not detected)

CONCLUSION

In conclusion, obtained results could make a useful contribution towards preventing ticks in cattle. Ivermectin injection is efficient means for tick control and according to serum levels and the effective level is ranged from 11-11.507 ppb and it should be repeated after 3 weeks. Also, HPLC method is a simple, sensitive, selective and reproducible for determination of ivermectin in serum samples of cattle. Interestingly ivermectin safe and has no adverse effect on liver and kidney functions as well as oxidant/antioxidant homeostasis if injected and repeated by recommended doses.

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