Field trial for control of resistant ticks in cattle population in Beni-Suef Governorate

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Abstract

A survey focusing on the resistance of four types of acaricides (organophosphates (OP), synthetic pyrethroids, macrocyclic lactones (ML) and ivermectin. Engorged females of ***Boophilus annulatus*** were collected from naturally infected cows where farmers had reported concerns about resistance to or failure of tick control. They subjected to in vitro resistance detection assays using the adult immersion test (AIT). An existing questionnaire including breed, tick resistance and insecticide profile (type, frequency, application, response) was recorded. Results revealed that out of 370 cattle examined, 208 (56.22 %) were found to be infested with ticks. The identification of ticks revealed that (83.0%) was ***Boophilus annulatus***, while the percentage of ***Hyaloma anatolicum*** was (5.77%). According to site of infestation, results indicated that the highest percentage was found in perineal region 65%, followed by head–neck 58 %, on udder46% and on other parts of body 7%. The analysis of the data showed that the acaricide formulations had different efficacies on the tick populations surveyed. Ivermectin presented the highest mean efficacies according to the AIT (99.74 ± 1.43). Meanwhile, deltamethrin presented intermediate efficacy (77.20 ± 15.53) Organophosphate (diazinon and malathion) showed the lowest efficacy levels observed in AIT (67.10 ± 4.70 and 61.23 ± 6.83, respectively) From this study it can be concluded that in vitro sensitivity of ticks must be carried routinely to ensure efficient use of insecticides. Adult immersion test is simple and reliable technique to identify tick resistant. Finely, ivermectin treatment in combination with regular spraying of animals and their surrounding environment is cost effective for control of ticks.

Key words: *Ticks, B.annulatus , cattle, resistance, insecticides .*

Introduction

Tick infestation is a major threat to profitable livestock production and among hard ticks**.** ***Rhipicephalus microplus*** is a major species responsible for significant losses to the cattle industry **(Ram *et al*., 2004** and **Jonsson, 2006)**. Ticks not only lead to anemia by feeding on blood of its host but also transmit various diseases (**Teglas *et al*., 2005)**. The most quantitative assessments of the economic losses of ticks and tick-borne diseases are from studies of ***Boophilus microplus***. These losses have been expressed either in terms of grams of live weight gain or milk production lost per tick engorging (for example 0.7g /tick) or in terms of total average financial loss (production losses plus cost of control) for animal per year (e.g. US$7.3/ head /year). The most important method of control for this tick is use of synthetic acaricides. For the application of acaricides several methods exist: dusting, hand spraying, mechanical spray race, hand dressing, systemic acaricides (pour-on, injection), and dipping **(Koney, 2004)**. However, uncontrolled applications of commercial acaricides may have accelerated the emergence of ticks resistance to several active ingredients available. Resistance is usually first recognized as a failure of treatment to eliminate tick burdens from cattle by insecticide when it is used at the recommended concentration and according to all of the recommendations for its use **(Luciana *et al.,* 2011)**. The resistance of ticks to acaricides possess an increasing threat to

livestock production in many countries because of their almost complete dependence on acaricides for tick control. Resistance has led to instability and increased costs in areas where the one host cattle ticks ***Boophilus microplus*** and ***B. decoloratus*** have acquired resistance to a variety of toxic chemicals. The point has now been reached where such resistance must be expected in these ticks within five to 10 years of the introduction of any new type of acaricide, unless control practices are changed. In Africa, resistance could ultimately lead to a much greater dislocation of the cattle industry than elsewhere because of the more serious problems caused by the three-host ticks ***(Rhipicephalus, Amblyomma*** and ***Hyalomm***) and the diseases they transmit. Many methods were developed for diagnosis of acaricides resistance in ticks and the most reliable and simplest one is the adult immersion test with some slight modification to reduce contamination between different concentrations. Many cattle smallholder and farmers in Beni-suef district have reported the failure of tick control in their herds and have requested assistance in choosing more effective pesticides. Thus, the objective of this research was to evaluate the susceptibility of field specimens of ***Boophilus annulatus*** to the acaricides used in the control of the parasite.

Materials and Methodes

Study area and period: This study was conducted in Beni-Suef district during the period from March to July 2012. A total of 370 cattle irrespective of their age, sex and breed were surveyed and examined for tick prevalence.

Questionnaire: Smallholder and farmers were interviewed to obtain information about history of tick infestation, acaricide of choice, source of insecticide, route of application and frequency, how to calculate the concentration of recommended dose and tick resistance. Information were gathered verbally from each subject after explaining the purpose of the research and then recorded on the questionnaire. The use of OP compounds was more frequent in agricultural fields and the same insecticides were generally used for tick control. The use of acaricides was comparable in all the areas surveyed and expressed as the mean percentage of farmers using tick control as 3.7–18.0 % (routine treatment), 36.8–65.4% (occasional treatment) and 24.5–42.0% (no treatment). The farmers using acaricides only when they found ticks on their animals and largely contributed to the "no treatment" group. The mode of application was spray and injectable route. Amongst the respondents 34% used pyrethroids, 22% ivermectin, 28% organophosphates during the last five years.

Collection and Preparation of Ticks.

Two stages stratified sampling methods were adopted to collect live engorged females of ***B. microplus*** ticks from animals and from the sheds. Animals were clinically inspected for tick infestation. Adult ticks with a length equal to or greater than 4 mm were collected from naturally infected cows had a history of insecticidal resistance with maximum of three ticks / animal, none of which had received tick treatments for at least 30 days. Ticks were placed in identified plastic flasks free of acaricide residues with lids containing small holes. They were transported to the Animal Health Laboratory within 36 hours of collection for the evaluation of the in vitro efficacy of acaricides through the adult immersion test (AIT). Only samples with no oviposition were analyzed. In the laboratory, all females were separated, carefully washed and then dried on filter paper. Engorged females weighing between 160 and 300 mg with no signs of injury, were subjected in the tests.

Acaricides: The following commercial acaricides were purchased and stored at 4°C; (Diazinon 60 % EC, Alstomect –(Alstore) UK) diluted with distilled water 0.1 % ;Malathion 57 % EC, diluted with distilled water 0.1 %, Butox (50% deltamethrin, Intervet Schering-Plough Animal Health) at a final concentration of 0.0005% and Ivomec injection for cattle (ivermectin 10 mg/ml, Merial). These products were commercially available and used in accordance with the manufacturer's recommendations and following the commercial dosages of active ingredient used by farmers.

Adult Immersion Tests: The Adult immersion test was conducted according to **Drummond *et al*. (1973)**. Each group of 10 females was immersed for 10 minutes in 30 ml of the acaricide solutions. Commercial formulations of diazinon, malathion, deltamethrin were diluted in distilled water according to the label recommendations. For the evaluation of the avermectins (ivermectin) the females were immersed for 30 minutes in 30 ml of the solutions prepared according to the method proposed by **Sabatini *et al*. (2001)**. All solutions described were kept under constant agitation during the immersion period. Because of the large number of engorged females, only the control was prepared in triplicate and the field populations were analyzed with two replicates. Each replicate was performed with ten ***B. microplus*** females with weights as homogenous as possible. The females of the control groups were immersed in distilled water for 30 minutes. After immersion in the pesticide solutions or distilled water, all the engorged females were fixed with two-sided adhesive tape in petri dishes (90 mm diameter), which were identified by group, weight, pesticide, and testing date. The petri dishes containing the engorged females were incubated in a climate-controlled chamber at 27 ± 1°C and relative humidity of 85 ± 5%. After the oviposition period (14–18 days), the eggs were weighed and transferred to test tubes (150 mm in height × 15 mm in diameter closed with cotton balls). The identified tubes were placed in the climate-controlled chamber under the same temperature and relative humidity conditions described previously, where they remained until the larvae hatched. The hatching rate of the B. microplus larvae was based on visual estimation of the percentage of unhatched eggs remaining and of egg shells from which larvae had hatched.The effectiveness of the acaricides was determined by the effect on the estimated reproduction (ER) of the engorged females in a treatment group. The percentage control (% control) of ER afforded by the acaricides was determined by comparing the total ER of all treatment groups with that of the control group according to Drummond *et al*. (1973), calculated with the following formula.

$$ER = \frac{\text{Egg weight x \% hatched x } 20{,}000}{\text{Engorged female weight}}$$

Boophilus annulatus populations presenting a control percentage greater than or equal to 95% to the acaricide were considered susceptible. In vitro efficacy of 95% is the threshold for the approval of an acaricide product in Brazil according to federal regulations **(Luciana *et al*., 2011)**.

Data Analysis:
Data obtained were statistically computized using **SPSS (2006)**.

Field trial for control of ticks

Study area, period and animals: A three months study was carried out in a private farm at Beni-Suef region in the period from July to October (2012). The total number of dairy cattle (Friesian and Hybrid breed) in the farm were containd one hundred eighty six. They had previous history of tick resistance to different types of approved insecticides. The selection criteria were based on the owners' willingness to participate in the study. Animals were kept in 8 partially sheltered yards with earthy floor with minimal levels of hygiene or information related to appropriate measures of tick control. All the adult animals in the herds were qualitatively examined for the tick infestation. Each animal was examined for presence of ticks on the head, ears, neck, belly, back, legs, perineum and tail. Thirty animals found positive for tick infestation were tagged and separated from the rest of the herd. The tick infestation and distribution were determined quantitatively **(Urquhart *et al*., 1996** and **Sajid *et al*., 2009)**. Animals were randomly assigned into three equal groups (A, B and C) for therapeutic trial then transferred separately into three previously prepared shelters with earthy floor. Animals selected based on history of tick resistance. The age was one year and there was no history of application of acaricide since 30

days.

The treatment trial was conducted as described by **Sajid *et al.*, (2009)** with some modifications. Animals in groups A & B were treated once with Ivermectin 1% (Ivomec- a product of Marial , Private Limited) given subcutaneously at the rate of 0.2 mg/Kg body weight at the beginning of trial with recommended dose (1 ml / 50 kg S/C). Moreover, animals in group (A) had suitable hygienic measures besides they were sprayed with their surrounding environment by deltamethrin at recommended concentration (0.1 and 2 % respectively) in days 0, 28, and 56. Animals in group (C) were served as control and not received any treatment.The percent efficacy of ivermectin was calculated at days 1, 29, 57 and 75 post-treatment by using following formula.

Data analysis

The prevalence of ticks was determined using the following formula **(Thrusfield, 1995)**

$$\text{Prevalence} = \frac{\text{No. of existing cases during specified period x 100}}{\text{Population at risk during that specified time period}}$$

$$\text{Efficacy \%} = \frac{\text{No. of animals cured x 100}}{\text{No. of animals treated}}$$

Results and Discussion

In the surveyed areas , the housing condition were mostly bad (73.3%), this means that they do not even adopt to minimal standard in hygiene and all of the ground surface were clay (100%) that reflected on the hygiene status. Through the questionnaires survey, it could be observed that Butox acaricides were used in most of the properties (87.3%). Substitution of acaricide bases did not occur in systematic form in all farms and when it occurred, the reason given was mainly price and ease of purchase of the acaricides in the local market. The frequency of treatments to control the cattle ticks was weekly in 43.7% of the farms and the method of acaricide application most often used was spraying on the animals with manual pack pumps.

Table (1). The prevalence and frequent distribution of tick infestation in examined animals.

Breed	Cattle		Distribution (%) of tick infestation		
	Examined	Infested	B. annulatus	H. anatolicum	Mixed infestation
Friesian	273	187 (68.5%)	156 (83.4%)	8 (4.28%)	23 (8.42%)
Balady	97	21 (21.65%)	17 (80.95%)	4 (19.05%)	-
Total	370	208(56.22%)	173 (83.0%)	12(5.77%)	11.06(2.98%)

Table (2). The logistic regression module to demonstrate the association between the prevalence of ticks and some factors.

Factor	SE	OR	95% CI
Breed	0.22	1.59 *	(1.22 - 2.08)
previous history of tick infestation	1.90	7.31	4.39-12.7
Housing condition	1.30	4.21*	(3.19-9.17)

SE: Standard Error, OR: Odd ratio, CI: Confidence Interval.

* Indicated OR > 1 and could be considered a risk factor.

Table (3). Efficacy of different acaricides based on populations of ***Boophilu annulatus*** collected from naturally infested animals.

	Tick wt. (mg)	Min. efficacy (%)	Max. Efficacy (%)	Mean efficacy (% ± SD)
Ivermectin (1%)	135.6 ± 3.6	86.21	100	99.74 ± 1.43
Deltamethrin	135.2 ± 5.2	51.35	100	77.20 ± 15.53
Diazinon (60 %)		61.22	76.84	67.10 ± 4.70
Malathion (57%)	129.6 ± 7.0	48.35	70.5	61.23 ± 6.83
Control	126.4± 5.4	0 ± 0	0 ± 0	0 ± 0

Table (4). The efficacy of different treatments on tick infestation in the examined groups (A, B &C)

Group (n=10) Days	A	B	C
1	0 (0 %)	0 (0 %)	10 (100%)
29	0 (0 %)	1 (10 %)	10 (100%)
57	0 (0 %)	3(30 %)	10 (100%)
75	1 (10 %)	8 (80%)	10 (100%)

Table (1) revealed that the prevalence of tick infestation was reported to be 208 (56.22%) out of 370 animals included in the study. Nearly similar percentage of Ixodid tick infestation in cattle was recorded by **Kamal (2012)** during months of June and July (58.33 % and 62.00 respectively). Moreover, results revealed that ***B.annulatus*** was the predominant spp., affecting cattle (83.0 %) followed by mixed infestation by (***B. annulatus and H. anatolicum***) and ***H. anatolicum*** (2.98%and 5.77 % respectively.,) which was in accordance with higher percentage (86.84 %) recorded by **Kamal (2012)** in Beni-Suef region followed by ***H. annaloticum*** exacavatum (5.66%).

Regarding the predilection sites of tick's infestation in different body parts of examined animals, results indicated that the highest percentage was found in perineal region 65%, followed by head –neck 58 %, 46 % on udder and 7% on other parts of body. **Kamal (2012)** reported that the neck and dewlap of infested cattle were the most predeliction sites of ***B.annulatus*** ticks followed by thigh region, under the tail and the back region.

Regarding the risk factors such as breed, previous history of tick infestation and housing conditions (Table 2) showed a highly significant in tick's prevalence ($p < 0.01$) in Beni-Suef districts. To quantify these relationships, the logistic regression model was adopted. The result showed that breed, previous history of tick infestation and housing conditions could be risk factors for presence of tick infestation (OR = 1.59, 7.31, 4.21 respectively). Results revealed that Friesian breed, animal with previous history of insecticidal resistance and poor housing conditions were the highest risk factors for tick infestation. Our findings on the general spectrum of ticks were similar to those of other authors **as Jonsson *et al*., (2000).** Considering previous studies on tick infestation in domestic ruminants and results of current study, unless adequate and sustainable tick control measures are put in mind. Tick infestation will remain the main drawback to livestock industry in Egypt. Lack of tick control measures on examined farm and the poor animal husbandry practices may be potential factors for the observed high tick loads. Moreover, the limited scope of awareness regarding the impact of ticks, non-existence of acaricide

uses and lack of adequate veterinary infrastructure in the area may also account for the widespread existence of tick species in the area.

Table (3) revealed that the mean efficacy of the acaricides and their respective standard deviations against ***B.annulatus*** collected from naturally infected cows. Ivermectin, presented the highest mean efficacies according to the AIT (99.74 ± 1.43), Meanwhile, deltamethrin presented intermediate efficacy (77.20 ± 15.53). Organophosphate (diazinon and malathion) showed the lowest efficacy levels observed in AIT (67.10 ± 4.70 & 61.23 ± 6.83 respectively). These results augmented by **De Castro (1997), Luciana *et al*.(2011)** and **Campos and Oliveira,(2005)**. On the other hand, **Luciana *et al*. (2011)** recommended the AIT proposed by **Drummond *et al*. (1973)** for the evaluation of the susceptibility to acaricides in cattle tick populations, since it is fast, simple and a relatively cheap method for the detection of acaricide resistance in the field .The uncontrolled use of these products in the study area may promote the complete failure of tick control within a short period of time **(Jonsson and Hope 2007)**.

Table (4) showed that the post-treatment quantitative assessment of tick burden revealed that the average number of ticks in the control group (C) remained constant. Meanwhile. following Ivermectin treatment in groups (A & B) on day 1, tick count decreased from 100% to (0%), Meanwhile at days 29, 57 and 75, in group (A) tick counts were (0, 0 & 10 % respectively). While in group (B) tick counts were (10, 30 & 80 % respectively). Nearly similar findings were obtained by **Soil *et al.* (1989). Miller *et al*. (1997)** who concluded that the most important aspect of this study is that it provides a model for development of other sustained release delivery systems using macrocyclic lactone compounds.

From this study. it can be concluded that in vitro sensitivity of ticks should be carried routinely to ensure efficient use of insecticides. Adult Immersion test is simple and reliable technique to identify tick resistant and finely ivermectin treatment in combination with regular spraying of animals and their surrounding environment is much cost effective for control of ticks.

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محاولات حقلية للسيطرة على قراد الأبقار المقاوم للمبيدات الحشرية في محافظة بني سويف.

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الملخص العربى

أجريت دراسة للسيطرة على قراد الأبقار المقاوم للمبيدات الحشرية في محافظة بني سويف مع التركيز على وجود مقاومة لأربعة أنواع من مبيدات القراد (الفوسفات العضوية (OP)، البيريثرويد الاصطناعية (SP)، ما كروسيكلكmacrocyclic (ML) و (بالإيفرمكتين) . تم جمع الإناث معظمهم من بو أفلس انيولاتس B.annulatus من الأبقار المصابة طبيعيا حيث وجد مقاومة القراد لبعض انواع من المبيدات الحشرية و بتعرض هذا القراد لفحوصات المختبر في الكشف عن ظاهرة المقاومة باستخدام اختبار الغمر للكبار (AIT).من خلال دراسة 370 من الماشية وجد ان 208 (56.22٪) مصابة بالقراد . وتحديد القراد كشفت أن (83.0٪) كان بو أفلس انيولاتس ، في حين بلغت نسبة هيالوما اناتولكم H.anatolicum (5.77 ٪). ووفقا لموقع الإصابة على الحيوان أشارت النتائج إلى أن تم العثور على أعلى نسبة كانت على منطقة العجان 65٪، تليها 58٪ على الرقبة والرأس، و46٪على الضرع وغيرها 7٪. تحليل البيانات أظهرت أن مبيدات القراد لها تأثيرات مختلفة على تجمعات القراد وفي الوقت نفسه أظهرت فعالية متوسطة دلتامثرين قدم (77.20 ± 15.53) الفوسفوري (دياز ينون ومالاثيون) تركيبات مختلفة . كان على تجمعات القراد التي شملها الاستطلاع. آيفرمكتين، قدم أعلى كفاءة وفقا لAIT (99.74 ± 1.43)، ومستويات أقل فعالية لوحظت في AIT (67.10 ± 4.70 و 61.23 ± 6.83 التركيب.) من هذه الدراسة يمكن أن نستخلص أنه ينبغي القيام بإجراء إختبار الحساسية للقراد في المختبر بشكل روتيني لضمان الاستخدام الفعال للمبيدات الحشرية، (اختبار الغمرللكبار) هو تقنية بسيطة وموثوق بها لتحديد مقاومة القراد للمبيدات الحشرية كما وجد ان العلاج بالإيفرمكتين مع الرش بالمبيدات الحشرية للحيوانات والبيئة المحيطة بها فاعلية من حيث الكفاءة من أجل السيطرة على القراد وبتكلفة مناسبة .