

***In vitro* efficacy of diazinon and amitraz on *Boophilus decoloratus* tick at Sebeta Awas district, Ethiopia**

Siede Gashaw¹, Alemayehu Regassa¹ and Yeshawork Begashaw^{2*}

College of natural and computational science, Hawassa University, School of Veterinary Medicine, P. O. Box 05, Hawassa, Ethiopia

National Animal Health Diagnostic and Investigation Center, P. O. Box 04, Sebeta, Ethiopia.

*Corresponding author email: yeshadessie@gmail.com

Abstract

This study was conducted in Oromiya region, western Shewa Zone, at Sebeta Awas district, Ethiopia to determine the *in vitro* efficacy of Amitraz 12.5% and Diazinon 60% against *Boophilus decoloratus* using adult immersion test. A total of 180 engorged adult female ticks of *B. decoloratus* were collected from local cattle under extensively managed herds and immersed in Amitraz 12.5% and Diazinon 60% at field recommended concentration and in distilled water for control groups for 1 minute and then incubated at $27 \pm 1^\circ\text{C}$ and relative humidity of 85% for 7 days. The oviposition response of *B. decoloratus* in both groups were observed at regular interval. The mean mass of eggs laid by *B. decoloratus* of the treated group and those of untreated groups was compared to estimate the efficacy of each tested acaricide. Thus, *B. decoloratus* treated with Diazinon 60% at field recommended concentration died and did not lay eggs while some of *B. decoloratus* which were treated with Amitraz 12.5% at field recommended concentration survived and as a result eggs were found in seven days incubation time. There was a statistically significant variation ($p < 0.05$) between the two acaricides in the overall oviposition control of *B. decoloratus* tick species. On the other hand those treated with water as a control group were able to survive and lay many eggs. Diazinon 60% at field recommended concentration was better in overall mean percent control (C%=100) than Amitraz 12.5% (C%=98.27) of *B. decoloratus* at field recommended concentration. The results of the study suggested that both Amitraz and Diazinon provide higher oviposition inhibition on *B. decoloratus* tick. Further study in relation with the *in vivo* trial is recommended.

Keywords: Acaricides; *Boophilus decoloratus*; Efficacy; *In vitro* testing

Introduction

Ixodidae Ticks are very important vectors for transmission of many bacterial, viral and protozoan pathogens of human, livestock and wild animals (Estrada-Peña *et al.*, 2004). Tick infestation presents a serious challenge to farmers of ruminants in both developed and developing countries (Jongejan and Uilenberg, 2004). Ticks which are considered to be most important to the health of domestic animal in Africa comprise about seven genera. Among these the main tick genera found in Ethiopia includes *Ambylomma*, subgenus *Rhipicephalus* (*Boophilus*), *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* (Walker *et al.*, 2003). Among 60 tick species found infesting both domestic and wild animal of Ethiopia, 30 species have been widespread and are important parasites of livestock and cause significant economic losses to the livestock industry (Gebre *et al.*, 2004). *Boophilus decoloratus* is a one-host tick, which is indigenous to Africa and presumably evolved as a parasite of ungulates in East Africa and may have found its way south with the migration of indigenous tribes and their livestock. Cattle are its main domestic hosts although heavy infestation may also occur on horses and wild ungulates. Other domestic animals appear to be much less important as hosts (Mekonnen *et al.*, 2002). *Boophilus decoloratus* showed no marked preference for any particular attachment site and it completes the parasitic phase of its life cycle within three weeks on the same host. The short life cycle allows the tick to pass through several generations in one year (Norval, 1994).

In tropical Africa, tick and tickborne diseases are economically very important diseases next to trypanosomosis (Tiki and Addis 2011). *Boophilus decoloratus* transmits *Babesia bigemina*, which causes red water in cattle (Heyne, 1986). As *B. decoloratus* is a one-host tick, it can be effectively controlled by three-weekly acaricide treatment of cattle. Zebu cattle develop a considerably better host resistance to this tick than European breed cattle and require fewer acaricide treatments (Norval, 1994). This tick species is important not only as vectors of various pathogens but also because it quickly develops resistance to a wide range of acaricides. The development of resistance in this species was usually the main reason for the introduction of new acaricides (Walker, 1991).

Ethiopia, despite owning the largest livestock number in Africa due to disease derived by microbial and parasitic agents, food shortage, less productive local livestock breeds, poor management system (Zijlstra, 2015), the livestock sector is not contributing to the economy of the country as expected. The contribution

of livestock to the national economy with regard to foreign currency earnings through exporting live animal, meat, offal, partially and completely finished leather and leather products is significant (CSA, 2013). Ticks harm the hosts both directly and indirectly. Direct harm results from blood loss, damage to hides and skins, serving as route for secondary infection, production of toxin and causing paralysis (Walker *et al.*, 2003). Indirectly, ticks cause economic loss by playing an important role as vectors for transmission of several tick-borne diseases (TBDs), reduced productivity and fertility, anemia and death (Eyo *et al.*, 2014).

The aim of tick control campaign is not to control all ticks simultaneously, but a definite species because of its particular role (FAO, 2004). The currently available tools for tick control consists of acaricides relying on treatment with different application methods and/or formulations, tick resistant animals, tick vaccines, TBDs vaccines and management interventions. The successful implementation of rational and sustainable tick control program in grazing animals is dependent up on a sound knowledge of the ecology or epidemiology of the parasite as it interacts with the host in specific climatic, management and production systems (Walker, 2011). In most situations, however, efficient and reliable control of ticks and TBDs are still based primarily on intensive use of acaricides, often without the local understanding of those responsible factors for tick distribution dynamics (Brito *et al.*, 2011). Tick treatment relying on different application methods have been the main method of tick control in Africa, leading to numerous problems; environmental pollution, development of resistant tick strains and escalating costs (Walker, 2011).

Resistance is generally first recognized as failure of a drug to control parasitism but the formal definition of resistance is a shift in the target species susceptibility to a drug (Sangster, 2001; Corley *et al.*, 2013). World Health Organization Scientific Group (1965) has developed the definition of resistance in broad terms as “the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject”. Such a general definition could be accepted as a basis for discussions on acaricide resistance (Rao *et al.*, 2014).

To alleviate these problems, the most frequently used techniques to detect resistance in cattle tick are the adult immersion test (AIT), larval packet test (LPT), and larval immersion test (LIT). However, for the success of any tick

management strategy, it is necessary to use a test that is practical, quick, economical and reliable to detect presence of resistance in target population (FAO, 2004). The escalation of acaricide resistance in ticks has encouraged the establishment of different acaricide resistance testing methods. The commonest reason for controlling ticks on livestock is to prevent and control important tick borne diseases such as anaplasmosis, babesiosis, cowdriosis and theileriosis.

Some livestock herds may be subject to serious threat from two or more of these diseases and this has led to substantial use of tick control systems, mostly relying on chemicals that kill ticks. This has contributed greatly to development of the livestock industry in Africa (Latif and Walker, 2004). Likewise, in Ethiopia, over the past decades ticks are mainly controlled by using variety of acaricides; including organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids. However, with the most widespread, under or over concentration and frequent use of organochlorine and organophosphate compounds, ticks are likely to develop resistance in Ethiopia (Mekonnen, 2001). Continuous studies on dynamics of tick population with the efficacy status of acaricides against the most abundant and important tick species in particular area are necessary to carry out efficient tick control and/or tick burden reduction (Gebre, 2001). Hence the objective of the present study was to evaluate the efficacy of Amitraz (12.5%) and Diazinon (60%) acaricides against adult engorged female ticks of *B. decoloratus* under *in vitro* condition.

Materials and methods

Study area

The study was conducted from November 2016 to April 2017 at Sebeta located in the Oromia special zone, Oromia region, Ethiopia Addis. Sebeta is located at 25 km south west of Addis Ababa at a latitude and longitude of 8°54'40"N and 38°37'17"E, respectively. It has an elevation of 2356 m.a.s.l and has annual mean rain fall range from 860 to 1200 mm. The mean annual minimum and maximum temperature is 8°C and 19°C, respectively (CSA, 2011). In and around Sebeta town, different species of livestock are kept. The farming system is a mixed crop-livestock production system where draft-oxen are used for plowing to produce crops. The livestock population of the district are 6395, 1702, 1123, 1157, 922 2016, 6520 cattle, sheep, goat, horse, mule, donkey and poultry respectively (Sebeta Awas Agricultural Office, 2016).

Study population

Local cattle found in and around Sebeta town were target population. Animals were selected based on the following selection criteria: on their infestation level with engorged adult female ticks and those cattle which did not receive acaricide treatment one month before the trial. Totally from 40 cattle herds, 120 cattle were selected for female adult engorged tick collection. All animals were kept under extensive management system.

Sample size and sampling method

For the Adult immersion Test, the general practice has been to use a minimum of 10 engorged female ticks for each acaricide and the same for controls, but 20 ticks for each trial would be preferred (FAO, 2004). Thus, for this test a total of 180 engorged adult female *B. decoloratus* ticks with no signs of injury and color changes were collected and three groups of twenty ticks were randomly formed (Group I for Amitraz, Group II for Diazinon and Group III for control/water). This set up was then covered and incubated in an incubator at temperature of 27°C±1 and relative humidity (RH) maintained at 85%. Three replicates were used for each acaricide.

Acaricides

The choice of acaricides used was based on their commercial availability and patronage by farmers, veterinary clinic and drug stocks at Sebeta Awas districts. According to the information obtained from the farmers, the most frequently used acaricide were Amitraz 12.5% and Diazinon 60%. Amitraz 12.5% was manufactured by Adami tulu pesticides processing Share Company (Ethiopia) and Diazinon 60% was manufactured by KAFR EL ZAYAT pesticides and chemicals Company Egypt. These acaricides were stored and used according to the manufacturers' guideline.

Study methodology and design

The study areas and herds with high tick infestation were selected and ticks were removed carefully by hand from the body sites. All visible and fitted engorged adult female ticks for trial were collected from 40 naturally infested local cattle herds. From each herd, 3 or 4 cattle, based on their infestation level with engorged adult female ticks, a total of 120 cattle were selected. At each

collecting site, cattle were restrained, and adult engorged female ticks of any species (as it is difficult to identify the tick species at collection site) were collected from the sampled cattle (Ducoenez *et al.*, 2005).

Adult immersion test (AIT)

The AIT was used to test fully engorged females, of size greater than 4.5 mm (Drummond *et al.*, 1973), under standard laboratory conditions of 28°C and 85% relative humidity. Acaricidal products containing the following active ingredient as the single Amitraz (12.5%) and diazinon (60%) were tested separately using the AIT.

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. Each test was performed in duplicate with 20 females per group, with an average tick weight of 250 mg. The ticks were immersed for 1 minute in the solution containing the acaricide diluted in distilled water, after which they were dried with absorbent paper and transferred to Petri dishes.

The indicated concentration for Diazinon is 0.06% while that of Amitraz is 0.025%. The formula, $V_1C_1 = V_2C_2$ was used to prepare the concentration of acaricides, where V_1 and V_2 are the volume of the acaricide to be drawn from the stock product and the final volume after reconstitution, respectively, C_1 and C_2 are the stock product concentration and the required final concentration after preparation, respectively. For all the preparations, the final volume was 1000 ml.

A controlled bioassay design was used to evaluate the efficacy and egg laying suppression effect of Amitraz and Diazinon against *B. decoloratus* under *in vitro* condition. The FAO (2004) modified protocol for the AIT and as suggested by (Drummond *et al.*, 1973) was used to conduct bioassay using a commercial acaricides at the recommended field concentration by the manufacturer.

To estimate the efficacy of each acaricide, both groups (treated and control) were then tested using the egg laying test (ELT) method (Drummond, *et al.*, 1973 and modified by FAO, 2004) which involves the comparison of the egg mass of ticks treated with acaricide and the egg mass of untreated ticks and finally estimates the percentage control value, using the following formula:

Percent control = $(\frac{MEC - MET}{MEC}) * 100$

MEC

Where, MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively.

Data management and analysis

All collected data were entered into Microsoft Excel 2007. Statistical analyses were carried out using SPSS version 20. Data were analyzed using descriptive statistics in the first step; Percent control (%C) obtained with Egg Laying Test (ELT) for each acaricide were used to evaluate its effectiveness whereas, independent sample t-test was used to examine mean Percent Control between acaricides. A p-value less than 0.05 at 95% confidence intervals was considered as significant.

Results

The oviposition response of female engorged *B. decoloratus* after immersion in Amitraz 12.5% and Diazinon 60% in three replicates is presented in Table 1.

Table 1. Mean oviposition of engorged female *B. decoloratus* after immersion in manufacturers' recommended concentration of Amitraz 12.5% and Diazinon 60% EC after seven days of incubation.

NT	Tt	NIFT	EW	NTS	Av.EMPTG	PC
1	Diazinon (60%)	20	4.90	0	0	100
	Amitraz (12.5%)	20	4.95	10	0.010	98.72
	Control (water)	20	4.80	20	0.781	0.00
2	Diazinon (60%)	20	5.05	0	0	100
	Amitraz (12.5%)	20	4.92	9	0.018	98.19
	Control (water)	20	4.85	19	0.995	0.00
3	Diazinon	20	4.80	0	0	100
	Amitraz	20	5.01	8	0.022	97.90
	Control	20	4.95	20	1.050	0.00

NB: NT (No of trial), Tt (Treatment), NIFT (Number of immersed female ticks), EW (Engorgement weight (gram)), NTS (Number of ticks survived after 7 day incubation, Av.EMPTG (Avg.egg mass per treatment group (gm) and PC (Percent control (%C)

In the trial, none of *B. decoloratus* treated with Diazinon laid eggs, while few *B. decoloratus* treated with Amitraz laid small batch of eggs with mean weight of 0.017 gm. There was a statistically significance variation ($p < 0.05$) between the two acaricides in the overall oviposition control of *B. decoloratus* tick species (Table 2).

However, *B. decoloratus* tick species in the control group laid relatively large batch of eggs with mean weight of 0.942 gm. The overall mean %C of Amitraz and Diazinon, and their respective standard deviations as well as their minimum and maximum mean efficacy during the three replication of the trial is presented in (Table 3). Diazinon 60% showed evidence of better effect on oviposition of *B. decoloratus* ($p < 0.05$).

Table 2. T-test analysis of mean percent *B. decoloratus* oviposition control between Amitraz 12.5% and Diazinon 60% EC at recommended concentration.

Ticks	Acaricide	Mean %	N	SD	t-value	Df	p-value
<i>B. decoloratus</i>	Diazinon 60%	100	3	0	4.43	4	0.0114
	Amitraz 12.5%	98.27	3	0.667			

N=Number of trial; %C=Percent control; SD=Standard Deviation; df=difference

Table 3. Overall mean percent oviposition control of Amitraz 12.5% and Diazinon 60% EC at field recommended concentration and their standard deviation against adult female *B. decoloratus*.

Acaricide	minimum efficacy	maximum efficacy	mean efficacy \pm SD
Diazinon 60%	100	100	100 \pm 0
Amitraz 12.5	97.19	98.72	98.27 \pm 0.677

Discussion

Several authors have studied the efficacy of Amitraz and Diazinon on different tick populations using AIT. The results showed different susceptibility levels. In most of the studies, Amitraz revealed high degree of tickicidal efficacy that agreed with the present finding. Similarly, a closely comparable finding, %C of 98 to 100, was reported by Mekonnen *et al* (2001) at Sebeta, Ethiopia. In Wolaita and Dawuro zone, Ethiopia, Asha and Eshetu (2015) also reported mean efficacy of 91.79% for Amitraz at the same dilution rate. In South Africa, Mekonnen *et al* (2002) also reported 100% efficacy for Amitraz. Moreover, Souza *et al* (2003) in southeast Brazil and Eshetu *et al* (2013) in Borena, Ethiopia

obtained mean Amitraz efficacy of above 95%. On the other hand lower efficacy was reported by Mendes *et al* (2001) who showed an average efficacy of 77.4% and Furlong *et al* (2007) reported efficacy of 47.9% for Amitraz. In northeast region of Brazil, a low efficacy of Amitraz, with efficacy of 48.4% and 30.9% was reported by Brito *et al* (2011) and Campos and Oliveira (2005), respectively.

In most of the studies, Diazinon revealed lower degree of tickicidal efficacy than Amitraz. However, it is higher in the present finding which is 100%. Eshetu *et al* (2013) in Borena, Ethiopia obtained mean Diazinon efficacy of about 80.1%. In Wolaita and Dawuro zone, Asha and Eshetu (2015) also reported mean efficacy of 65.3% for Diazinon. There is no high efficacy variation between the two presently tested acaricides in this study. But there is a slight variation in oviposition response. In the trial, none of *B. decoloratus* treated with Diazinon (60%) laid eggs, while few *B. decoloratus* treated with Amitraz (12.5%) lay small batch of eggs.

The use of acaricide at inappropriate concentration is one of the prime factors which affect the efficacy of an acaricide and contributes to failure of tick control. During this study, farmers complained of failure of acaricides to kill ticks after being treated and reported that the acaricide was not working. Especially, this coincided with the period during which heavy tick burdens were present on cattle. *In vitro* laboratory tests, however, indicated that those acaricides that were mostly used for tick control in the study area had high acaricidal efficacy of 98.3% for Amitraz and 100% for Diazinon. The result in this study is suggestive of good performance of both acaricide against *B. decoloratus* and the reason for the complaint by the livestock keepers in the area could be due to faulty preparation, dilution, storage and application of acaricides in accordance with the manufacturer's recommendation

Conclusion

The current study revealed that Diazinon 60% at field recommended concentration provides relatively a higher oviposition response inhibition of *B. decoloratus* than amitraz 12.5% at field recommended concentration. In the present study, the two acaricides have conserved their efficacy on *B. decoloratus* tick species in the study area. Further tests using different tick species and other efficacy evaluation methods involving larval and lymphal stages as well as *in vivo* efficacy trial at field level should be conducted to assess the residual ef-

fect of these acaricides. Awareness creation to farmers on the proper usage of acaricides is also recommended

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Asha, A. and Eshetu, E., 2015. *In vivo* and *in vitro* acaricidal efficacy evaluation on cattle ticks in selected areas of Wolaita and Dawuro zones, Ethiopia. *J. Biol.Agr. Healthcare.*, 5(18), 116-122.
- Brito, L.G., Barbieri, F.S., Rodrigo, B., Rocha, M.C., Oliveira, S. and Elisana, S., 2011. Evaluation of the efficacy of acaricides used to control the cattle tick, *Rhipicephalus microplus*, in dairy herds raised in the Brazilian southwestern Amazon. *Vet. Med. Int.*, 201(6), 2-13.
- Campos, D.A., and Oliveira, P.R., 2005. *In vitro* valuation of acaricides efficiency to *Boophilus microplus* (Canestrini, 1887) Acari: Ixodidae) from bovines at the region of Ilh'eus, Bahia, Brazil. *Cienc. Rural*, 35(6), 386–392.
- Corley, S.W., Jonsson, N.N., Piper, E.K., Cutullé, C., Stear, M.J. and Seddon, J.M., 2013. Mutation in the Rm -AOR gene is associated with amitraz resistance in the cattle tick *Rhipicephalus microplus*. Academic Science. USA.
- CSA, 2011. Federal Democratic Republic of Ethiopia, Central Statistical Agency, Agricultural Sample Survey Report on Livestock and Livestock Characteristics. Volume II, 2007/2008 Central Statistical Agency, Addis Ababa, Ethiopia.
- CSA, 2013. Federal Democratic Republic of Ethiopia, Central Statistical Authority, Agricultural sample survey (2012/2013), Report on livestock and livestock characteristics (Privet and Peasant Holdings), Addis Ababa, Pp. 9-20.
- Drummond, R.O., Ernest, S. E., Triveno, J.L., Gladney.W.J. and Graham, O. H., 1973. *Boophilus Anulatus* and *Boophilus microplus* laboratory tests for insecticides. *J. Economic entomol.* 66, 130-133
- Ducoenez, S., Barré, N., Miller, R. J. and de Garine-Wichatitsky, M., 2005. Diagnosis of amitraz resistance in *Boophilus microplus* in New Caledonia with the modified Larval Packet Test. *Vet. Parasitol.*, 130, 285-292.
- Eshetu, E., Dinede, G., Matios, L. and Tolosa, T., 2013. *In vitro* efficacy evaluation of amitraz 0.025% and diazinon 0.06% against *Rhipicephalus pulchellus* and *Ambly-*

- omma gemma* in Borena pastoral area, Southern range land of Ethiopia. *J. Parasitol. Vector Biol.*, 5(6), 72-76.
- Estrada-Pena A., Bouattour, A., Camicas, J.L. Walker, A.R., 2004. Ticks of domestic animals in the Mediterranean region: a guide to identification of species. University of Zaragoza, Spain
- Eyo, J.E., Ekeh, F.N., Ivoke, N., Atama, C.I., Onah, I.E., Ezenwaji, N.E. and Ikele, C.B. 2014. Survey of Tick Infestation of Cattle at Four Selected Grazing Sites in the Tropics. *Global Vet.*, 12, 479-486.
- FAO, 2004. Acaricide resistance: diagnosis, management and prevention, in Guidelines Resistance Management and Integrated Parasite Control in Ruminants, Animal Production and Health Division, Agriculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy, Pp. 25-77.
- Gebre, S., 2001. Problem of Acaricide resistance for the control of ticks. Proceeding of the Ethiopian Society of Animal production 9th Conference.
- Gebre, S., Mekonnen, S., Kaaya, G.P., Tekle, T. and Jobre Y., 2004. Prevalence of Ixodid ticks and Trypanosomosis in camels in Southern Ethiopia. *Ethiop.Vet. J.*, 8 (2), 23-24.
- Heyne, H., 1986. Differentiation of *B. decoloratus* and *B. microplus*. *J. South Afri. Vet. Assoc.*, 57, 251-252.
- Jongejan, F. Uilenberg, G., 2004. The global importance of ticks. *Parasitol.*, 129, S3-S14.
- Latif, A.A. and Walker, A.R., 2004. An introduction to the biology and control of ticks in Africa Pp. 15-17.
- Mekonnen, S., Bryson, N.R., Fourie, L.J., Peter, R.J., Spickett, A.M., Taylor, R.J., Strydom, T. and Horak, I.G., 2002. Acaricide resistance profiles of single- and multi-host ticks from communal and commercial farming areas in the Eastern Cape and North-West Provinces of South Africa. *Onderstepoort J. Vet. Res.*, 69, 99-105.
- Mekonnen, S., Hussein, I. and Bedane, B., 2001. The distribution of ixodid ticks (Acari: Ixodidae) in central Ethiopia. *Onderstepoort J. Vet. Res.*, 68 (4), 243-251.
- Mekonnen, S., 2001. *In vivo* evaluation of Amitraz against ticks under field conditions in Ethiopia. *J. S. Afr. Vet. Assoc.* 72 (3), 44-45.
- Mendes, M.C., Verissimo, C.J. and Kaneto, C. N., 2001. Bioassays for measuring the acaricides susceptibility of cattle tick *Boophilus microplus* in São Paulo state. *Anim. Plant Sci.*, 68 (2), 23-27.

- Norval, R.A.I., Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., 1994. Infectious Diseases of Livestock with special reference to southern Africa, Volume 1. Oxford University Press, Cape Town. Pp. 3-24.
- Rao, Z. A., Muhammad, A. Z., Douglas, D. C., John G. and Zafar, I., 2014. Acaricide resistance in cattle ticks and approaches to its management: The state of play. College of Veterinary and Animal Sciences, Jhang, Pakistan. *Vet. Parasitol.* 203, 6–20.
- Sangster, N.C., 2001. Managing parasiticide resistance. *Vet. Parasitol.*, 12, 98(1-3), 89-109.
- Souza, A. P., Sartor, A.A. and Bellato, V., 2003. Efficacy of acaricides in dairy cattle herds in southern region of Parana state, Brazil. *Revista de Ciencias. Agrovet.*, 2 (2), 131-135.
- Tiki, B. and Addis. M., 2011. Distribution of Ixodid Ticks on Cattle in and Around Holeta Town, Ethiopia. *Global Vet.*, 7(6), 527-531.
- Walker, A.R., 2011. Eradication and control of livestock ticks: biological, economic and social perspectives. Royal (Dick) School of Veterinary Studies, University of Edinburgh, summer hall Place, Edinburgh EH9 1QH, UK. Cambridge University Press 2011. Pp. 945-959
- Walker, A.R., Bouattour, J.L., Camicas, A., Estrada-Pena, I.G., Horak, R.G., Latif, A.A. and Preston, P.M., 2003. Ticks of domestic animals in Africa: a guide to identification of species, *Biosci. report*, Pp. 1-221.
- Walker, J.B., 1991. A review of the Ixodid ticks (Acari: Ixodidae) occurring in South Africa. *Onderstepoort J. Vet. Res.*, 58, 81-105.
- Zijlstra, J., 2015. Business Opportunities Report Dairy #2 in the series written for the, Ethiopian Netherlands business event 5–6 November 2015, Rijswijk, The Netherlands.