ISSN: 1119-1104

CLINICAL EVALUATION OF NEEM-AZAL® 1% T/S AGAINST DOG TICKS AND FLEA INFESTATION IN JOS METROPOLIS NORTH CENTRAL NIGERIA

[*1]Dogo, G.I.A., [2]Weka, R.O., [2]Egwu, K. and [3]Hubertus, K.
[1]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria.
[2]Parasitology Division, National Veterinary Research Institute, Vom Plateau State, Nigeria.
[3]Trifoilio-M Lanau GmbH, Germany
Email: gonidogo@gmail.com

(Received 12th August 2013; Accepted 19th December 2013)

ABSTRACT
Dogs are the common successful canids and pet animals adapted to human habitation worldwide. They are usually infested with parasites that could harm their owners. The aim of the study was to clinically evaluate the effect of Neem-Azal® 1% T/S against Dog ticks and fleas infestation in jos metropolitan Veterinary Clinics and environs. A total of 36 days, infested with ectoparatites in six locations were physically examined and treated with Neem-Azal® 1% T/S. Out of a total of 877 ticks examined infesting dogs *Amblyomma lepidum* was 133(15%) and *Rhiphicephalus sanguineus* was 7444 (85%). The only Flea species encountered was *Ctenocephalides canis* 155 (100%). The geometric mean tick count reductions at Day 3, 10, and 21 were 45%, 65%, and 100%, respectively. For fleas at day 3 it was 100% and percentage of tick and flea-free dogs at Day 10 were 70% and 100% respectively and the differences were highly significant at Day 21 ($P<0.0001$) except dogs treated at ECWA Rural Veterinary Clinic group experienced ticks re-infestation 21 days post treatment ($P>0.05$). An improvement of the hair coat of all dogs treated with Neem-Azal® 1% T/S shampoo was observed and a dog that showed signs of myiasis was healed. The product is therefore recommended as a suitable routine treatment of Dogs against ticks and fleas infestation in Jos Metropolis of North-central Nigeria.

INTRODUCTION

Dogs are the common successful canids and pet animals adapted to human habitation worldwide (Ugbomoiko, 2008). Dogs have contributed to the physical, social and emotional well0being of their owners; particularly children (Dohoo *et al.*, 1998, Robertson *et al.*, 2008). The domestic dogs are also used as house-guards and for hunting in many parts of the world including Nigeria. In spite of the beneficial effects, close bonds of dogs and human, ectoparasites have remained a major threat to the health of dogs. Many arthropods, such as the ticks and fleas, live as bloodsucking ectoparasites on domestic dogs (Segun, 1998). These ectoparasites cause distress, severe dermatitis or act as vectors of various disease pathogens in domestic dogs, other animals and humans (Dagnone *et al.*, 2003; Gonzalez *et al.*, 2003; Molyneuz, 2004). In most household settings, treatments to eliminate these parasites are, if done at all, is often applied in advanced stages of disease, causing distress on pets and their owners (Irwin 2002; Morrison, 2001).

Therefore, ectoparasitic infestations in dogs have received much attention from several workers the world over (Gonzalez *et al.,* 2004, Durdeen *et al.,* 2007, Nithikathkul *et al.,* 2005). In Nigeria, a number of studies on ecroparasites of domestic dogs have been reported. For example, Chukwu (1985) reported that from some parts of the eastern region, 2.1% dogs had *Echidnophaga gallinacean* while 26.3% had *Ctenocephalides canis.* Ugochukwu and Nnadozie (1985) found that 30% of the dogs examined were infested by ticks, 27.6% by lice, 25.9% by fleas (*Ct. canis)* and 13.3% by mites. In the Jos Plateau area of northcentral Nigeria, James-Rugu and Iwuala (2002) reported that 65.7% of dogs had ticks including *Rhipicephalus sangujineus, Boophilus decoloratus, Haemophysalis leachii and Amblyomma lepidum* while 28.3% were infested with flea which were *Ct. canis* and *Xenopsylla cheaqpis.*

As blood is the sole feed of engorging ticks and lice, scientists have attempted to deliver acaricides systematically to ticks in tick-afflicted animals (Landau, 2009). Although synthetic organic pesticides appear to provide a solution to the problem of the ectoparasite control, it has become apparent that repeated application and excessive reliance on synthetic pesticides can be an inadequate method of control. Some of which is the appearance of increasing resistant strains of numerous pests, deleterious effects on the health and environment (toxic residues and adversely affecting targeted organisms), and at the same time such pesticides are expensive to export for developing poor countries with limited foreign currency (Hassall, 1990; Li *et al.,* 2003). Therefore, basic research has been directed at bio-active substance which has satisfactory properties concerning their effects on the target pests, not expensive to produce and environmental friendly. From accumulated research data, it is apparent that this goal will be achieved through the use of natural pesticides.

Recently, plant extract has proven effective in controlling many of serious pests especially in tropic and subtropics countries. Also they are biodegradable, effective at very low concentrations, very low in mammalian toxicity and potentially compatible with natural enemies. The use of plant species to control insect pests has been in practice for centuries to a limited extent. At present, agrochemical companies and research laboratories have been focused on the study of natural products for the development of new insecticides (Addor, 1995). Hence the discovery of active compounds that are more selective and less persistent and prevent ectoparasite from developing resistance has been shown to be beneficial for both the environment and agriculture product consumers, although natural products cannot automatically be assumed to be without risk.

One group of compounds that has demonstrated significant toxic effects on some pests of modern man have been discovered in the Neem tree (*Azadiracta indica).* Neem-based products from *Azadiracta indica* have been successfully used for pest control in agriculture and gardening long ago in India and more recently in European countries and in the USA (Neem Foundation, 2007). The natural abundance, prolificacy and traditional use of neem in Asia and Africa for medical, fertilizer and pest control purposes make it an ideal renewable resource for many and varied biotic agents which have been explored and continued to be detected in it.

The fruit and seed kernels, particularly rich in bioactive azadirachtin and other limonoids (tetranortriterpenoids), are the

main source for a large number of products used for the control of pest insects, mites, fungi and soil-borne nemabolites of the Neem tree exhibit a wide range of biological activities in insects. The inhibition of tick eggs production and embryogenesis in ectoparasites feeding and ovipositional deterrence, reduced fitness, with low toxicity and sterility in a number of species of *hemimetabolous* and *holometabolous* insects has been reported (Guerrini, 2000; Landau *et al.*, 2009). Furthermore, many arthropod ectoparasites are susceptible to both the insecticidal, growth regulatory and repellent activity of neem extracts (Laucantoni *et al.*, 2006). The complex neemlimonoid spectrum and its multiple mode of action also prevent ectoparasites from developing resistance.

Surprisingly, registered neem products for the control of pathogens ectoparasities or disease vectors affecting human and animal health are few. The moderate interest of chemical and pharmaceutical industries for developing neem-based products for human health stands in sharp contrast to the wide traditional use of neem preparations by populations of different cultures, India, in other Asian countries and Africa (Habluetzel, 1999). Evaluation of acadiractin on ectoparasites of animals have been scarce (Guerrini, 2000), hence this preliminary work was carried out to evaluate the potential effects of Neem-Azal 1% T/S, a natural pesticide and insecticide, developed by Trifolio – M GmbH, Germany from neem plant for the control of ectoparasites of pet animals in Jos, northcentral, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in Jos Plateau State which is located in Nigeria's middle belt, with an area of 26.899 square kilometers and an estimated population of about three million people. It is located between latitude 9^{o} 0' to 9^{o} 40' North and longitude 8^{o} 30' East of the equator. The altitude ranges from around 1,200 meters (about 4000 feet) to a peak of 1,829 metres above sea level in the Shere Hills range near Jos. Years of tin mining have also left the area strewn with deep gorges and lakes. Though situated in the tropical zone, a higher altitude means that Plateau State has a near temperate climate with an average temperature of between 18 and 22^{o}C. Harmattan winds cause the coldest weather between December and February. The warmest temperatures usually occur in the dry season months of March and April between 26 and 29^{o}C. The mean annual rainfall varies from 131.75cm (52in) in the southern part of 146cm (57in) on the Plateau. The highest rainfall is recorded during the wet season months of July and August (Odumodu, 1983)

Study Design

Preliminary field trials were conducted to clinically evaluate the efficacy of the plant extract Neem-Azal®1% T/S a Neem oil preparation, *(Azadirachta indica)* on dogs infested with ectoparasite (Ticks and Fleas) in Jos metropolis. A total of 36 dogs infested with ectoparasites in Jos metropolis in six settings namely, (Jos Veterinary Teaching Hospital (10 dogs), Ginza Veterinary Clinic (9 dogs), ECWA Rural Veterinary Clinic (5 dogs), Veterinary Clinic Vom (5 dogs), Households A (4 dogs) and Household B (3 dogs) were examined and treated.

Owners Consent

Ethically, the purpose and benefits of the trial were explained to the dog owners and their consent was sought before trials commenced. In addition, free wash as an incentive and the hope that the animals

would be free of tricks following application elicited maximum cooperation from the owners.

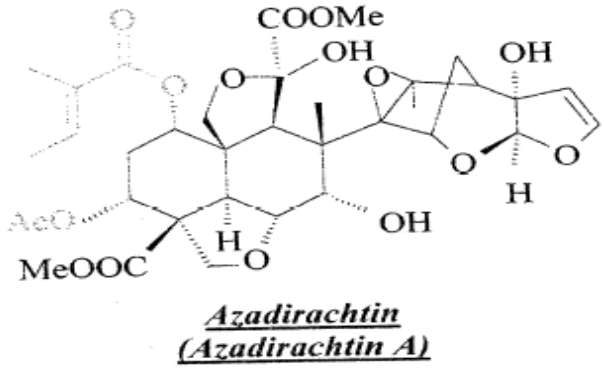
Ticks and Fleas Identification

Ticks were placed in petri dish containing 70% alcohol and examined using low power dissecting stereo microscope ZEISS Model, Stemi DV4 (Germany). Ticks were identified using taxonomic keys of morphological characters described by Walker *et al.*, (2003). Fleas were identified according to Soulsby, (1982).

Topical Treatment using Neam-Azal ® 1% -T/S

Tick infested dogs were visited over a period of three weeks, at weekly interval, on day 0, 3, and 10 consecutively according to manufacturer's instruction. On each occasion water was applied to the hair of the dog to make it wet, after which 20mls of the Neem-Azal® T/S shampoo (Fig.1.). The chemical structure of the active ingredient of the product is stable under field conditions (Fig. 2). The preparation was message thoroughly with gloved hands on the dorsal and ventral aspects of the dogs body avoiding the natural orifices (eyes, mouth, nose and anus) including the ear and inter digital spaces between the dogs claws. After 120 minutes, the body was rinsed with water and allowed to dry. In each of the pens visited, 60ml of Neem-Azal® 1% T/S was also diluted in 1 litre of water and sprayed on the walls of the pen and the pen was washed. For trials done in the Clinics, the dogs were washed on day zero and then they were followed up at home to be sure of the housing and kennels on days 0, 3, 10 and 3 weeks post treatment. The kennels and environment were also sprayed repeatedly with the preparation on each visit. Retention and repellency of ecoparasites was evaluated by counting the ticks present on the dogs on each visit and the percentage of reduction was calculated and recorded.

Fig.1. Neam-Azal ® -T/S Shampoo in 1liter, 250ml and 100ml container by *Trifolio-M Lanau GmbH, Germany*



Azadirachtin (Azadirachtin A)

Fig. 2. Chemical structure of active ingredient of Neem-Azal ®-T/S

Statistical Analysis

Statistical analysis was performed using the SPSS 17.0 statistical software (SPSS Inc. Chicago, IL, USA) to analyze the effect of Neem-Azal® 1% TS on the ectoparasites and a one-way ANOVA in order to establish their distribution in the localities. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The results of the clinical evaluation of Neem-Azal® 1% T/S in six locations in Jos metropolis are presented on Table 1. All dogs (100%) had at least one species of

ectoparasites viz; *Amblyomma lepidum*, *Rhiphecephalus sanguineus* and *Ctenocephalides canis* on their body.

This study clearly showed that both ticks and fleas live on the bodies domestic pets in Jos metropolis. This is in agreement with a survey conducted by Nithikathkul *et al.* (1996) were both fleas and ticks lived on the bodies of domestic dogs. The ticks observed on the dogs were ticks that belong to the general *Amblyomma* and *Rhipicephalus*. The numbers of *Rhipicephalus sanguineus* examined were more than *Amblyomma lepidum* ticks as presented in Table 1. The present study reiterate earlier reports that the brown dog tick (*Rhipicephalus Sanguineus)* is the most abundant and widespread tick in the World and a well-organized vector of may pathogens affecting dogs and occasionally humans (Gonzalez *et al.*, 2004; Nithikathkul *et al.*, 2005). It has been established that the tick is found on dogs living in both urban and rural areas, being highly adapted to live within human dwelling and being active throughout the year in tropical and subtropical regions (Dantas-Torres, 2010). *Ct. canis* was the only flea species found. This may be due to the dogs' surveyed being confined to local environment which are similar to findings reported by Nithikathkul *et al.* (2005). From this study, there were differences in the prevalence of ticks and fleas in each domestic dog which might be due to the differences in animal immunity or the different location, relative prevalence and density of ticks and fleas. |These differences might be due to the dissimilar temperature and/or moisture in each area that effect the growth and reproduction of ectoparasites and tick control strategies.

There was a significant drop on the number of ticks due to repeated application of the product as revealed by Dogo *et al.* (2011b). Interestingly, all dogs that had wound on the ear were healed within 7 days of application.

Studies are now available which confirmed the wound healing efficacy of Neem oil when applied on exposed skin parts (Dua *et al.*, 1995; Dogo *et al.*, 2011b). However, during the 21 days post application, all the four dogs at Household A and ECWA Rural Veterinary Clinic were heavily re-infested with ticks. This might be due to the fact the houses and environment in the study area were surrounded by thick bushes and tall grasses, where the ectoparasites may have been harbored and eventually resurfaced (Service, 1996; Gonzalez et al., 2003). This report indicate that strange as it seems (e. g, when you see a single dog infested with over a 100 ticks), most of the ticks are not on the dog but in the environment, where it is under direct influence of several biotic (e.g. predators) and a biotic (e.g., weather condition) factors. Furthermore, it was observed that houses with gardens were more suitable biotic for *R. sanguineus* (Shimada *et al.*, 2003). The dogs in ECWA Rural Clinic might also be infested with ticks during and following treatment. This could not be farfetched from the fact that during treatment the shampoo was diluted (60 ml in one litre of water) before each application on the dogs and kennel. Hence when one compares the result with all the other cases, the shampoo appears to be more effective in its concentrated form than the diluted form.

It also confirms that some dog breeds appear to be resistant than others (Louly, 2009) to infestations by *R. sanguineus.*

Table 1: Neem-Azal® 1% T/S trails from Day 0,3,10 and 21 on Dogs infested with ectoparasites in Jos Metropolis North central, Nigeria

Location	Number of Dogs	Ticks or Fleas Species seen	Number identified/ frequency (%)	Treatment day(s)	Efficacy (%)
Household A	4(All Male)	*Rhipicephalus*	102(11.6)	0,3,	45
		Sanguineus		10,21	100
		Ctenocephalides	45(29,0)	0, 3	100
		Canis			
Household B	3(Male 1 and Female 2)	*Rhipicephalus sanguineus*	15(17,1)	0, 3	85
Veterinary Clinic, Vom	5(Female 3 and Male 2)	*Rhipicephalus*	120(1.3.7)	10	100
		Sanguineus		0,3	70
		Ctenocephalides	33(21,3)	10	100
		Canis		0,3	100
Jos Veterinary Teaching Hospital	10 (Female 5 and Male 5)	*Rhipicephalus Sanguineus*	200(22,8)	3	65
				10	95
		Ctenocephalides	77(49,7)	21	100
		Canis		3	100
Ginza Veterinary Clinic	9(Male 6 Female 3)	*Rhipicephalus Sanguineus*	113(12, 9)	3	60
				10	100
		Ambyomma lepidum	78(8,8)	3,10	70
ECWA rural Veterinary Clinic	5(Male 1 Female)	*Rhipicephalus Sanguineus*	250(28.8)	0,3	20
				10	45
		Ambyomma Lepidum	45(5,1)	21	*0

***Ticks re-infestation 21 days post treatment suggestive of no residual effect on the ticks (P>0.05)**

These and other "on-host" ecological parameters can also vary according to diverse factors, at both population (e.g., dog population density and proportion of dogs treated with ectoparasiticides or tick repellants within a population) and individual levels (e.g., dog population density and proportion of dogs treated with ectoparasiticides or tick repellants within a population) and individual levels (e.g., dog population density and proportion of dogs treated with ectoparasiticides or tick repellants within a population) and individual levels (e.g., age, breed and lifestyles) (Dantas-Torres *et al.*, 2009; Tinoco-Gracia *et al.*, 2009). In South-eastern Brazil, the prevalence and mean density were much higher among dogs living in houses with grassy yards which is similar to these cases as compared with dogs in household B, Jos Veterinary Teaching Hospital, Vom Veterinary Clinic and Ginza Veterinary Clinic where there were no re-infestation post treatment. The surroundings of such buildings were cemented and bushes/grasses were very far and not cited near the areas.

It is also important to note that although some dog owners bathed their dogs with

acaricide (Diazinon and Coumaphos); however, those dogs were still infested with ectoparastites upon investigation. This possibly demonstrates that routine bathing with common acaricide leads to resistance and therefore cannot control the ectoparasitic infestations. Diazinon and Coumaphos are organophosphate acaricides, but some previous studies have shown the occurrence of resistance in some ectoparasites against them (Hassa11, 1990; Li, 2003).

In the course of this study, it was discovered that most dogs brought to the clinic on a daily basis with one form of complain or the other had tick infestation eith moderate or heavy with or without clinical signs. Those that were heavily infested had various kinds of skin infections, anemia and were debilitated (One of the dog that was severely emaciated and had icterus and died during the course of trial (Veterinary clinic Vom). This is common as compared with reports from other countries that several arthropods that lived as ectoparasite on domestic dogs can cause severe dermatitis or act as vectors of pathogenic agents, resulting in serious diseases in dogs especially life threatening anemia in young or debilitated animals (Araujo et al., 1998; Kordick et al., 1999). Nonetheless, most dogs with ectoparastic have no clinical symptoms. The findings in this study collaborates with Egbo *et al.* (2011) who reported the acaricidal activity of Neem-Azal 1% T/S washing emulsion in dog under field conditions in Zaria.

CONCLUSION

Neem-Azal® 1% T/S has demonstrated some repellent activities and is effective against ticks and fleas infestation on dogs' which may cause direct damage to the animal. This could probably be due to Neem-Azal® 1% T/S mode of action on eliminating target pests but does not harm the host because of the unique emulsifiable and spreading property. Use of natural additive gives Neem-Azal® 1% T/S the status of a total natural acaricide and the ability of this natural product to control arthropods at relatively low concentrations represents an alternative to the use of synthetic pesticide, chemical or at least a compliment in the control of disease and vectors. In addition to the very favourable toxicological properties, the healing properties; less expensiveness and readily availability of materials for pest control are amazing characteristics of this product. In conclusion, Neem-Azal® 1% T/S was effective against ectoparasites of dogs and is therefore recommended for use in Veterinary Clinics in northcentral Nigeria.

ACKNOWLEDGEMENT

Authors wish to thank the management of National Veterinary Research Institute, Vom and Prof. (Mrs.) L. H. Lombin (MFR) now Dean, Faculty of Veterinary Medicine, University of Jos for approving the grants for this study and to all staff of Parasitology division of National Veterinary Research Institute, Vom for their technical support.

REFERENCES

Addor, R.W. (1995). Insecticides In: Godfrey, C.R.A. (Ed), Agro-Chemicals from natural products. Marcel Dekker In. C., N.Y. (1-63).

Arauju, F.R., Silva, M.P., Lopes, A. A., Ribeiro, O. C., Pires, P. P., Carvalho, C. M., Balbuena, C.B., Villas, A. A. and Ramos, J. K. (1998). Severe Cat Flea infestation of dairy calves in Brazil. *Veterinary Parasitology.* 80: 83-86.

Chukwu, C. C. (1995). Prevalence of Fleas on Dogs in Anambra State of Nigeria. *International Journal of Zoonoses.* 12(3): 192-195.

Dagnone, A. S., Autran, H. S., Morais, M. C. Vidotto, F.S. and Jojima, O.V. (2003). Ehrlichiosis in Anaemic, thromnocytopenic or tick infested dogs from a hospital population in South Brazil. *Veterinary Parasitology*, 117(4): 285-290.

Dantas-Torres F., Melo, M.F., Figueredo, L.A. and Brandao-Filho, S.P. (2009). Ectoparasite Infestation on Rural Dogs in the Municipality of Sa'o Vicente Ferrer Pernambuco, Notheastem Brazil. *Review Brasilian Parasitology Veterinarinar*. 18: 75-77.

Dogo, G.I., Tanko, J., Salami-Shinaba, J.O., Onovoh, E.O., Shamaki, D., Agbede, R. I.S., Kleeberg, H. and Schwarz-Fiedlerv, S. (2011a). The Use of Neem-Azal® 1% T/S in Controlling Natural Fleas infestation on Kano brown goats in Vom, Nigeria. *Vom Journal of Veterinary Science*. 8: 49-53.

Dogo, G.I., Nnabuife, H., Kleeberg, H., Schwarz-Fieldlerv, D., Salami-Shinaba, J.O. and Agbede, R.I.S.(2011b). In-vitro acaricidal effect of Neem-Azal T/S 1% on *Boophilus decoloratus* Larvae. *Vom Journal of Veterinary Science*. 8: 21-24.

Dohoo, I.R., McDonell, W.N., Rhodes, C.S., Elazhary, Y. L. (1998). Veterinary Research and Human Health. *Canadian Veterinary Journal*. 39: 549-556.

Dau, V.K., Nagpal, B.N., Sarma, V.P. (1995). Repellent Action of Neem cream against mosquitoes. *Indian Malariology*, 32: 47-53.

Durdeen, L. A., Judy, T.N., Martin, J.E. and Spedding, L.S. (2005). Fleas Parasitizing Domestic Dogs in Georgia, U.S.A. Species Composition and seasonal Abundance Veterinary Parasitology. 130(1-2): 157-162.

Egbo, A. P., Agbede, R.I.S., Dogo, G.I., Tanko, J. T., Shamaki, D. and Kleeberg, H. (2011): Assessment of acaricidal activity of Neem-Azal® 1% T/S Washing Emulsion Using Dogs under field Conditions in Zaria. *Nigerian Journal of Parasitology*, 32(2): 239-245.

Garboui, S.S., Jaenson, T.G., Palsson, K. (2006). Repellency of Mygg A Natural Spray (Para-menthane-3, 8-diol) and RB86 (Neem Oil) against the tick *Ixodes ricinus* in the field in East-central Sweden. *Experimental and Applied Acarology*, 40: 271-277.

Gonzalez A., Dolores C., Castrol and Gonzalez, T. (2004). Ectoparasitic species from *Cantis familiaris* (Linne) in Buenos Aires Province, Argentina. *Veterinary Parasitology*, 120(1-2). 123-129.

Guerrini, V. H. (2000). Effect of Azadiractin. *Research*, 4: 133-138.

Hassall, K. A., (1990). The Biochemistry and uses of Pesticides. 2nd Edition. Macmillin Press Ltd., London, 536.

Irwin, P. J. (2002). Companion Animal Parasitology. Clinical Perspective. *International Journal of Parasitology*, 32: 591-593.

James-Rugu, N. N. and Iwuala, M.O.E. (2002). Ticks of Ngerian Livestock with different fur conditions and colour shades. *African Journal of Natural Sciences*, 3: 102-106.

Kordick, S. K., Breitschwedt, E.B., Hegarty, B.C., Southwick, K.L., Colitz, C.M. Hanock, S.I., Bradley, J. M., Rumbough, R., Mcpherson, J.T., MacCormack, J.N. (1999). Co-

infection with Multiple Tick-borne pathogens in a Walker Hound Kennel in North Carolina. *Journal of Clinical Microbiology*, 37: 2631-2638.

Li, A.Y., Davey, R.B., Miller, R.J. and George, J.E. (2003). Resistance to coumaphos and Diazinon in *Boophilus microplus* (Acari:Ixodidae) and evidence for the involvement of an oxidative mechanism. *Journal of Medical Entomology*, 40: 482-400.

Louly, C.C.B., Soares, S., Silveira, D., Neto, O., Silva A., Borges, L. (2009). Differences in the susceptibility of two breeds of dogs, English Cocker Spaniel and Beagle, to *Rhipicephalus sanguineus* (Acari:Ixodidae). *International Journal of Acarology*, 35: 25-32.

Landau, S.Y., Provenza, F. D., Gardner, D.R., Pfister, J.A., Knoppel, E.L., Peterson, C.C., Kababya, D., Needham, G.R., Villalba, J.J. (2009). Neem-tree (*Azadirachta indica*) Extracts a feed additive against the America Dog tick (*Dermacentor variabilis*) in Sheep (Ovvis Aries) Veterinary Parasitology. 165: 311-317.

Lucamtoni,L., Gisti, F., Cristofaro, M., Pasqualini, L., Esposito,F., Lupetti, P., Habluetze, A. (2006). Effect of Neem extract on blood feeding, Ovi-position and Oocyte Ultra-structure in *Anopheles stephensi* Liston Dipteria; Culicidae. *Tissue and Cell*, 38(6): 361-371.

Molyneux, D.H. (2004). 'Neglected' Diseases but unrecognized successes-Changes and Opportunities for Infectious disease control. *Lancet*, 364: 380-383.

Morrison, G. (2001). Zoonotic Infestion from Pets. Understanding the risk and Treatment. *Postgraduate Medicine*, 110: 24-26.

Walker, A. R., Bouattour, A., Camicas, J. L., Estrada-Pena, A., Horak, I. G., Latif, A. A., Pegram, R. G. and Preston, P. M. (2003). Ticks of domestic animals in Africa. A guide to identification of ticks on livestock in Africa. *ICTTD special publication* 231.