Acaricidal Efficacy of *Cassia sieberiana* (*In-vitro* and *In-vivo*) against *Sarcoptes scabieivar cuniculli* on Experimentally Infested Rabbits(*Oryctolagus cuniculus*) in Maiduguri, Nigeria.

Abstract

The present study discusses about Acaricidal Efficacy of *Cassia sieberiana* (*In-vitro* and *In-vivo*) against *Sarcoptes scabiei var cuniculli* on Experimentally Infested Rabbits (*Oryctolagus cuniculus*) in Maiduguri, Nigeria.Eighteen (18) Rabbits of both sexes and of post-weaned ages (8-10 weeks) were used for the study, the animals were purchased from a commercial rabbit breeder and on their arrival placed in a clean and well ventilated cages at large animal clinic Faculty of Veterinary Medicine, University of Maiduguri.The choice of the plant *Cassia saberriana* selected for the study is based on their current and past use in traditional medicine, local and traditional knowledge has been the starting point for many successful drug development projects in the past and such approach is generally based on a detailed observation of how local populations uses the plants. Further studies on Methanol extract and other solvents and parts of the plant such as the leaves, flowers and the pods should be tried to demonstrate the effectiveness of the plants medicinal used or in other organisms such as gastrointestinal helminths.

Keywords: gastrointestinal helminths, Acaricidal Efficacy, Veterinary Medicine, rabbit breeder

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Introduction:

Rabbit has emerged as a key livestock that is increasingly being raised by farmers in many parts of our country and the globe at large; this is because of its small space utilization and

use of fiber and domestic remnants from leafy vegetables (Kumar *et al*., 1998; Abdul R. and Ismail, 2008). However disease and inadequate technical knowledge amongst animal health providers on those diseases are the major challenge facing the sustainability of rabbit farming (Soundararajan *et al*., 2005).

Sarcoptes scabiei commonly known as itch mite is an ectoparasite that burrows into the skin and causes a disease commonly known as mange in animals and scabies in humans. Such animals affected include wild and domesticated ruminants, dogs, cats, apes, as well as laboratory animals including rabbits among others (Suchow *et al*., 2002).

The genus *Sarcoptes* is a part of larger family of mite collectively known as scab mites comprising of one specie *Sarcoptes scabiei* with further identification by variety of names indicating the host specie, e.g*S. scabiei var hominius* in humans and *S. scabiei var cuniculii* in rabbits. The complete stages of life cycle are found on the host, where the female oviposit into the tunnel made in the *stratum corneum* of the skin causing intense itchy skin rashes, hypersensitivity and inflammation (Jofre *et al*., 2009). The life cycle is 21 days but can be delayed duringextreme weather and infestation is by contact (Soulsby 1982; Hofing *et al*., 1994;Wardhana *et al.*,2006). Sarcoptic mange in rabbit has been described as an uncommon disease (Scott *et al*., 2001), however it has been reported from different countries (Radi *et al*., 2004). Wild animals have been reported to transmit sarcoptic mange in different animals including rabbits experimentally as well as naturally (Arlian *et al*., 1984; McCarthy *et al*., 2004 andHeukelbach *et al.*, 2005).

Due to resistance developed against synthetic acaricide, its toxicity and environmental contamination (Currie *et al*., 2004), there is need for exploration of plant extract with medicinal properties as alternate in treatment. More planthas been shown to possess acaricidal properties

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against ectopararites;(Crude *Aloe vera* gel, Oyelami *et al.*, 2009;Methanolic extract of *Vitex negundo* dried stem and leaves, Khan *et al*., 2012; *Cassia sieberiana*, Biu *et al*., 2013and Crude watery extract of *Onobrychis Ptolemaica,* Shahatha *et al.*, 2020;), thus a trial of *Cassia sieberiana* using a dusting technique of powdered extract will be evaluated on mite.

MATERIALS AND METHODS

Source of Experimental Rabbits;

Eighteen (18) Rabbits of both sexes and of post-weaned ages (8-10 weeks) were used for the study, the animals were purchased from a commercial rabbit breeder and on their arrival placed in a clean and well ventilated cages at large animal clinic Faculty of Veterinary Medicine, University of Maiduguri. They were routinely screened for ecto, endo and haemo-parasites using standard methods (Soulsby, 1982; Currie *et al*., 2004). They were suitably housed in cages provided with sawdust as bedding, feeding with variety of vegetable and commercial grower feed, and water were provided *ad libitum*. And they were allowed to acclimatize to laboratory conditions for two (2) weeks prior to commencement of the experiment. The experimental procedure was in accordance with ethical regulation committee of the Faculty of Veterinary Medicine, University of Maiduguri. At the end of the study period all infected rabbits will be immediately treated for sarcoptic mange.

Collection of mites for the study;

Sarcoptes scabiei var cuniculi were collected from naturally infested rabbits in Maiduguri, Nigeria. The scabs containing mites were placed in a Petri dish and incubated at 35°C for 48 hours at Veterinary Parasitology and Entomology Research Laboratory. Under a

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stereomicroscope the motile larvae are distinguished with having six legs while the nymphs and adult have eight legs according to (Soulsby 1982) were all used for the experiments.

Collection and Identification of *Cassia sieberiana*;

The plant *Cassia sieberiana* were collected within University of Maiduguri campus, and identified based on its botanical features as described by Hassan *et al*, (2007) and further authenticated by a Botanist at the Department of Biological Sciences, Faculty of Science, University of Maiduguri and a voucher specimen number LCMC 228 was deposited in the herbarium.

Preparation of *Cassia sieberiana* stem extracts

Freshly harvested stem bark of the plant were cut into smaller pieces to enhance drying under shade at room temperature (±27°C). Dried plant was pulverized with pestle and mortar to obtained a fine powder as described by Tiwari *et al*., (2011), thus 100g of powdered plant material was extracted in 1000mls of distilled water to obtain the aqueous extract separately at 60°C for 8 hours in a Soxhiet extractor as described by Tiwari *et al*. (2011). The extract was concentrated on an aluminum tray using hot air oven (40-50°C) to remove the solvent, leaving behind the dried extract which was weighed and stored at room temperature of 27°C in sealed plastic containers until required for use, as described by Biu *et al*. (2013).

Phytochemical screening of aqueous extract of *Cassia sieberiana*;

Small portion of the extract was tested for the presence of secondary metabolites such as simple sugar, carbohydrates, soluble starch, tannins, phlebotannins, cardiac gylcosides, terpenoids,

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saponins, flavonoids and alkaloids using the methods described by Brain and Turner (1975); Silva *et al*.,1998; Trease and Evans (2002) and Sofowora (2008).

Chemical Acaricide;

Permethrin is a synthetic pyrethroid used in treatment of lice infestation and scabies in humans and livestock. It is a yellow to light orange-brown, low melting solid or viscous liquid. Elimite^{R} was purchased which is a brand name of permethrin cream available in 50mg/ig for topical used marketed by Prestium Pharmacy Inc. indicated for treatment of infestation with *Sarcoptes scabiei*, and has a wider pharmacodynamics against Lice, ticks, fleas, mites and otherarthropods. And the extract powders were also converted in to paste/gram according to their concentrations respectively for uniformity.

Acaricidal Bioassays;

In-vitro Acaricidal evaluation;

The bioassay response of the extract acaricidal study was based on that of Khater *et al.*,2007 and Politi *et al*., 2012. A dose dependent response was conducted using graded doses of the extract being tested, as described by Singh *et al*.,2014. Control group A: (normal control) was not treated but merely maintained at the same condition as the treated groups. Control group B: (positive control) was treated with 0.05% permethrin for 5 minutes, while treatment groups (C-I) were immersed in 10 mls of each extract concentration (2.5mg/ml, 5mg/ml, 7.5mg/ml,10mg/ml,12.5mg/ml, 15mg/ml, and 17.5rng/ml) in a Petri dish followed by a gentle agitation for 5 minutes, before drying on a tissue paper.Each group was then transferred into a petri dish padded with Whatman's No.1 filter paper and incubated in a desiccator maintained at

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room temperature (27-28°C) and 85-90% RH (12/12h photoperiod) for 48hours. The effect of each extract concentration leading to mortality was monitored and death was confirmed by observing loss of motility and reflex after exposing to light for 48hours, dark discoloration of the cuticle and absence of movement Politi *et al.*,2012 and Singh *et al.*,2014. Numbers of dead and live mites were counted, post-exposure time was also considered and these were used to calculate the adult mortality rate (AMR) as corrected by Abbots formula and recommended by food and agriculture organization of United Nations (FAO 2004).

The *In-vivo* Acaricidal evaluation;

Eighteen (18) New Zealand White and crossed rabbits aged at post-weaned (8-10 weeks) with an average weight 0.8kg were classified into six equal groups of three (3) each randomly, the first group 'A' were uninfected untreated (normal control), group 'B' were designed as infected untreated (negative control group), group 'C' were infected and treated with orthodox acaricide (positive control), group 'D' were uninfected and treated with orthodox acaricide,group 'E' uninfected and treated with Cassia extract while group 'F' were infected and treated with Cassia extract (Extract test control) using concentration that shows lethal concentration (LC99) value 24 hours' post-treatment in-vitro, according to Haussain (2002). The infestations were carried out on the dorsal back surface of the neck about 4x2cm after scratching the fur and until appearance of hyperemia. Mite suspensions were applied using an applicator, after induction of infection the rabbits were caged separately and monitored. The pathological changes in the infected area were observed and recorded on days 7, 14, 21, 28 and 35. Confirmation of infestation by collecting deep scrapings to re-harvest mites and their developmental stages, and the index scoring value used to describe lesions by Jensen *et al.*,2002 were used.

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Data analysis;

All data obtained were subjected to accurate statistical package, Probit analysis were conducted on mortality data in bioassay tests, while analysis of variance (ANOVA) with Duncan's multiple range test were carried out on biological data.

RESULTS

Varying degree of infestation was observed during the study period, the criteria for grading the intensity of infestation is presented in Table 1. Out of the 15 rabbits, 1 rabbit (6.7%) did not show any signs of infestation at all. Among the rabbits showing infestation,13.3% showed slight degree of infestation, moderate infestation was observed in 13.3% of rabbits. However heavy infestation as observed by high levels of pruritus, scaling and alopecia was observed in 66.7% of the rabbits (Table 1). All animals in the negative control (n=3) did not showed any level of infestation during the study period.

At day 7, only erythematous rash was observed on the infected area, no significant change in the behavior of the infected rabbits was observed. At day 14, the skin at the infected area becomes more erythematous and some raised papules were visible even with naked eye. Change in behavior of the infected rabbits was observed, they became restless and scratched the infected area. At day 21, infected area appears moist with serous exudates due to rapture of vesicles and papules from constant scratching, infected rabbits become more restless due to severe pruritus and show less interest in feeding. Microscopic examination of the skin scraping showed few adults mites and large number of larvae and nymphs. At day 28, the yellowish scab appeared on the infected area and in some rabbits with severe pruritus, lesion becomes hemorrhagic and signs of secondary infection with pyoderma were observed. Animals are more

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restless with skin scrapings showing large number of adult mites. At day 35, persistent excoriation and resultant sero-hemorrhagic exudation making the skin of the affected area thick and crusty. At this stage, histopathological changes were seen in the skin of the infected area, necrosis and degeneration of the epidermis was evident.

Table 1; Outcome of induction of infection:

Days	Group A (n=3)	Group B (n=3)	Group C (n=3)	Group D (n=3)	Group E (n=3)	Group F (n=3)
7	N	-	-	-	-	-
14	N	+	++	++	++	+
21	N	+++	++	+++	++	+++
28	N	+++	+++	+++	++	++
35	N	++	+++	+++	+++	++

N = Normal skin and wool

+++ = heavy infestation

++ = moderate infestation

+ = slight infestation

Table 2; Phytochemical components of *Cassia sieberiana* leaf aqueous extract

Components	Tests	Scoring
Alkaloids	Dragendorff's	++
Terpenes	Liebermann-Buchard	++
Saponins	Frothing	+++

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Tannins	Ferric chloride	+
Glycosides	Salkowski's	++
Anthraquinones	Borntrager's	+
Combined anthraquinones	Sulphuric and Borntrager's	-
Flavonoids	Pew's	+++
Reducing sugar	Fehling's	++
Ketones	Standard	-
Pentoses	Standard	+
Monosaccharides	Barfoed's	-
General Carbohydrates	Molisch's	++

Keys

- = Not detected

+ = Low concentration

++ = Moderate

+++ = High

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Table 3; In-vitro mortality percentage of *S.scabiei* treated with *Cassia sabierriana*

	Post treatment(hours)								
Concentrations	24			48			72		
	D	L	M%	D	L	M%	D	L	M%
-ve control	0	10	0	0	10	0	3	7	30

+control	10	0	100	10	0	100	10	0	100
2.5mg	0	10	0	3	7	30	4	6	40
5.0mg	0	10	0	3	7	30	5	5	50
7.5mg	0	10	0	2	8	20	4	6	40
10.0mg	0	10	0	2	8	20	4	6	40
12.5mg	2	8	20	7	3	70	8	2	80
15.0mg	0	10	0	2	8	20	3	7	30
17.5mg	0	10	0	2	8	20	3	7	30

Keys

D = Dead

L = Live

M% = % of Mortality

Table 4;Index scoring of *S.scabiei var cuniculli* infested rabbits with extract LC_{99}

	Days post treatment				
	7	14	21	28	35
-Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
+Control	1.65±0.02	1.65±0.03	1.44±0.05	1.24±0.07	1.09±0.07
LC99	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean±S.D

DISCUSSION AND CONCLUSION

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Discussion; Almost all the mammalian host are known to be parasitized by itch mite *Sarcoptes scabiei*, they include a wide range of domestic mammals; pets, livestock and dairy animals, wild animals and humans (Kuizheng 1994; Daszak *et al*.,2000; and Deshmukh *et al*., 2010).

In the current study, scabies was successfully transferred to laboratory rabbits.66.7% of the rabbits showed heavy infestation, 13.3% showed moderate infestation and 13.3% also showed slight infestation while 6.7% did not showed any degree of infestation. It was also observed in the current study that scabies mite is site specific, because they were introduced on the body of rabbits behind the neck back and could only produce disease at the extremities, this finding is in accordance with Licois *et al.*, 2006, whom reported occurrence of lesions at the extremities and hinders feed consumption in laboratory animals. Also Mendlowitz *et al.*, 2002, reported that lesions when present cause alopecia and dermatitis on the face, nose, lips, feet and external genitalia appear as light brown, thick crusty with foul smelling exudates that are erythematous and may lead to secondary bacterial infections.

The choice of the plant*Cassia saberriana*selected for the study is based on their current and past use in traditional medicine, local and traditional knowledge has been the starting point for many successful drug development projects in the past and such approach is generally based on a detailed observation of how local populations uses the plants. Phytochemical screening indicates varying degree of organic compounds which accounts for the medicinal usage of the plant *Cassia saberriana*, Mahato *et al*., 1992; Ojo *et al*., 2006 and Buratai *et al*.,2011. In this study, *In-vitro* assay showed that at 12.5mg/ml 24hours post exposure the extract is capable of killing 2(20%), 28hours 7(70%) while 72hours 8(80%) but other concentrations have not shown effectiveness on mites even beyond 72hours. Moreover,the LC_{99} which is 12.5mg/ml when used *In-vivo* on experimentally infested rabbits did not show effectiveness even beyond 35days of

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treatment. This may be due to varying concentration of the components as indicated in Table (2) or the solvent (aqueous) used in extraction, which does not agree with the findings of Biu *et al*., 2013 that reported the effectiveness of *Cassia sieberiana* on *Hyalomma* ticks and Shahatha *et al*., 2020,reported that Crude watery extract of *Onobrychis ptolemaica*at 10 mg/ml showed complete termination of scabies mite in sheep, whereas lemon oil at 50 and 100% have shown effectiveness to kill 99% of mites after (1) one hour exposure and Methanolic extract of *Vitex negundo* dried stem and leaves *in vitro* showed 90% acaricidal activity, while *in vivo* showed 69, 73, 75, 77 and 78% acaricidal activity respectively.

In conclusion, Sarcoptic mites can be experimentally transmitted between different livestock host irrespective of the specie as earlier proven by Sabiha *et al*., (2016). However, this work shows that the organisms are site specific thus can only produce scab on the extremities of the ear, mouth, nose and paws. Also the plant *Cassia saberriana* despite the chemical component present and capable of killing *Hyalomma*ticks as reported by Biu *et al*., (2013) cannot control scabies mite at varying concentration, further studies on Methanol extract and other solvents and parts of the plant such as the leaves, flowers and the pods should be tried to demonstrate the effectiveness of the plants medicinal used or in other organisms such as gastrointestinal helminths.

Reference

Arlian LG, Runyan RA, Estes SA. (1984a) Cross infestivity of Sarcoptes scabiei. Jornal of American Academic Dermatology;10(6):979-86.

Ar1ian LG, Runyan RA, Sorlie LB, Estes S.A. (1984b) Host-seeking behavior of Sarcoptes scabiei. Journal of American Academic Dermatologiest. 11(4):594-598.

UNDER PEER REVIEW

Biu, A. A., Abdulkadir, M. A., Konto,M., Mohammed, A., Fadimatu, M., and Emmanuel, S. Acaricidal activity of aqueous extract of Cassia sieberiana DC(C'aesalpiniaceae) onHyalomma KOCH,(Acari:Ixodidae) Larvae, (2013) Journal of science and multidisciplinary research ;5(2) :37-42.

Brain, K. R., and Turner, T. D. (1975).The practical evaluation of pharmaceuticals. Wright sciencetechnical, (1st ed., pp.190-191): Bristol.

Evans, W. C. (2002). Phytochemicals. In: Trease and Evans pharmacognosy. (13 ed., pp.144-148). London,:Braillier, Tindal and Causel. experimental model for study of human and animal scabies. PLoSNègI

Kumar A., Dogra A. and Guleria J.S. (2010): Problems and constraints of rabbitry in India: A study of Himachal Pradesh. Global Journal of Science Frontier Research 10 (8): 40-46.

Kurnar, N.(1998): Studies on rabbit mange with special reference to evaluation of recent chemotherapeutic agents against rabbit mange. M.V.Sc. Thesis, Punjab Agriculture University, Ludhiana, India.

Licois D (2006): Pathology in Recent Advances in rabbit science. Published by Institute for Agricultural and Fisheries Research (ILVO) Animal Science Unit; Belgium.

Scott, D.W., Miller, W.H., Griffin, C.E.(2001): Dermatoses of Pet rodents, rabbits and ferrets. Pages 1415-1458. In: Scott, D.W., Miller, W.H., Griffin,C.E . (Eds).Muller and Kirk's small animal dermatology.W.B. Saunders, Philadelphia, PA.

UNDER PEER REVIEW

Silva, L. G., Lee, 1.S., and Kinnghom, D. A. (1998).Special problems with the extraction of plants in natural product isolation (Cannel RJD). (pp.343-364): Human press Iric.999, review drive suite 208 Totowa, New Jersey, USA,072512.

Sofowora, A. (2008) Medicinal Plants and Traditional Medicine in Africa. (pp.289). Ibadan, Nigeria.: Spectrum Book Ltd..

Soulsby, E.J.L. (1982). Helminths, arthropods and protozoa of domesticated animals, 7th Edition. Bailliere Tindall, London, 482-486.

Soundararajan,C., Iyue, M.,(2005): Incidence of mange infestation in rabbits. Journal of Veterinary Parasitology 19:2. Structural mechanisms of inactivation in scabies mite serine protease Diego, California.

Radi, Z.A. (2004). Outbreak of Sarcoptic mange and malasseziasis in rabbits (Oryctolagus cuniculus). Comp. Med. ,54(4) :434-437.

Suckow, M.A., Brammer, D.W., Rush, H.G., Chrisp, C.E.,(2002): Biology and diseases of rabbits. Pages 349-350.In :Fox,J.G., Anderson, L.C., Loew, F.M.,survey of taxonomic richness. Zootaxa.;3 148:237.

Singh PM, Fischer K. A (2014):Scabies mite serpin interferes with complement mediated neutrophil functions and promotes staphylococcal growth. PLoSNegl Trop Dis. ;8(6):e2928. we PM, Reynolds SL, Fischer K.(20 14): Parasitic scabies mites and associated system. PLoS One. 7(7):e40489.

Trease, G. E., and Evans, W. C. (2002).Pharmacognosy. (15 ed., pp. 13 5-488): Saunders. Trop Dis.4(7):e756.

UNDER PEER REVIEW

Wesonga O. and Mahato M. (1992): Rabbit Encephalitozoonosis in Kenya Laboratory Animals 26:219-221. ilberJ.L- (1999): 'Pathology of the Rabbit' Armed Forces Institute of Pathology,Washington DC .Modifiedin 2000.http://www.morfe.coni JPATHORABB1T.pdf.

Abdul Rahman A.M., and Ismail. 2008. Future Designin an Energy Efficient Building as an Identity of a Malaysian Tropical.

Wardhana, Manurung, dan Iskandar. 2006. Skabies: Tantangan Penyakit Zoonosis Masa Kini dan Masa Datang. Wartazoa. 16 (1): 40-52.

Khan M.A., Shah A.H., Maqbol A., Khan N., Rahman Z.U. 2012. Miticidal activity of methanolic

extract of Vitex negundo-Lam against Sarcoptes scabiei in animals and man. *Journal of Animal and Plant Sciences* 22 (Suppl. 2): 102-107.

Shahatha S.S. 2020. Epidemiological, diagnostic and therapeutic study for mange in sheep of Anbar Province-Iraq. *Iraqi Journal of Veterinary Sciences* 34: 1-7 (in Persian with summary in English). doi:10.33899/ijvs.2020.163587.

Oyelami O.A., Onayemi A., Oyedeji O.A., Adeyemi L.A. 2009. Preliminary study of effectiveness of Aloe vera in scabies treatment. *Phytotherapy Research* 23: 1482-1484. doi:10.1002/ptr.2614.

Currie B.J., Harumal P., McKinnon M., Walton S.F. 2004. First documentation of *in vivo* and *in vitro* ivermectin resistance in Sarcoptes scabiei. *Clinical Infectious Diseases* 39: e8-e12. doi:10.1086/421776.

Deshmukh V.V., Varshney, Chaudhary, and Desai. 2010. Clinical Management of Scabies in Rabbit Bunnies. *Polivet*, 11:112-114.

UNDER PEER REVIEW

Kuizheng, C., Zuomin, Jaming, Rongbin, Chang-Cai, Youngsen, and Gong. 1994. Preliminary Investigations of Ectoparasitic Infestation in Domestic Rabbits. *Chinese Journal of Veterinary Science and Technology*, 24: 16-17.

McCarthy J.S., Kemp D.J., Walton S.F., Currie B.J. 2004. Scabies: more than just an irritation. *Postgraduate Medical Journal* 80: 382-387. doi:10.1136/pgmj.2003.014563

Heukelbach J., Wilcke T., Winter B., Feldmeier H. 2005. Epidemiology and morbidity of scabies and pediculosis capitis in resource-poor communities in Brazil. *British Journal of Dermatology* 153: 150-156.doi:10.1111/j.1365-2133.2005.06591x.

Currie, BJ.; Harurnal, P.; McKinnon, M. and Walton, F. (2004) : First documentation of in vivo and in vitro ivermectin resistance in Sarcoptes scabiei. Clin Infect Dis 39(1): e8-e12.

Mendlowitz L (2002): 'Rabbit Diseases' Comparative Medicine program, website:http://www.radi1.missouri.edu/info/dora/RABBPAGE/rabbit.htm Modified on October 18, 2002.

Jofre, L., Naomi, I., Neira, P., Saavedra, T., Diaz, C. (2009):Acarosis and related zoonoses. RevistaChilena de Infectologia 26:248-257.

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