Epidemiology, genetic divergence and acaricides of *Otodectes cynotis* in cats and dogs

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Abstract

Otodectes cynotis mite is a common parasite of cats and dogs, survives in the ear canal and causes otitis externa, itching and severe complications. The microscopic examination of ear swabs, skin scraps and faecal samples of 289 cats and 223 dogs revealed that mono-specific and mixed infestations of *Otodectes cynotis* in cats were (24.56%) and(6.57%) while in dogs were (7.17%) and (4.48%) respectively. The highest rate of infestation was in young cats and the lowest was in elder dogs. The mixed infestations were found in combination with Sarcoptes, Demodex, Dermatophytes, Ticks, Fleas, Ascarids, Dipylidium and Isospora. The RAPD-PCR proved the genetic divergence between cat and dog isolates whereas they are morphologically similar. Selamectin-pour on, Doramectin-subcutaneous injection and Ivermectin-Ear drops were evaluated two weeks post treatment. The rate of success in cats were (96.66%) ,(90.00%) and (83.33%) and in dogs were (77.77%), (75.00%) and (66.66%) respectively. It is concluded that Selamectin pour on is the best acaricide against *Otodectes cynotis* in both cats and dogs. It is also needed to prepare a vaccine in the future to prevent the infestation with *Otodectes cynotis* and its complications.

Keywords: Otodectes, Epidemiology, Genetic divergence, Acaricides, Egypt, Parasite, Pet animal.

Introduction

Otodectes cynotis mites are non burrowing, white and very active parasites. They infest several species of animals including cats and dogs (Scott et al., 2001). It causes severe mechanical irritation due to the presence of the mite inside the ear leading to a higher activity of ceruminal glands and, consequently, the establishment of a favorable environment for secondary infections by bacteria or fungi (August, 1988). The infestation with Otodectes cynotis mite is termed otodectic mange (Sweatman, 1958). It is clinically observed as the infested animals show discomfort, intense itch, excessive waxy material or pus and even audition interference, depending on the level of parasitism (Gotthelf, 2000). Although the importance of mites in cats and dogs as it causes external otitis, information regarding their prevalence and the factors influencing their survival is lacking (Gram et al., 1994 and Sotiraki et al., 2001). The rates of infestation of *Otodectes cynotis* were studied in cats and dogs at different age groups. The morphological and genetic divergences between cat isolates and dog isolates of *Otodectes cynotis* were also studied. Finally, the complications of *Otodectes cynotis* infestations were recorded and the three acaricides were also evaluated in cats and dogs for treatment of *Otodectes cynotis* infestations.

Material and methods

Animals : 289 cats and 223 dogs were examined along two years in the Teaching Hospital of Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University. Age, breed, sex, clinical signs, complications and history of previous medications were registered for each examined animal.

Samples: Ear swabs and waxy materials from ears, skin scrapings and faecal samples were collected from the examined cats and dogs.

Acaricides: Selamectin pour on (Revolution®, Pfizer company), minimum dose is 6 mg/kg body weight applied topically on skin once, Doramectin injection (Dectomax®, Pfizer company), 1 ml/50 kg body weight injected subcutaneous once, Ivermectin (Iveen®, Adwia company) as ear drops once every 3 days, were evaluated for treatment of cats and dogs infested with Otodectes cynotis mites.

Ear swabs: Ear swabs from ear canals of both ears of the examined cats and dogs were collected and examined as described by Richard and David (2001) and Stephen and Dwight (2006) with some modifications. Blackish way material of ear canal was collected by a disposable ear cotton swab. The collected materials were gently mixed with Tap water and examined microscopically under low, medium

Table-1: Mono-specific and mixed infestations of *Otodectes cynotis* in Cats and Dogs.

Animal	Age	*Otodectes cynotis* infested animals						*Otodectes cynotis* Non-infested animals	Total
		Mono-specific infestation with Otodectes cynotis		Mixed infestations of Otodectes cynotis		Total			
		(No)	(%)	(No)	(%)	(No)	(%)		
Cat	Young	30	10.38	12	4.15	42	14.53	85	127
	Adult	25	8.65	5	1.73	30	10.38	74	104
	Elder	16	5.53	2	0.69	18	6.22	40	58
	Total	71	24.56	19	6.57	90	31.14	199	289
Dog	Young	9	4.03	6	2.69	15	6.72	98	113
	Adult	5	2.24	3	1.34	8	3.58	59	67
	Elder	2	0.89	1	0.44	3	1.34	40	43
	Total	16	7.17	10	4.48	26	11.65	197	223
Total		87	16.99	29	13.00	116	22.65	396	512

and high magnification power lenses. Otodectes cynotis mite were counted per slide and their motility were recorded before and after treatment.

Skin scrapings: Skin scraping was done in cats and dogs infested with Otodectes cynotis mite and suffered from hair loss and skin encrustation. Skin scrapings were done according to Richard and David (2001) with some modifications. Skin was scraped with a scalpel to collect skin tissue scraps. Skin scraps were gently mixed with sodium hydroxide 10 % solution and examined under a microscope to detect mites or dermatophyte. In few cases flea and ticks were visually detected during collection of skin scraps.

Faecal examination: Faecal smears were collected from cats and dogs infested with Otodectes cynotis mite and suffered from diarrhea. Faecal samples were collected and examined as previously described by Chandler et al.(2004).

Morphological and genetic divergences of Otodectes cynotis isolates between Cats and Dogs: Otodectes cynotis mites detected in ear swaps of Cats and Dogs were morphologically studied regarding body and leg sizes as described by Lohse et al. (2002). DNA from ear swaps containing Otodectes cynotis mites was extracted and measured. RAPD-PCR using three different primers was done according to (Hugh and Annette, 1994). The three primers were H-12 (5`-ACGCGCATGT-3`) (primer-1, P1) , T-20 (5`-GACCAATGCC-3`)(primer-2, P2) and V-07 (5`-GAAGCCAGCC-3`) (primer-3, P3), the primers and RAPD-PCR kits were produced by Gene tech company, Egypt. Three RAPD-PCR reactions were done for cat isolate DNA of Otodectes cynotis and the same reactions were done for dog isolate DNA of Otodectes cynotis using the three primers. One control negative reaction was carried out. Haem-III (DNA marker) was loaded onto gel to know DNA bands molecular weight. Each reaction was represented by small ependolf tube. Then each small ependolf tube containing separated reaction was spun to collect all reagents with each other. A 40 µl of paraffin oil was added to each reaction. Then was incubated and was labeled in small ependolf tubes in a set of PCR with the following program: -

Step-1: Intial denaturation at (94C°/ 3 minutes).
Step-2: Denaturation at (94C°/1 minute), annealing at (27C°/1 minute) and extension at (72C°/1 minute), (repeated 39 cycles).
Step-3: Final extension at (74 C°/10 minute)
Step-4: The reaction was preserved at 4 C°/ overnight

Results of RAPD-PCR reactions were detected by running of RAPD-PCR products with loading buffer in 1.5% agarose gel in 1X TAE buffer. Positive result was seen as bands on gel.

Evaluation of Acaricides: Each *Otodectes cynotis* mites infested cat and dog was examined before and two weeks post treatment by examination of ear swabs from both ears. Three acaricides , Selamectin pour-on, Doramectin injection and Ivermectin ear drops were evaluated for treatment of *Otodectes cynotis* infestation. The evaluation was depending upon the number of *Otodectes cynotis* mites per swab, the status of mites (either living or dead or absent) and level of improvement of clinical signs especially amount of ear waxy material and degree of itching.

Results

As summarized in tables-1 and 2, and figure-1. The mono-specific and mixed infestations of *Otodectes cynotis* in cats were (24.56%) and (6.57%) while in dogs were (7.17%) and (4.48%) respectively. The highest rate of infestation was in young cats and the lowest was in elder dogs.

The Mixed infestations of *Otodectes cynotis* mites with Sarcoptes, Demodex, Dermatophytes, Ticks, Fleas, Ascarids, Dipylidium and Isospora were detected. Complications of *Otodectes cynotis* infestations were observed as ear haematoma, in-co-ordination and imbalance, ear scratch and bleeding,

Table-2.: Evaluation of Acaricides.

Animal	Selamectin-pour on			Doramectin-injection			Ivermectin-ear drops			Total
	No of animals			No of animals			No of animals			
	Treated	Recovered	success (%)	Treated	Recovered	success (%)	Treated	Recovered	success (%)	
Cat	30	29	96.66	30	27	90.00	30	25	83.33	90
Dog	9	7	77.77	8	6	75.00	9	6	66.66	26
Total	39	36	92.30	38	33	86.84	39	31	79.48	116

deafness, fever and death.

The morphological characters of *Otodectes cynotis* mites from cats and dogs regarding the body and leg sizes were similar. The RAPD-PCR proved the genetic divergence between cat and dog isolates whereas they are morphologically similar. Result showed that lane-1(Haem-III: DNA marker), lane-2(cat isolate with primer-1), lane-3 (cat isolate with primer-2), lane-4 (cat isolate with primer-3), lane-5 (control negative), Lane-6(dog isolate with primer-1), lane-7(dog isolate with primer-2), lane-8 (dog isolate with primer-3).

The evaluated Selamectin pour-on, Doramectin subcutaneous injection and Ivermectin-Ear drops showed variable rates of success for treatment of *Otodectes cynotis* mites two weeks post treatment. The rate of success in cats were (96.66%) ,(90.00%) and (83.33%) and in dogs were (77.77%), (75.00%) and (66.66%) respectively. Causes of treatment failure were recorded as presence of pus, presence of excessive amount of ear waxy material, The eggs of mite resist acaricides, presence of the source of infection such as the place or another in-contact animal.

Discussion

Otodectes cynotis mites can survive in ear canal of both cat and dog and as well as surrounding environment. It feeds on epidermal debris inside the ear and is very contagious and, they cause serious ear irritation and damage if not treated (OIE, 2005).

Rates of mono-specific, mixed and the collective infestations of *Otodectes cynotis* in cats and dogs as illustrated in table-1, shows that the rate of mono-specific infestation with *Otodectes cynotis* was the highest in young cats(10.38%) and the lowest in elder dogs (0.89%). Also the mixed infestation of Otodectes cynotis with other parasites and dermatophytosis was the highest in young cats (4.15%) and the lowest in elder dogs (0.44%). The results showed that the mono-specific and mixed infestation of *Otodectes cynotis* in cats were (24.56%) and (6.57%), in dogs were (7.17%) and (4.48%) respectively. It is very clear that the infestation of *Otodectes cynotis* in cats is higher than that in dogs, which may prove the higher susceptibility of cats to Otodectes cynotis. The other parasites infested cat and dog at the same time of *Otodectes cynotis* infestation also dermatophytosis accompanied *Otodectes cynotis* especially in cats. Xhaxhiu et al.(2009) found infestation rate of *Otodectes cynotis* in dogs as (6.7%), also they recorded the mixed infestation with three species of ectoparasites as 38.1% of *Otodectes cynotis* infested dogs. Also Ugbomoiko et al. (2008) reported the Overall prevalence of ectoparasites was 60.4% and of intestinal helminths 68.4% in Nigerian dogs. Chee et al.(2008) found *Otodectes cynotis* infestation as (22.3%), they reported mono-specific and mixed infestations at the rate of (83.0%) and (17.0%) respectively in *Otodectes cynotis* infested dogs. Rodriguez-Vivas et al. (2003) reported the infestation of *Otodectes cynotis* in Mexican dogs was (3.5%).

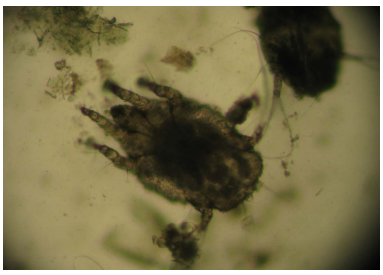

Figure-2: *Otodectes cynotis* adult (100 X) (Ventrodorsal view).

Although the Cat and Dog isolates of *Otodectes cynotis* were morphologically similar in body and leg sizes , the RAPD-PCR showed the genetic divergence between cat isolates and dog isolates that was proved by presence of different RAPD-PCR bands pattern using three primers as illustrated in figure-6. The genetic divergence may explain the higher infestation rate of *Otodectes cynotis* in cats. We thought that there were two genetically different types of *Otodectes cynotis*, one in cats and another in dogs. That was reported by Lohse et al. (2002), they characterized the second internal transcribed spacer (ITS 2) of the rDNA of 16 *Otodectes cynotis* isolates from 11 cats, two dogs, one arctic fox and two ferrets originating from four different continents. In addition, mites from dog, cat and arctic fox were investigated morphologically. Sequence comparisons revealed five different, but closely related genotypes.

Complications of *Otodectes cynotis* were recorded (table-1) as ear haematoma, in-co-ordination and imbalance, ear scratch and bleeding, deafness, alopecia, fever and death. That increased the importance of early treatment of *Otodectes cynotis* and change the concept of treatment to be prevention of *Otodectes cynotis* by vaccination. The three different acaricides with different three routes of administration were evaluated in both *Otodectes cynotis* infested cats and dogs as shown in (table-2). Selamectin-pour on , Doramectin-subcutaneous injection and Ivermectin-Ear drops were evaluated in Otodectes cynotis infested animals. The ear swabs were examined in treated cats and dogs before and 2 weeks post treatment and number of *Otodectes cynotis* mites per swab, the status of mite either living or dead or absent, improvement of clinical signs especially amount of ear waxy material and itch were taken in consideration to evaluate acaricides.

The rate of success of Selamectin-pour on , Doramectin-subcutaneous injection and Ivermectin-Ear drops were recorded at the two weeks post treatment (in table-2) in cats as (96.66%) ,(90.00%) and (83.33%) and in dogs as (77.77%), (75.00%) and (66.66%) respectively. The highest rate of success was achieved by Selamectin-pour on in Cats and Dogs while the lowest was reported for Ivermectin-Ear drops in cats and dogs. We thought the possible causes of treatment failure were presence of pus that might interfere with action of Acaricides so we advise to clean ear canal and evacuate pus to enhance treatment success. Presence of secondary bacterial and/or fungal infection which prolonged the time needed for healing of ear skin. Presence of excessive amount of waxy material of ear that hinder arrival of acaricides to mite. Presence *Otodectes cynotis* eggs that might resist acaricides so newly developed living larva and numph were seen after two weeks post treatment. Presence of the source of infection (place or another animal) which could help in re-infection of already treated Cat or Dog, (Six et al., 2000, Curtis,2004, Krieger et al.,2005, Ghubash,2006, Maggie et al.,2007).

We concluded that *Otodectes cynotis* is more prevalent in cats than dogs. The genetic divergence is clear between cats isolates and dogs isolates of *Otodectes cynotis*. The Selamectin-pour on is the best acaricide for treatment *Otodectes cynotis* infestation in both cats and dogs. The future view should be concentrate on the preparation of a vaccine to prevent complications of *Otodectes cynotis* in cats and dogs.

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