

**UNIVERSITY OF AGRICULTURAL SCIENCES AND
VETERINARY MEDICINE OF BANAT TIMISOARA**

FACULTY OF VETERINARY MEDICINE TIMISOARA



PhD THESIS

**RESEARCH ON ANIMATED PATHOGENS
INVOLVED IN CARNIVORES OTITIS**

SCIENTIFIC,

PROF. DR. ROMEO TEODOR CRISTINA

The holder of Pharmacology and Pharmacy disciplines

PhD,

ASIST. DR. MED. VET. DÉGI JÁNOS

Discipline of Infectious Disease

2009

This PhD thesis contains:

Introduction
Abstract, (rom, eng)
Abbreviations
General part (119 pages)
Special part (176 pages)
Tables: 36
Figures full color: 89
Bibliography: 355 references

SUMMARY

Thesis title:

Research on animated pathogens involved in carnivore's otitis

Part I

CHAPTER 1

BIBLIOGRAPHICAL STUDY

The bibliographical study is organized on **seven chapters** that cover:

- notions about dog and cat ear anatomy and histology,
- aspects related to the hearing physiology,
- otitis definition and classification,
- analysis of the causes and agents that contribute to the occurrence of the otitis to carnivorous animals,
- diagnosis and treatment of the external otitis,
- precepts about oto-toxicity and
- otitis surgical management.

The analyzer, established by PAVLOV, defines the Unitarian design of „sense organs” structure and activity, and describes a morfo-functional system composed by three sub-systems functionally incorporated: the receiver, the centripetal control channel and central section. The last constituent distinguishes and stores the information's received by way of the first two constituents, and elaborates the distinctive perceptions (awareness states). The analyzer has an exact morfo-functional specialization, every sense of the organism being dependent on the activity of a certain analyzer as a result of living matter evolution adaptation.

The stato-acoustic analyzer, named also the acoustic-vestibular analyzer, is constructed of complex systems with the purpose of receiving, transmitting and analyzing the static and acoustic stimuli, and their conversion in acoustic-vestibular perceptions. The receiver or peripheral section is represented by the Outer Ear, Middle Ear and Inner Ear.

The external otitis is a frequent disease found in dogs (with an incidence ranging from 3.9 to 20%), and cats (ranging from 2 to 6.6%). The circumstances that predispose the ear to inflammations consist in any

alterations at the level of the External auditory canal. The external otitis can be related with other dermatologic diseases, particularly with the allergic and immunological intermediate diseases (alimentary allergic dermatitis, atopic dermatitis, contact dermatitis), or sistemic diseases (endocrinopathies such as hypothyroidism or Sertoli cells tumor). Bacterial infection, foreign bodies, parasites (*Otodectes cynotis*, *Demodex canis*, *Sarcoptes scabiei*, *Notoedres cati* and ticks), fungi, yeasts (*Malassezia pachydermatis*), or neoplasia can be eventual grounds.

The chronic external otitis may cause secondary lesions to the external auditory canal (epithelial hyperplasia and tissue ossification with chronic inflammation), this perpetuates the infectious and makes the medical intervention heavier because of the external auditory canal area obstruction. Moreover, secondary ulcerations and infection with pyogenes bacteria, yeasts and/or funguses are usually present. The apocrin glands grow in size, number and secretions during external chronic otitis, while sebaceous glands diminish their number and become less active. External otitis of the dog is a disease with a multifactorial etiology.

The metabolism products (toxins, enzymes and fatty acids), produced by the bacteria and yeasts during growth, can aggravate the inflammatory processes.

The objective in the therapy of otitis should be the elimination of the grounds that support the disease (removal of the foreign bodies, etc) and the identification of the agents that predispose such as allergies, otacariosis, systemic dermatopathies, etc. This schedule should be followed according to the inflammation type by the application of suitable substances in order to clean and dry the auditory canal, to melt the cerumen and then to dose out the external ointment or, if it is necessary, a general path medication. The systemic action reduces the inflammation and eliminates the microorganisms which are involved in the start of the disease. The success of the external otitis therapy in dogs depends on the complex ointments that have antibacterial, anti-mycotic, and anti-inflammatory activity.

Part II

OWN RESERARCHES

CHAPTER 2

Epidemiological researches regarding dog external otitis

The external auditory duct inflammation plays an important part in canine pathology, because it affects a relative great number of animals, 6 to 20%, having also problems regarding the therapy.

In the study that was carried out from **September 2003 to March 2007**, were studied **781 dogs** of **48 breeds**. The frequency of external otitis to the investigated dog population formulated by incidence was of **178 cases** (absolute value), 22.79%, respectively (frequency-relative value). 24 breeds of dogs were found with external otitis (plus half breeds). In this study there was observed an increase diagnostic of the auricular affection to the following breeds: Cocker spaniel, German shepherd, Poodle, Boxer, Rottweiler, beside other studied breeds that were diagnosed with auricular affection. The external otitis was not diagnosed in **24 breeds** of dogs; this thing is explained by the reduced number of this type of breeds who were examined in veterinary cabinet. Although there is a predisposition to this disease there was no external otitis diagnosed to Collie, Saint Bernard, Belgian shepherd, Kuvasz, etc.

Clinically speaking, external otitis was divided in acute and chronic. Although, in the same study it has been noted also the location of the inflammatory process, unilateral or bilateral. According to the inflammation type, external otitis was divided in external erythematose-ceruminose otitis and external purulent otitis.

So, the frequency of acute external otitis expressed as an absolute value were about **115 cases**, dogs belonged to **23 breeds**, and as a relative value, the proportion was about **64.60%** of all cases with external otitis.

The frequency of external unilateral otitis was about **74.89%** and the frequency of bilateral external otitis was about **25.10%**, represented as incidence rate.

External erythematose-ceruminosae otitis had the following frequency: frequent-German shepherd (32.83%), Cocker spaniel (25.47%), Poodle (11.50%) and Boxer (5.47%).

The external suppurative otitis had a frequency of 23.07% of number of external otitis and 4.85% of the examined canine population.

It is noticeable the fact that the highest frequency of dog external otitis, found in adult animals, between 2 and 5 years and males, respectively. As for seasonal incidence of external otitis in dogs, after the study we carried out, it was observed an increase of the otitis frequency during the periods with high variations of temperature and humidity associated with high levels of rainfall. Thus, in September 2004-august 2005, the frequency of external otitis in dog registered high values in October (15.73%), April (14.04%), November (13.48%), May (11.23%) and December (10.11%), respectively. we noticed that external otitis was spread to sexes as it follows: to males (54.49%), by comparison with data gathered from females (45.5%). Subsequent laboratory examination, the etiology of external otitis in dogs, was of parasitary, bacterian and mycotic origin.

External parasitary otitis, according to the study carried out; there were **28 cases, 15.73%** respectively, of the total cases with external otitis.

The bacterian otitis were diagnosed to **36 dogs** with external otitis, the frequency of this type of otitis had been of **31,30%** while the incidence of mycotic otitis was about **54 cases, 46.95%** expressed as a relative value, respectively, from the total number of all external otitis cases.

The frequency of composite external otitis (bacterian and mycotic) was about **25 cases**, representing **21.73%**. Other types of otitis diagnosed were external allergenic otitis, that has an incidence of **35 cases, 1.06%** from the total population of examined dogs, respectively, and 5.07% external otitis, external otitis produced by foreign bodies respectively, **156 cases** recorded, that belonged of a number of **16 breeds, 4.75%** reported to the canine dog population examined and **22.64%**, reported to the number of dogs with external otitis.

CHAPTER 3

Epidemiological researches about external otitis in cat

The study of external otitis in cats was carried out during **September 2003 to September 2004**, in this time there were examined **298 cats** both in Timisoara and Arad cities, from **7 breeds**, including the common cat (European type). Cats were examined in different veterinary laboratory from FMV infectious clinic Timisoara.

From the total of examined animals external otitis was diagnosed to **19.48%** of the cases. The research regarding the involvement of the age and sex of the cat affected by the external otitis, show that these parameters have a relative influence in triggering the pathological process.

The most cases (**46.55%**) were diagnosticated to adult animals, with age ranging between 1 to 5 years, followed by the young animals with a frequency of **22.41%**. Less observed cases (**17.24%**) were observed to the animals between 5 to 10 years, and to the older animals the percentage is even lower (13.79%).

Other parameters pursued in this study were the pathological process location and clinical aspects of external otitis.

If we refer to the external otitis localization, it can be observed that the bilateral otitis is net superior (**72.41%**), by comparison with the unilateral ones (**27.58%**).

This aspect can be related to the fact that external otitis on cats are predominant of parasite nature, and this is characterizes by this type of evolution.

Regarding the clinical aspect there is a high frequency of external erythematose-ceruminose otitis (**72.41%**), by comparison with the suppurative ones (**27.58%**).

The most examined breeds of cats' were Persians (23.08%), British shorthair (22.22%), European common (21.74%), Burmese (20.31%) and Blue Russian (20%). Cases less diagnosed with otitis were the Norwegian forest (8.33) and Siamese (6.25%) breeds. To the cats the external otitis causes are of parasite and infectious nature, and foreign bodies and allergenic phenomenon's are in less measure responsible for the occurrence of otitis.

The parasitary origin otitis are predominant in external etiology of otitis, their frequency is about **43.10%**, followed by otitis of infectious origins (**41,37%**).

The mycotic otitis had a greater frequency (**29,31%**), than the bacterian ones (**6,89%**). The foreign bodies represent the lowest factor that produces external otitis (**5,17%**), followed by the ones of allergenic nature (**10,34%**).

Literature indicates that among the factors that can influence the trigger of external otitis both in dogs and cats, there are climatic factors. Because of this reason, we monitored the cat external otitis cases during a calendar year, in order to observe the influence of the factors mentioned above.

The most frequent cases were registered during March (18.96%), October (15.51%), and November and February (10.34%), respectively. These results can be related with the fact that, during periods mentioned above, humidity and relative temperature variations were high, fact that certainly influences the microclimate at

the level of the ear, being a support for yeasts and bacteria. Another explanation would be that during October and March respectively, there is a correspondence with the mating period of the cat, when there is a crowd of animals and the contact between individuals is tighter so the transmission of some external parasites is increased, such as *Otodectes cynotis*.

CHAPTER 4

Researches regarding the microbial flora in dog external otitis

Dog external otitis is a disease with a multifactorial etiology. The situations that predisposes the ear to the inflammation of the external auditory canal are local factors or it may be the consequence of a systemic disease such as atopic dermatitis, hypothyroidism, food allergy, etc. These factors determine cerumen gland irrigation, fact that leads to a growth of the cerumen quantity and so the ventilation of the external auditory canal diminishes, possibly there is created favorable micro organisms' proliferation conditions that inhabit this anatomic area. The purpose of the clinical bacteriological examination is to supply rapid and accurate information about the presence of the bacteria forms or about an infectious process. Samples were taken from a number of **118 dogs** of different breeds and ages, diagnosed with acute and chronic external otitis, having pathological auricular discharges.

Samples were taken with sterile cotton pads, set on thermo resistant plastic or wood stem, previously moistened in saline serum. After that samples were introduced in a sterile tube if there was the possibility of processing them immediately. In cases when the procession was not possible, the exudate sampled from external auditory canal was put into a transport media. For transport there were required special sterile tubes used for micro organisms transport, that contain special transport media (Stuart and Amied), from BIOCLINICA laboratory from Timisoara and Arad.

In order to isolate and identify the micro organisms there were used standard associate procedures with rapid identification assay for some staphylococci and streptococci strains such as BACTIDENT – STAPH (Merck) and SLIDEX STREPTO – KIT (Biomeriex), and multitask systems API 20E, respectively, provided by the Infection Disease Department of the Faculty of Veterinary Medicine, Timisoara. There was also tested the behaviors of all isolated bacterial and yeasts strains, by using the difuziometrical method.

The external bacteria otitis was diagnosed to **36 dogs** with external otitis, the frequency of this type of otitis is about **31,30%**, while the fungal otitis was found in **54 cases, 46,95%**, respectively, formulated as a relative value, of the total number of cases with external otitis. According to the achieved data, bacteria were isolated in about 44.02% of the cases and yeasts were isolated in about 55.97% of the infectious external otitis.

The most frequent isolated bacteria were *Staphylococcus intermedius* – **42 strains** (26.41%,), *Pseudomonas aeruginosa* **16 strains** (10,06%), group G streptococcus **11 strains** (15%), *Staphylococcus aureus subs. aureus* **10 strains** (6,28%), and rarely isolated bacteria were *Proteus spp.* **7 strains** (4,40%) and *Corynebacterium spp.* **3 strains** (1,88%). Also the bacteria were isolated from pure culture in about 67.41% of the cases with bacterian external otitis and in mix culture about **32,58%**, respectively.

In the case of mixt culture the frequency of **two bacteria** culture was of **96,66%**, while on the three bacteria culture were represented only 3.33% according to the tinctorial affinity of the isolated bacteria to the Gram coloration, the Gram positive bacteria had a clear higher share (**71,91%**) versus Gram negative bacteria (**28,08%**).

Yeasts from *Malassezia* genus were isolated in clear culture in about **68,42%** and **21,05%** respectively of the cases in association with bacteria from Staphylococcus type. The number of *Malassezia pachydermatis* stains isolated from dogs diagnosed with external otitis was about 57 (35.84%). It is to be observed the association with bacteria *Staphylococcus intermedius* type on a rate of 21.05%, followed by the G group streptococci (3.50%).

The yeasts from *Candida* genera had a decreased share by comparison with *Microsporium canis* who were isolated on a share of **3,14%**, representing **five strains** of all pure culture cases.

The response to the antibiotic was tested to all strains isolated from external otitis, no matter the clinical progress. Analyzing the acquired data it can be observed that the majority of the isolated strains prove to react very well to the hardly use antibiotics or to the new antibiotics used in animal therapy. From the analysis of these data, it can be established that the most frequent isolated bacteria, *Staphylococcus intermedius* respectively, responded well tot he neomycin treatment (90.47%), ciprofloxacin (80.95%), fluorfenicol (69.04%), enrofloxacyl (73.80%), amoxiclav (66.66%), and cefalexin (59.52%). The strains presented resistance to tetracyclin (47.61%) and gentamycin (38.09%).

The majority of the isolated strains of *Pseudomonas aeruginosa* proved to be sensible to a reduced number of antibiotics such as: fluorfenicol (87,5%), ciprofloxacin (85,71%) enrofloxacin (62,5%) and gentamycin (50%). The resistance phenomenon was notified for lincomycin (75%), amoxyclav and cefalexyn (68,75%), ceftiofur (62,5%), and neomycin (56,25%). As it regards the *Proteus* spp., it was observed a increased sensibility to ciprofloxacin (85,71%), gentamycin, enrofloxacin, fluorfenicol (71,42%), neomycin (57,14%), spectinomycin and cefalexyn (42,85%). There was observed resistance to tetracyclin, lincomycin (71,42%), rifampycin, cefalexyn (57,14%) and amoxyclav (42,85%).

Staphylococcus aureus display an increased sensibility to amoxyclav, ciprofloxacin, fluorfenicol (80%), rifampycin and enrofloxacin (70%) and tetracyclin (60%). Resistance was observed to gentamycin, neomycin (50%), spectinomycin (40%), respectively.

Group G streptococci and *Corynebacterium* spp isolated from dogs external otitis manifested sensibility to enrofloxacin (81,81%, 66,66% respectively), ciprofloxacin (81,81%, 100% respectively), fluorfenicol (72,72%, 100% respectively), ceftiofur (63,63%, 66,66% respectively). In the same time there was observed a sensibility of group G *Streptococcus* to neomycin (66,66%) and bacteria from *Corynebacterium* genera were sensible to cefalexyn (72,72%). The resistance process of the *Streptococcus* strains of group G was observed to appear for tetracyclin, lincomycin (54,54%), and to gentamycin, spectinomycin and neomycin (45,45%).

CHAPTER 5

Researches regarding the auditory canal microbiological flora of cats with external otitis

To establish the microbial flora of cats with auricular diseases, there were studied **58 cats** that came to the veterinary clinics to be diagnosed on a suspicion of auricular trouble during September **2005-September 2007**, in Timisoara and Arad cities. Cats taken into study belonged to different breeds and different ages, from 2 months to 14 years. Samples were collected from cats observed at FMV Timisoara, Infectious Disease Clinic, and to private veterinary laboratories in the two cities.

The samples were collected from healthy cats who were the subject to a complex anamnesis and clinical examination.

From the total of **58 cats**, there were 27 males and 31 females. The cats were living either indoor or outdoor, and have had the same owner. The examinations were carried out to the Parasitical Diseases Clinic and Infectious Diseases Department, respectively as well as to the BIOCLINICA-Arad.

For the bacteriological examination and fungal examination there was used calf defibrinated agar blood 5-10%, agar BHI media, incubated at 37⁰C, in anaerobiont conditions, and for the fungal examination were used agar Sabouraud dextrosed type II medium with a supplement of 0.05% cloramphenicol and MYCOSEL agar, incubated at temperatures from 25 to 35⁰C, in anaerobiont conditions. The subsequent identification supposed the use of multitask type media like API 20E and API STAPH, API STREP, and also using morphological and biochemical characteristics. The cerumen samples for the parasitological examination were treated with KOH 10% glycerinate, to clarify the probe from the microscope blade and subsequently subject to a microscopic examination using growing resolutions to distinguish eventual mites, present on the collected samples. Regarding the positive bacterial cultures, there was observed a growth of diverse bacteria species. The most frequent isolated bacteria was *Pasteurella* spp. (15,49% of the cases for cats), followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (12,67%), and *Staphylococcus* spp. (11,26%) respectively.

The bacteria isolated with a reduced incidence was *Proteus* spp. (5,63%), and group G *Streptococcus* (2,81%), respectively. If we take in consideration the type of otitis, clinically classified in erythematose-ceruminal external otitis and discharging external otitis, an increased frequency was observed to the discharging ones (78.87%), by comparison to the erythematose-ceruminal ones (21.16%). To the incipient external otitis, namely the erythematose-ceruminal otitis, the bacteria were isolated in a reduced number, the most frequent bacteria being the *Staphylococcus* spp. (20% of the cases of erythematose-ceruminose external otitis). The complex otitis, chronic and recurrence discharging external otitis, were isolated a high number of bacteria, belonging to different species; the most frequent were: *Pasteurella* spp. (19,64%), followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (16,07%), *Staphylococcus* spp. (8,92), *Proteus* spp. (7,14%), and group G *Streptococcus* (3,57%). By making an analysis for positive cultures in connection with fungus and yeasts growth, we noticed a high percentage of *Malassezia* spp. (29.57%), followed by *Microsporum canis* (9.85%).

If we refer to the clinical aspects of the otitis, erythematose-ceruminal form, there was a prevalence of dermatofits infections with *Microsporum canis* (44.66%) followed by yeasts such as *Malassezia* spp. (33.33%). As for the chronical, complex, discharging phases, the dermatophites were absent, while the yeasts of *Malassezia* spp. were present on a 28.57% ratio. Of the total of isolated strains, in pure culture there were

isolated only 12.67% of the micro organisms, while in the mixt cultures the percentage was about 87.32%. In pure culture, for the erythematose-ceruminal external otitis type to the cat, there were isolated only dermatophites and yeasts from *Malassezia* spp., 7/7 strains, 2/21 strains respectively.

All micro organisms strains, were tested for the response to the antibiotics, by using general antibiotics: trimethoprim, amoxiclav, gentamycin, enrofloxacyl, tetracyclin, lincomicyl, spectinomycin, neomycin, ciprofloxacyl, ceftiofur, cefalexyn and fluorfenicol.

The most frequent isolated bacteria was *Pasterurella* spp. which is sensible to ciprofloxacyl (90.90%), enrofloxacyl (81.81%), amoxiclav and cefalexyn (72.72%) respectively. The resistance phenomenon was observed to a number of 10 antibiotics out of 12 tested. An increased resistance was observed to trimethoprim, tetracyclin and lincomicyl (45,45%).

Staphylococcus aureus was sensible to ciprofloxacyl (88.88%), fluorfenicol (77,77%), amoxiclav and enrofloxacyl (66,66%), ceftiofur (55,55%), respectively. The resistance was notified to the majority of the tested antibiotics, especially to lincomicyl and spectinomycin (55,55%), gentamycin and tetracyclin (44,44%), respectively.

Another important ethiological factor, isolated from cats' external otitis, is *Pseudomonas aeruginosa*, and following the response test to antibiotics was observed an increased response to ciprofloxacyl (88,88%), fluorfenicol (77,77%), amoxiclav, ceftiofur and cefalexyn (55,55%), and trimethoprim (44,44%) respectively. The multiple response phenomenon was observed to a large lot of the tested antibiotics, because, it is well known the fact that the drug resistance comes out frequently to this type of bacteria. Of the antibiotics to whom the resistance was observed are: spectinomycin (66,66%), trimethoprim and neomycin (44,44%), lincomicyl and ceftiofur (33,33%), respectively. If we refer to the *Staphylococcus* spp strains a greater sensibility was recorded to enrofloxacyl and ciprofloxacyl (87,5%), amoxiclav and neomycin (75%), ceftiofur (50%), respectively. to this bacteria, the resistance was observed to gentamycin, lincomicyl and spectinomycin (37,5%).

Out of bacteria less frequent, isolated from the cat external otitis, group G *Streptococcus* and *Proteus* spp., had a response to amoxiclav and enrofloxacyl (50%), spectinomycin, neomycin, ciprofloxacyl and ceftiofur (50%), in case of streptococci strains.

The response of the yeasts and dermatophites isolated from cat external otitis was tested with ketoconazol, clotrimazol, nistatin and amphotericin B. For *Malassezia* spp strains an increased sensibility was observed against ketokonazol (87.1%), followed by clotrimazol (66.66%) and nistatin (52.38%). The sensibility against dermatophites was obvious in the case of ketokonazol (71.42%) and of the nistatin (42.85%). The resistance shown to amphotericin B, for the yeasts of *Malassezia* spp, with a frequence of 42.85%, and in case of *Microsporium canis*, the resistance was observed to clotrimazol and amphotericin B (57.14%), respectively.

CHAPTER 6

Research on the correlation between clinical course and microbial flora of the external otitis in dogs

Another study was aimed to establish a correlation between the clinical aspects of external otitis in dogs and microorganisms isolated from these cases of the disease.

The research was pursued in particular the role detect bacteria, yeasts, fungi and predisposing factors in the pathogenesis of external otitis in dogs.

Therefore, increased attention was given to detecting and correctly assessing the dominant primary factors, in particular, when they made reference to the diagnosis and management of recurrent and persistent external otitis. Conduct preventive and curative in cases of otitis external identified was aimed at normalizing the number of bacteria in all cases of external otitis.

Clinical and laboratory study, on establishing the correlation between clinical aspects of external otitis in dogs and microorganisms isolated from these cases of the disease was made. The canine population, was sufficiently representative and revealed the presence of bacteria *S. aureus subsp. aureus* of group G streptococci, *Pseudomonas aeruginosa* and *Proteus* spp., associated in all cases of EO with suppurative inflammation.

In cases of erythematous ceruminous EO, most frequently microorganisms isolated were *S. intermedius* and the genus *Malassezia* yeasts in pure culture or in combination. High frequency of the genus *Malassezia* yeasts and bacteria of the genera *Pseudomonas* and *Proteus* suggested previously treated with antibiotics incorrectly set.

Increasing the amount of yellow and fluid exudate, characteristic of Gram-negative infections (*Pseudomonas aeruginosa* and/or *Proteus*) or moderate otic secretions, dried darker shade associated with Gram positive (*Staphylococcus spp.*) were always associated with the affected ear.

Other Gram negative bacteria have brought abundant secretions, wet, smelling, or yellow blade.

Malassezia pachydermatis produced consistently wax, chocolate brown. Cerumen which was black-brown color was due to the presence of *M. pachydermatis* infection, alone or in combination with other bacteria (usually by coagulase-positive staphylococci or mite *Otodectes cynotis*).

CHAPTER 7

Research on the implication of *Malassezia pachydermatis* in the external otitis in dogs

Another study in the research of external otitis in dogs was the frequency screening of the genus *Malassezia* yeasts as potential zoonotic risk factor. The study was focused only on the ear duct in dogs, without referring to other sites, wishing it to be an accurate picture on the extent of the infection in the canine population in the west of the country, the early stage of a study with zoonotic risk to dog owners.

Results of cytological examination of otic exudation originating from dogs with external otitis confirmed, in most cases, an important fungal and bacterial activity. It is possible that some cases of acute OE early to see only clinical ceruminous secretion and it is associated cytological with an increased number of epithelial cells exfoliate. A pure yeast infection (*Malassezia pachydermatis*) will develop a wax abundant, dark brown color, sweet-smelling.

Mixed infections, bacterial and fungal are frequently encountered. The presence of more than **5 items** levuriforme and bacterial elements in **25 microscopic field**, at high resolution (considered the objective X 40), suggesting a significant microbial activity warranting therapeutic intervention. Coloration Gram highlighted levuriforme cells belonging to the genus *Malassezia* in **38 dogs**.

Malassezia in association with bacteria were evident in 14 dogs, while one in **21 dogs** *Malassezia*. The microscopic field at a high resolution, included more than **10 levuriforme cells** to **38 dogs** of **118 examined**, while in **75 dogs** in **10 cells** we detected levuriforme of the genus *Malassezia*, *Malassezia* for the 5 samples are absent, (as confirmed by negative cultures).

Microscopic examination of exudative ear has provided valuable guidance on choosing the most suitable culture medium. The genus *Malassezia* yeasts were isolated in pure culture in 32.20% of cases of external otitis. If *Malassezia* strains isolated, 55.26% of cases were in association with *Staphylococcus intermedius*, *Pseudomonas spp* and 5.26% with a rate of 2.63% in group G streptococci, *Proteus spp*, *S. aureus* and *Candida spp*.

During the study on the frequency of the presence of *Malassezia* genus yeasts in the external ear canal of dogs with acute and chronic otitis, strains were isolated lipid-dependent, the strains which did not require added fat for cultivation and development.

Of the **38 strains** isolated from dogs with otitis pipeline external audit of this study, **30 strains** were grown on agar Sabouraud only type II, gentamycin dextrose with added 0.05%, while **8 strains** increased on Sabouraud agar with added olive oil.

On the surface environment of calf blood agar with 5% in **21 of cases** (after 48 hours incubation at 37 ° C and under conditions of aerobiosis) was not detected any bacterial colony, but noted the presence of the very fine point of pseudo-hemolysis, like grains of sand and discoloration environment (characteristic of specific growth *Malassezia*).

CHAPTER 8

Research on the frequency of infestation with the cat *Otodectes cynotis*

Occasionally, cats can be the source of many diseases in their life. They can transmit disease directly to other animals and on humans. These may include some important parasitic diseases caused by ectoparasites, which in many cases can be zoonotic, or some unusual risk.

Thus, mites with regular progress may be an important source for transmission of various diseases, also can trigger various phenomena of hypersensitivity and dermatological diseases, and, especially, may be the main cause of anemia with prolonged treatment in young animals.

The most common ear mite of the cat is *Otodectes cynotis* that colonize the external ear canal and external ear. Given these issues, the objectives of this study was to determine the degree of infestation of the cat external ear with *Otodectes cynotis* mites.

The research was conducted during January. 2005 – December 2007, in places Timisoara and Arad, **298 cats**, belonging to different races (European, British Shorthair, Persian, Burmese, conjoined twins, Russian Blue and Norwegian Forest), aged between 3 months and 14 years.

Cats were initially subjected to a thorough clinical examination, which included the dermatological and otoscopic examination. Following these tests, of all cats examined, diagnosed with external otitis were **58 cats**. Of cats diagnosed with EO, *O. cynotis* for mites, **25** were diagnosed positive for cats, representing **43,1%** of cases affected by infection in the ear. 7 cats were diagnosed with severe clinical signs EO, which, in addition to mites were isolated and Gram positive and Gram negative.

These cats have come specifically scratching the ears and shaking the head. Internal surface of the auricular pavilion erythema was associated with secretions Scrapers, and variable amounts of brown, with coarse appearance. These clinical signs can be considered typical for cases associated with *O. cynotis*.

During this study, cats with EO were monitored in terms of infestation with *O. cynotis* accumulation in one ear (left or right), or both ears. The results showed infestation accumulation in his right ear where the **32%** of cats diagnosed with EO parasitic, respectively, **24%** of cats, the left ear. Mites were identified in both ears, with a frequency of **44%**.

When referring to the sex of infected animals, we found significant differences between males (**56%**) and females (**44%**).

Age, as shown in the results of this study demonstrate that young animals are more affected (**72%**) compared with other age groups. It may be a crucial factor in the infestation with *O. cynotis*.

On race infected animals, we observed that cats of European race are more affected (**84%**) than those of pure races (**16%**).

The length and type of hair, the study achieved a certain measure affecting infestations these mites. Cats with short hair (**84%**) were most affected, followed by those with coarse (**64%**), respectively, at a remarkable distance from the smooth hair (**24%**). This may be associated with the fact that in cats most commonly is this pilosity type.

After analyzing the data, the living environment of cats is an important factor taken into account, both in triggering and maintaining infestations in cats. Thus, animals kept outside the home, are more susceptible to infestation with *O. cynotis*, the degree of infestation was **84%** of cats diagnosed with parasitic EO. Cats kept indoors, or in an environment isolated from the outside, seem to be much less susceptible to infection. In the study conducted, these cats were diagnosed only at a rate of 16% of all affected cats.

Among the factors studied were related to frequency and ear cleaning that contact with animals, either within species or other species. It is known that as the ear cleaning is done more frequently, the possibilities of developing a disease are lower and the diagnostic of a potential problem occurs more rapidly, helping to establish a therapy in time, reducing the possibility of complicating different infections.

All cats have been diagnosed parasite OE caused by *O. cynotis* were most frequently cleaned in the ear once a week. Finally, another factor described, was the contact with other animals. By monitoring this parameter, we could demonstrate its role in the transmission and spread of infestation with *O. cynotis* in a population of cats.

Most infected cats had contact with other cats (56%), and a certain part with dogs (44%). There were no reported infestations in cats which had contact with any animal.

CHAPTER 9

Research on the importance of cytological examination of otic exudation in external otitis in dogs

Systematic diagnostic procedures in external otitis in dogs should include details of history, clinical examination, cytological examination otoscopy and which are necessary to identify the causes and factors that contributed to the disease. Also recommended, conducting cultural and antibiogram tests and, biopsy examination in all recurrent and refractory cases.

Information obtained by cytological examination of exudative ear, are immediate and high therapeutic value, that growth under laboratory outstanding share in the total cost of veterinary care. Cytological

examination reveals the presence, number and characteristics of three important elements: bacteria, yeasts, and inflammatory cells (lymphocytes and neutrophils).

In this regard a study was made with reference to the importance of this examination in the diagnosis of external otitis in dogs. Otic smear examination required exudation was obtained from **10 dogs** with normal ears of **14 dogs** with acute and chronic external otitis, which is or therapeutic intervention performed. After a complete medical history, clinical and otoscopic examination the exudate from the ear was examined.

Installation material was obtained by using tube samples, provided with sterile hydrophilic cotton ball. If the pipeline ear was dry, sterile swab was wetted with sterile isotonic solution (NaCl 0.9%). This minimizes the cellular destruction exudation during collection and processing. Even when the channel was sufficiently wet, it was considered necessary wetting pad.

To prevent contamination, ear exudate samples were collected through a clean and sterile otoscope cone. In the study conducted Gram used color as allowed assessment tinctorial affinity of bacterial cells. Before staining, smears were fixed by repeated passage through flame, heat setting to determine the melting wax, which made the exudative to join the blade of a microscope.

Colored smear was examined initially at low resolution, X6 and X10 objectives to determine that all smear or colored areas suitable and if there are areas with high cell or are present uniform color areas. After that increased resolution from X20 objective to assess cellularity and cell composition (inflammatory cells, epithelial cells, spindle cells, etc..) of otic exudative.

Then individual cells were evaluated and compared with other cells in the exudate using X40 objective (to increase resolution, the smear applying cedar oil). The study aimed to establish quantitative reference and correlate them with clinical data.

Results of cytological examination of otic exudation from dogs with external otitis confirmed, in most cases, a significant microbial activity. Thus, **42,85%** of cases of external otitis were found in number yeast cells increased, while bacteria (hull and / or bacilli) were present in only **14,28%** majority of cases. The presence of neutrophils, the lymphocytes and phagocytes particularly bacteria show a real infection and not abundant growth of commensal microorganisms. In one case it was found increased number of epithelial cells exfoliate, (but where microbial activity was not well represented). It is possible that some cases of acute OE, early to see only clinical ceruminous secretion and smear it is associated with an increased number of keratinocytes. Cytological examination of exudative ear dogs collected from the ear, may represent an important and necessary investigation to identify causes leading to external otitis.

CHAPTER 10

Research on the effectiveness of otic antiseptic solutions in erythematous-ceruminous otitis in dogs

The objective of this study was to evaluate clinic, therapeutic, antimicrobial activity and tolerance of two antiseptic solutions for use in dogs with external erythematous ceruminous otitis. An important aspect in the management of otitis in dogs covered the study clinical activity, antimicrobial and antiseptic solutions Scrapers tolerance in dogs with OE, which has been made between October 2006-1 mar. 2007.

In this period **120 dogs** were examined in the Clinic of Infectious Diseases of the FMV Timișoara and various veterinary offices in Timișoara. Canine patients were examined clinically for diagnosis of external otitis. Confirmation of EO was determined by history, clinical examination and by examining the external auditory duct with otoscope, in 50 dogs from many breeds.

In this study were included dogs belonging to every race, regardless of sex, with EO Er-C, associated with the yeast of the genus *Malassezia*, or bacteria. For this study were excluded dogs with parasitic OE with *Otodectes* mites, than those treated with antifungal, antibacterial and anti-inflammatory substances, topical or systemic means. In order to test two otic antiseptic solutions, the 50 dogs were divided into two groups: **25** were treated with OTISEPT and **25** were treated with OTTO CLEANS.

The difference in chemical composition of both products has motivated us to choose these two products manufactured in our country and available on the market of veterinary medicine.

The clinical condition of the ears was assessed on the first day (day 0) after 7 days (day 7) and after 14 days of treatment (day 14). The assessment was based on five clinical parameters: the amount of exudate, erythema, stenosis, and abraded ear flag and pain.

For both products use, the main efficacy criterion was the reduction in population of microorganisms (yeasts and / or bacteria) in the external ear canal after 1 and 2 weeks of treatment respectively. Secondary criterion was to improve the clinical condition of dogs affected by EO.

After a week from the first administration in the OTISEPT treated group, clinical discomfort decreased by **64,00%** (16/25) of cases of OE, while the population of microorganisms was reduced to **56,00%** (14 / 25) of cases. In **32,00%** (8 / 25) of cases of OE considered for the study, OTISEPT treatment proved ineffective, clinical and microbiological aspects remain unchanged.

After 14 days of treatment, **92,00%** (23/25) of cases of dogs affected the clinical condition improved EO and initial clinical signs have disappeared, and at **8%** (2 /25) of cases of EO and clinical aspects population of microorganisms in the external ear canal remained the same after two weeks of treatment.

The population of microorganisms was reduced and ranged within normal flora of the external ear canal of dogs with normal ears. During the study were not detected phenomena of intolerance or secondary effects to the OTISEPT product.

In this study, both antiseptic otic solutions, used separately, twice daily for 2 weeks, have improved very well and have significantly reduced the clinical aspects of bacterial and fungal flora in most dogs with infectious OE.

Solutions tested, reduced signs of inflammation in the ear, shown by reducing redness, swelling and abraded the external ear canal. In addition, resulted in the suppression of pain, ear scratching, and head shaking that were present in most of the dogs.

The persistence of small amounts of exudate in the ear canal at the end of treatment, may reflect the fluidity of the product and remain inflated ceruminous gland activity. Therefore it is confirmed that regular use of antiseptic otic solutions is an important therapeutic component of the management of external otitis in dogs.

This effect, in large measure, lead to cerumen removal, bacterial toxins, the degenerate and scrape cells and free fatty acids, may all contribute to the occurrence of infection and stimulate the emergence of an inflammatory process.

CHAPTER 11

The risk of zoonotic pathogen involved in external otitis in dogs

Pets play an important role in society throughout the world. Although pets offer significant benefits, there is a potential danger associated with them. However, any animal owner and / or any service provider non animal health, not sufficiently aware of the potential transmission of zoonotic diseases from these animals.

All animals have a specific parasitic and microbial flora, which in some way have a zoonotic potential. As seen, the most common pathogens isolated from external otitis in dogs include *Staphylococcus intermedius*, *Malassezia pachydermatis*, *Pseudomonas aeruginosa*, *Proteus* spp. and beta hemolytic Group G streptococci.

To determine the impact of major zoonotic microbial etiological agents involved in the pathogenesis of external otitis in the dog, the pet owners to imagine a final study, and based on some recently published studies on the implications of *Staphylococcus intermedius*, *Malassezia pachydermatis* and other external otitis pathogens in humans and other pathological entities.

The study was conducted in collaboration with Pediatric ORL Clinic and Adult ORL Bega Timisoara Municipal Hospital, respectively with Bioclinica laboratory analysis of Timisoara, in the period May 2007 - May 2009.

Were selected for the study of human patients with purulent serous otitis and who presented for consultation and were dog owners.

After collecting data from history, was intended to establish a correlation between ear disease and the presence of owners with a condition similar to dogs or skin disorders.

Subsequently the dog owners were subjected to clinical examination, in the Infectious Diseases Clinic of the FMV Timișoara, to establish clinical status and to detect possible infections of the ears or skin.

Of the **42 samples** from human patients who have external otitis and which were involved in this study were isolated microorganisms potentially zoonotic namely *Staphylococcus intermedius*, beta hemolytic streptococci (group G) and *Malassezia pachydermatis*. Positive samples were in number 15 of 42, representing **35,71%**.

Positive for *Staphylococcus intermedius* were all **15 samples** (35.71%), beta hemolytic streptococci were present in **9 samples** (9/42), which is **21,42%** and yeasts from *Malassezia pachydermatis* species were present in **7 samples** (7/42) meaning **16,67%**. In **4 samples** were isolated by **two** germs associated with zoonotic

potential, namely in **3 cases**, *Staphylococcus intermedius* in combination with group G streptococci and in **one case** *Malassezia pachydermatis* and *Staphylococcus intermedius*.

Of the **52 samples** collected from dogs taken into study were isolated pathogens with considerable zoonotic risk but other bacteria were not quantified, (having no epidemiological importance in the study conducted).

Samples were derived from both dogs with skin disorders and from the clinically healthy. Thus the 52 samples were distributed as follows: 2 / 52 came from acute external otitis, 7 / 52 in chronic recurrent external otitis, 3 / 52 of piodermatitis, 4 / 52 in various allergic diseases, 8 / 52 of seborrheic skin disorders and 28/52 of clinically healthy dogs.

Also, there were isolated 27 strains of *S. intermedius*, 18 strains of group G streptococci and 22 strains of *Malassezia pachydermatis*. The percentage of strains of *S. intermedius* isolated by skin disorders were distributed as follows: clinically healthy 12/28, representing 23.07%, 7 / 8 in seborrheic samples (13.46%), 3 / 4 in samples from the disease Allergic skin and in small percentage in other skin disorders and ear.

CHAPTER 12

12.1. GENERAL CONCLUSIONS

From this PHD thesis have been cleared **42** general conclusions.

12.2. ORIGINAL CONTRIBUTIONS

Research results that have been making contributions of this personal thesis and original elements as follows:

- 1. Introducing the incidence of external otitis in dogs and cats in the west of the country.**
- 2. Structure presentation of microbial flora involved in external otitis in dogs and cats in the west of the country.**
- 3. Establishing a methodology supporting the cytological examination used in the diagnosis of external otitis in dogs.**
- 4. Microbiological assessment and management of two antiseptic solutions for the treatment of external otitis in dogs and cats.**
- 5. Highlighting the importance and role of *Malassezia* spp yeasts in the pathogenesis of external otitis in dogs and cats in western Romania.**
- 6. Establishing a correlation regarding the zoonotic impact of dog to man of *Malassezia pachydermatis*, *Staphylococcus intermedius* and *Streptococcus canis*.**

12.3. RECOMMENDATIONS FOR PRACTITIONERS

In this thesis emerged 30 recommendations for veterinary clinicians.

REFERENCES

This PHD thesis is based on 355 bibliographic titles, 31 web sites and 19 titles of original works from the published sentence topic.