Arq. Bras. Med. Vet. Zootec., v.58, n.6, p.1024-1029, 2006

Identification and antimicrobial susceptibility of bacteria isolated from corneal ulcers of dogs

[*Identificação e susceptibilidade antimicrobiana de bactérias isoladas de úlceras de córnea em cães*]

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ABSTRACT

A total of 22 clinical specimens were obtained from 19 dogs with corneal ulcer (16 unilateral and three bilateral) for isolation and antimicrobial susceptibility evaluation of the isolated bacteria. Bacterial growth was observed in 100% of the samples (n=22). *Staphylococcus intermedius* was the predominant species (35.5%), followed by *Corynebacterium xerosis* (19.3%). Gentamicin, ciprofloxacin, chloramphenicol and tobramycin had a high efficacy against all of the isolated bacteria. The results evidenced that 80.7% of the isolates were Gram positive cocci and Gram positive bacilli, and that those microorganisms were sensitive to gentamicin, ciprofloxacin, chloramphenicol and tobramycin.

Keywords**:** dog, corneal ulcer, bacteria

RESUMO

Utilizaram-se 22 amostras de material, obtidas de 19 cães com úlcera de córnea, sendo 16 unilaterais e três bilaterais, para isolamento e avaliação da susceptibilidade antimicrobiana das bactérias isoladas. Observou-se crescimento bacteriano em 100% das amostras (n=22). A espécie predominante foi *Staphylococcus intermedius* (35,5%) seguido de *Corynebacterium xerosis* (19,3%). Gentamicina, ciprofloxacina, cloranfenicol e tobramicina apresentaram alta eficácia contra todas as bactérias isoladas. Os resultados evidenciam que 80,7% dos isolados foram cocos e bacilos Gram positivos e que estes microrganismos foram sensíveis à gentamicina, ciprofloxacina, cloranfenicol e tobramicina.

Palavras-chave**:** cão, úlcera de córnea, bactéria

INTRODUCTION

Corneal ulcer is a common disease in domestic animals, especially dogs. Although the corneal ulcer usually has a traumatic origin, it may rapidly become contaminated with bacteria (Slatter, 2001). The corneal ulcer is characterized by a loss of corneal epithelium and activation of stromal fibroblasts, leading to swelling of the stroma, and migration of inflammatory cells, with a subsequently loss of corneal integrity and transparency (Suzuki et al., 2003). Once the ulcer is infected, the healing process is retarded and can lead to an endophthalmitis, or glaucoma (Gelatt, 2000). Therefore, treatment with antibiotics must be initiated when corneal ulcers are diagnosed.

Recebido em 23 de fevereiro de 2005
Aceito em 28 de julho de 2006
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For this purpose, an association of antibiotics should be used to improve their spectrum. Slatter (2001) and Gelatt (2000) recommended the use of ophthalmic preparations containing neomycin, bacitracin and polymyxin B. However, if a melting ulcer is present, gentamicin, tobramycin, ciprofloxacin and amikacin should be the antibiotics of choice (Slatter, 2001; Suzuki et al., 2003; Whitley and Gilger, 1999).

The antibiotic therapy is selected based on the culture and susceptibility of antimicrobial tests of the isolated bacteria (Moore et al., 1988). However, sometimes these tests are not performed, whether due to their cost or because the treatment must be initiated before their results. In these cases, the choice of the drug could be based on the incidence of pathogens at the location, and its probable sensitivity to the drug (Gelatt, 2000). Therefore, the aims of this study were to identify the microorganisms involved in corneal ulcers in dogs and their respective susceptibility to antimicrobials.

MATERIAL AND METHODS

The dogs included in this investigation were submitted to previous clinical and ophthalmic examinations, using a penlight, a direct ophthalmoscope and a slit-lamp. Corneal ulcers were detected by fluorescein stain (Fig. 1). This study included 19 dogs with unilateral or bilateral corneal ulcer. A total of 22 clinical specimens were obtained from 3 bilateral and 16 unilateral corneal ulcers, between October 2002 and February 2003. The samples were obtained in collaboration with six veterinary practices in the city of Fortaleza, Ceará. The age, breed, sex and clinical data of the animals were recorded and are listed in Table 1. Three dogs had already received antimicrobial treatment (two dogs were prescripted tobramycin three times daily and one dog chloramphenicol two times daily).

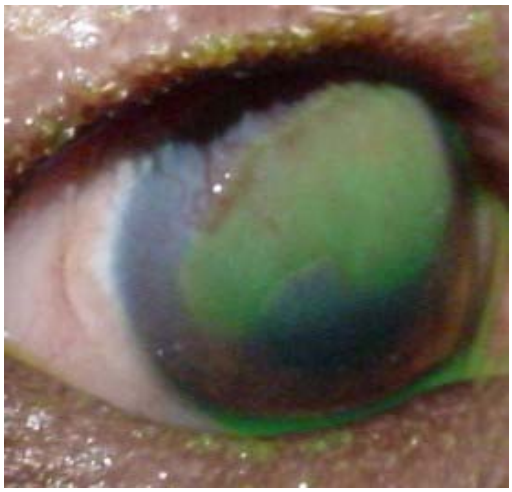

Figure 1. Eye of a Sharpei with entropion and corneal ulcer, evidenced by fluorescein stain.

Table 1. Ulcerative keratitis in dogs according to breed, sex, age, habitat, clinical data and affected eye

Breed	Sex	Age (months)	Habitat	Clinical data	Affected eye
Poodle (toy)	Male	72	Indoor	Renal failure	Left eye
Poodle (toy)	Female	15	Indoor	Healthy	Left eye
Poodle (toy)	Female	28	Indoor	Healthy	Right eye
Poodle (toy)	Male	1	Indoor	Healthy	Right eye
Pekinese	Male	60	Indoor	KCS	Left eye
Pekinese	Male	120	Indoor	KCS	Bilateral
Pekinese	Male	132	Indoor	KCS	Bilateral
Mixed breed	Female	55	Indoor	Healthy	Right eye
Mixed breed	Female	74	Indoor	KCS	Bilateral
Pinscher	Male	29	Indoor	Healthy	Left eye
Pinscher	Female	11	Indoor	Healthy	Right eye
English Cocker Spaniel	Female	42	Indoor	KCS	Left eye
German Shepherd	Male	1	Outdoor	Healthy	Left eye
York Shire Terrier	Male	54	Indoor	Dermatophytosis	Right eye
Sharpei	Male	13	Indoor	Entropion	Left eye
Boxer	Male	8	Outdoor	Demodicosis	Right eye
Schnauzer	Male	108	Indoor	Healthy	Right eye
English Bulldog	Male	27	Outdoor	Entropion	Right eye
Rottweiler	Female	48	Outdoor	Healthy	Right eye

KCS = keratoconjunctivitis sicca.

Clinical specimens were obtained from each corneal ulcer by corneal scraping after topical anesthesia with proparacaine 0.5%. The samples were placed directly into brain-heart-infusion (BHI) broth and transported to a medical mycology specialized center.

The clinical specimens were incubated at 37ºC. After 24 hours the samples were subcultured on blood-agar, chocolate-agar and MacConkey agar and incubated at 37ºC for 48 hours. The blood-agar plates were incubated in an atmosphere of 5% CO_2. The clinical specimens were daily examined for evidencing of microbial growth. The plates not presenting microbial growth within the incubation period (24-48h) were considered negative. The isolates were then purified, Gram-stained and identified by morphological characteristics and the use of standard biochemical tests (Koneman et al., 2001).

Bacteria were tested for antimicrobial susceptibility using the Kirby-Bauer disk-diffusion method, according to Performance… (1999). The following antibiotics were tested for all isolates: gentamicin, chloramphenicol, trimethoprim plus sulfadiazine, erythromycin, tobramycin and ciprofloxacin.

RESULTS

Bacteria were recovered from all of the 22 samples from corneal ulcers. A single bacterial species was isolated in 14 specimens (63.6%), while eight (36.4%) samples presented two or three different microorganisms.

A total of 31 isolates were recovered, the predominant group being Gram-positive cocci (51.7%), followed by Gram-positive bacilli (29%) and Gram-negative bacilli (19.3%). *Staphylococcus* spp. was the most frequently isolated genus (45.2%), followed by *Corynebacterium* spp., which represented 22.5% of the isolates. The results for each species are seen in Table 2.

Table 2**.** Bacterial isolates from corneal ulcers, presenting the number and percentage of each bacterial species

Organism	Isolates	
	#	%
Cocci Gram positive		
S. intermedius	11	35.5
S. aureus	2	6.5
S. cohnii	1	3.2
Streptococcus viridans group	2	6.5
Bacilli Gram positive		
Bacillus spp.	2	6.5
C. xerosis	6	19.3
C. afermentans	1	3.2
Bacilli Gram negative		
Enterobacter cloacae	2	6.5
Klebsiella pneumoniae	1	3.2
Escherichia coli	1	3.2
Pseudomonas aeruginosa	1	3.2
Acinetobacter lwoffi	1	3.2

Four antibiotics had high efficacy against the isolates: gentamicin (96.8%), ciprofloxacin (96.8%), chloramphenicol (93.5%) and tobramycin (90.3%). Trimethoprim plus sulfadiazine and erythromycin were effective only against 61.3% and 32.3% of the isolates, respectively. The results of susceptibility testing for each species are indicated in Table 3.

All animals received antimicrobial treatment (tobramycin, at least six times daily) associated with acetylcystein (20% solution) or autologous serum (nine dogs). The dogs with entropion were submitted to surgery to correct the eyelid disorder and the five dogs with keratoconjunctivitis sicca received concomitant treatment with topical cyclosporine. Conjunctival flap was performed in eight dogs to prevent corneal perforation and to improve corneal healing. In all animals the ulcerative keratitis healed between one and four weeks of treatment with minimum scar formation.

Table 3. Results of the antimicrobial susceptibility test, according to the isolated species, presenting the number and percentage of each bacteria resistant, intermediate or sensible for each drug tested

Drug	Sensitivity	Bacteria																							
		S. interm.		*S. aureus*		*S. cohnii*		*S. viridans* group		*C. xerosis*		*C. aferm.*		*Bacillus spp.*		*E. cloacae*		*E. coli*		*P. aerug.*		*K. pneum.*		*A. lowffi*	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Gentamicin	R	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	10	91	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
	Total	11	100	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
Chloramphenicol	R	2	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	9	82	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
	Total	11	100	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
Trimethoprim plus sulfadiazine	R	5	45	0	0	0	0	0	0	4	67	0	0	2	100	0	0	0	0	1	100	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	6	54	2	100	1	100	2	100	2	33	1	100	0	0	2	100	1	100	0	0	1	100	1	100
	Total	11	100	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
Erythromycin	R	10	91	2	100	0	0	2	100	4	67	1	100	2	100	2	100	1	100	1	100	1	100	1	100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	1	9	0	0	1	100	0	0	2	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	11	100	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
Tobramycin	R	1	9	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0
	S	10	91	2	100	0	0	2	100	6	100	1	100	2	100	2	100	0	0	1	100	1	100	1	100
	Total	11	100	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100
Ciprofloxacin	R	0	0	0	0	0	0	0	0	1	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	11	100	2	100	1	100	2	100	5	83	1	100	2	100	2	100	1	100	1	100	1	100	1	100
	Total	11	100	2	100	1	100	2	100	6	100	1	100	2	100	2	100	1	100	1	100	1	100	1	100

S. interm. (Staphylococcus intermedius); S. viridans (*Streptococcus viridans* group); *C. xerosis (Corynebacterium xerosis), C. aferm. (Corynebacterium afermentans); E. cloacae (Enterobacter cloacae); E. coli (Escherichia coli); P. aerug. (Pseudomonas aeruginosa); K. pneum. (Klebsiella pneumoniae); A. lwoffi (Acinetobacter lwoffi)*.
R=resistant; I=intermediate; S=sensible.

DISCUSSION

Salisbury et al. (1995) presented similar results of this study by studying dogs with keratoconjuntivitis sicca (KCS). They reported that 93% of the eyes presented bacterial growth. However, Gerding Jr. et al. (1988) recovered bacteria only from 66.2% of the samples from dogs with external eye diseases in the United States and only 6% of the positive specimens showed two or more microorganisms. These variations could be a result of the different sampling technique, since these authors used a moistened cotton swab while, in this study, the cornea was scraped with a proper spatula.

In this work, five dogs with corneal ulcer also presented KCS, which causes desiccation and inflammation of the conjunctiva and cornea. Tear deficiency decreases nutritional factors, growth factors, and antibacterial enzymes (lactoferrin, lysozyme, peroxidase), which encourage the growth of bacteria (Kaswan and Fullard, 1993; Whitley, 2000). This could explain a higher incidence of positive cultures from the cornea of dogs with KCS (93%) reported by Salisbury et al. (1995), even though they also used swabs for sampling collection.

Twelve breeds were represented in the present study. The most representative were poodle and pekinese (three animals each). From the three animals with bilateral corneal ulcer, one was a mixed breed and two were pekinese and all presented KCS. However, there was no significative difference between the breeds and the incidence of corneal ulcer.

The frequency of the corneal ulcer microorganisms found in this study is similar to previous studies related to different animals (Gerding Jr. and Kakoma, 1990; Kodikara et al., 1999). Gram-positive microorganisms (80.7%) predominated over Gram-negative bacteria (19.3%) and the genus *Staphylococcus* spp. was the most isolated (45.2%). Similar results were found in healthy dogs in São Paulo (43.9%) (Teixeira et al., 2002), from dogs with external eye diseases in Florida – USA (69.0%) (Salisbury et al., 1995), and Illinois – USA (39.3%) (Gerding et al., 1988). *S. intermedius* represented 35.5% of the isolates in this research. Gerding Jr. et al. (1988) reported that *S. intermedius* was also the most representative bacterial species, however, with only 17.3% of the isolates.

According to Gerding et al. (1988), the most effective antibiotics for *Staphylococcus* spp. were bacitracin, gentamicin and tobramycin; while chloramphenicol and erythromycin for *Streptococcus* spp.. However, data from the present study showed that erythromycin was not effective against the isolated *Streptococcus* spp.

Moore et al. (1995) considered chloramphenicol a less desirable initial choice to treat corneal ulcers since resistant *Pseudomonas* infections may develop soon after topical treatment is initiated. In addition, some strains of *P. aeruginosa* are resistant to gentamicin and close monitoring is really important when using this antibiotic (Moore et al., 1995). However, in this study there were no signs of *P. aeruginosa* resistance to chloramphenicol or gentamicin, probably due to a small number of samples or a less evident selective pressure.

The treatment for a corneal ulcer should be based on culture and antimicrobial susceptibility testing. However, very often the choice of an antimicrobial therapy has to be taken before obtaining results of the antimicrobial susceptibility tests. Therefore, knowledge of the most commonly isolated bacteria in the canine eye from a specific geographic area is important to provide the most efficacious antibiotic treatment. In this study, the results showed that 80.7% of the isolates were Gram positive cocci and Gram positive bacilli, and those species were sensitive to gentamicin, ciprofloxacin, chloramphenicol and tobramycin. Therefore, for those regions, they are the antimicrobials of choice when ulcerative keratitis is present.

ACKNOWLEGMENTS

The authors would like to acknowledge Professor André J. Monteiro from Departamento de Estatística e Matemática Aplicada at the UFC and Maria F.S. Teixeira, post-graduation student at UECE, for the collaboration given in the present work.

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