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Otodine®

Tris-EDTA and 0.15% chlorhexidine as the sole antimicrobial treatment in canine otitis externa.

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In vivo efficacy of a commercial ear cleanser.

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Abstract

The aim of this study was to evaluate the *in vivo* efficacy of an ear cleaner containing Tris-EDTA and 0.15% chlorhexidine (Otodine®) as the sole form of antimicrobial treatment. Nineteen dog ears with clinical signs of otitis externa were treated with Otodine® twice a day for 10 days. The ear canal was examined by otoscopy, cytology and microbiology before treatment (day 1), at the end of the treatment period (day 11) and one week after (day 18). In 18 cases (95%), a significant reduction in inflammation, exudation and discomfort was observed from day 1 to days 11 and 18 (one-way ANOVA t test, p range from 0.0564 to 0.9354). Fourteen cases (74%) were cured successfully as indicated by disappearance of all presenting symptoms, 50% or higher reduction of the clinical scores on both days 11 and 18, normal cytology and owner's satisfaction with treatment. The mid-term success rate was 63% since two of these dogs had relapses during the four weeks following the end of treatment. Four of the five dog ears not responding to treatment had a confirmed or suspected underlying disorder. The results showed that Otodine® can be used successfully as a first choice for treatment of otitis externa without any additional antibacterial or antifungal therapy. Considering the frequent recourse to antibiotics for treatment of otitis externa, the use of ear antiseptics as the sole form of antimicrobial treatment may be a useful therapeutic approach to minimize antibiotic usage and selection of antibiotic resistant bacteria in dogs.

Introduction

Otits externa (OE) is one of the most common reasons for pet owners to seek veterinary advice for their dogs. There are three components in the pathogenesis of OE: i) *predisposing factors* such as stenosis of the ear canal, excessive moisture and presence of irritating substances; ii) *primary causes* that initiate the inflammation process, such as parasites, foreign bodies, allergies and hypersensitivity disorders; and iii) *perpetuating factors* that prevent OE from resolving, such as overgrowth of bacteria and yeast as well as otitis media.1 Therapy traditionally consists of topical or systemic administration of antibacterials, antifungals, corticosteroids or any combination of these. The recent emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in small animals2-4 supports the need for alternative therapeutic approaches to eliminate such multi-drug resistant bacteria and to reduce the antibiotic selective pressure that favours their spread. Antiseptics represent a valid alternative to systemic antibiotics in dermatological infections that can be treated topically.

In contrast with systemic antibiotics, antiseptics act primarily at the site of infection without selecting for antibiotic resistance at body sites where most bacteria reside, for example the intestinal tract. A recent study has shown that an ear cleaner containing Tris-EDTA and 0.15% chlorhexidine (Otodine®, ICF, Cremona, Italy) has excellent *in vitro* activity against common pathogenic organisms associated with OE, including methicillin-resistant staphylococci.5 In the study reported here, the *in vivo* efficacy of Otodine® as the only form of antimicrobial treatment of canine OE was evaluated on the basis of clinical signs (inflammation, presence of exudate and discomfort), cytological examination and microbiological culture.

Materials and Methods

Selection of animals

The 17 dogs selected for this study attended the veterinary hospital Västra Djursjukhuset, Gothenburg, Sweden, between the 28th of September and the 16th of November 2009. The dogs were selected among consecutive cases showing clinical signs of OE. As an additional inclusion criterion, a written consent was required for participation in the study. Various small, large and mixed breeds were included in the selection, with Cocker Spaniels (n=3) and French bulldogs (n=3) being the only breeds represented by more than one dog. The dogs were between 14 months and 16 years of age, with an average of 4 years. Ten dogs were previously treated for OE and received antibiotic treatment for either OE or other infection within the last year.

One Cocker Spaniel and one French bulldog had bilateral OE, whereas the remaining 15 dogs had unilateral OE, leading to a total of nineteen ear cases (Table 1).

Treatment protocol

Each dog was treated twice a day for 10 days. The ear canal was cleaned with Otodine® and cotton wool, and subsequently filled with Otodine® and given a gentle rub into the base of the ear. After 5 min of exposure, the excess of product was removed using cotton wool. At the start of the treatment protocol, eight dogs were given orally prednisolone (Prednisolon Pfizer, 0.5mg/kg q24 for 3 days) or carboprofene (Rimadyl®, Orion Pharma, 4mg/kg q24 for 3 days) to relieve itch or discomfort (Table 1). One allergic dog (Alice) showing signs of pododermatitis was treated locally with Malaseb shampoo (DVM Pharmaceuticals, Inc.,USA). If signs of worsening were observed at any point during treatment, the case was recorded as a treatment failure and the dog treated with antibiotics and investigated for underlying disease.

Clinical and microbiological examination

The ear canal was evaluated by handled otoscopy and cytology immediately before treatment (day 1), at the end (day 11) and one week after the end (day 18). A scale from 0 to 3 was used to evaluate i) inflammation (0, inflammation; 1, light erythema; 2, moderate erythema and/or swelling; 3, severe erythema and/or swelling); ii) amount of exudate visible by otoscopy and on cotton swab (0, no exudate; 1, light exudate; 2, plenty exudate; 3, profuse exudate seen on direct inspection); and iii) discomfort (0, patient not bothered when having ear examined by otoscope or cleaned by cotton swab; 1, patient rejecting having ear examined; 2, patient vocalizing occasionally when having ear examined; 3, patient vocalizing constantly when having ear examined).

At each visit smears were prepared on glass slides and stained using Hemacolor® (Merck, Darmstadt, Germany). 50 High power fields (HPF) were evaluated on each slide, and an average number of microorganisms were calculated and rounded up or down to the nearest number divided by 10. The presence of at least five yeast organisms or 25 bacteria per high-powered field was considered suggestive of microbial overgrowth.6-7 A second sterile swab was used to collect exudate from the vertical part of ear canal and transported to the laboratory in transport medium (Copan Innovation, Venturi Transystem®, Italy). The swabs were inoculated onto blood agar (meat agar with 5% bovine blood) and Sabouraud agar with 0,1% chloramphenicol to evaluate the growth of bacteria and Malassezia, respectively. Bacteria were identified on the basis of colony morphology on blood agar, Gram staining and standard biochemical tests (cytochrome oxydase, catalase, coagulase, glucose and mannitol fermentation). The identification of suspected *Malassezia* colonies growing on Sabouraud agar was confirmed on the basis of cell morphology by phase-contrast microscopy.
Treatment success was defined as a 50% or higher reduction of the sum of the clinical scores on both days 11 and 18 accompanied by resolution of all presenting symptoms, absence of microbial overgrowth on cytological examination and owner's satisfaction with treatment outcome. Low numbers of *Malassezia* (≤ 10 yeast organisms per field) exceeding the cut-off value proposed for abnormal cytology (≥ 5 yeast organisms per field) by Ginel et al.6 were disregarded in the absence of any signs of inflammation, exudate and discomfort. In order to evaluate medium -term success of treatment, all cases regarded as cured on day 18 were followed up for a period of four weeks by phone interviews to the owners.

The owners were asked by the clinician in charge for the study whether their dog showed any residual signs of OE and if the infection had been cured in their opinion.

Statistical analysis
A one-way ANOVA with t-test was calculated using PROC GLM (SAS version 9.1) to refute or accept the null-hypothesis that the clinical scores of inflammation, exudation and discomfort did not differ between the first visit (day 1) and the two control visits (days 11 and 18).

Results
Overall nine dogs (47%) had an underlying problem contributing to OE. Two dogs had stenotic ear canals, one dog had atopy, one dog had food allergy and two dogs had an unknown allergic component. Two dogs were being investigated to find an underlying disease. One dog had recently gone through a double-sided Zepp operation on the ear canal in order to eliminate its predisposing factor. Two dogs had stenotic ear canals as consequence of chronic inflammation or breed (Molly) or breed predisposition (Theo), while the rest of the dogs presented with acute signs of exudative OE. (Table 1).
The distribution over time of the clinical scores for inflammation, exudate and discomfort are shown in Figures 1, 2 and 3, respectively. The sum of the scores for all dogs on day 1 (n=118) was reduced by 70.3% on day 11 (n=35) and by 67% on day 18 (n=39). All cases but one showed a statistically significant improvement on the basis of the clinical scores (one way ANOVA with t test, p range from 0.0564 to 0.9354), as well as a reduction in the number of microorganisms observed by cytological examination (Table 2).

Treatment was successful in 14 (74%) of the 19 cases. Treatment failure was observed in only one (Molly) of the seven dogs without history of otitis and such a dog had stenotic ears. Four of the five cases considered as treatment failures had an underlying cause contributing to the infection not being cleared (Table 1).

Based on microbiological culture, one case was sterile throughout the study, 12 were sterile on day 11 and eight were sterile on day 18. For all sterile samples the cytology results indicated absence of microbial overgrowth. The relationships between clinical scores, cytology and laboratory culture results are shown in Table 2. Significant improvement of clinical signs and concomitant detection of bacteria were observed in three and five cases on days 11 and 18, respectively (Table 2). In five cases, pure cultures of ubiquitous bacteria that are generally not associated with canine OE, namely *Bacillus*, *Acinetobacter* and *Branhamella*, were obtained on days 11 or 18 despite the fact that these bacteria were not isolated on day 1. These microbiological findings were considered as clinically irrelevant since they were not associated with persistence of the clinical symptoms and rod-shaped cells typical of these species were not visualized by cytology. Treatment of these cases was recorded as successful, also in consideration of the fact that no relapse was observed within 4 weeks after the end of treatment. Despite the clinical improvement observed on both days 11 and 18, one case (Theo) was regarded as a treatment failure due to persistence of original symptoms according to the owner's experience and additional detection of large numbers of *S. pseudintermedius* by microbiological culture.

S. pseudintermedius and *Malassezia* were the most prevalent organisms isolated from 17 and nine samples, respectively.

Other bacteria included *Corynebacterium auriscanis* (n=5), coagulase-negative *staphylococci* (n=5), *Pseudomonas aeruginosa* (n=4), *Bacillus spp.* (n=3), *Acinetobacter spp.* (n=2), *Branhamella spp.* (n=2), *Streptococcus canis* (n=1) and *Enterobacteriaceae* (n=1). Nine out of nineteen samples (47%) had two concurrent microbial components and one case (5,2%) was sterile throughout the study.

Discussion

The present study indicates that OE can be controlled using Tris-EDTA and chlorhexidine in approximately three out of four cases without the use of antibiotics or antifungals. All dogs but one improved their clinical symptoms along the treatment period, and 14 of them had resolved infection one week after the end of treatment on the basis of clinical examination, cytology and owner's satisfaction. Only five of the 10 culture-positive cases observed at this time point were considered treatment failures. The other five dogs showed no clinical signs and the bacteria detected in their ear canal were considered as either commensals or contaminants. In support of this interpretation, it should be noted that most bacterial isolates recovered from these dogs on day 18 (*Bacillus*, *C. auriscanis*, *Acinetobacter* and *Branhamella*) were not isolated from the same dogs on days 1 and 11 (Table 2). These bacterial species are ubiquitous and generally not regarded as primary pathogens in canine OE. Altogether the results confirm that culture should not be used as the sole means of monitoring response to therapy.7

The isolation frequencies of the different microorganisms found in this study were similar to those previously reported by other authors.8

Results

Good cure rates (67 to 88%) were observed in cases associated with *S. pseudintermedius* and *Malassezia*, which are the most common microorganisms associated with OE. Considering that the presence of exudate in the infected ear canal may result in antimicrobial dilution and interfere with in vivo antimicrobial activity, the results of this clinical study are comparable to the laboratory findings of a previous study of Otodine®, which showed effective in vitro killing of microorganisms commonly encountered in OE.5 Since both *S. pseudintermedius* and *Malassezia* were shown to be highly susceptible in vitro (MBC = 23/0.8 µg/ml of chlorhexidine/Tris-EDTA), failure of treatment with these microorganisms is likely due to specific host or disease factors rather than to bacterial resistance. The treatment failure observed in the only case associated with *P. aeruginosa* was also unexpected on the basis of *in vitro* susceptibility data since this Gram-negative species was previously reported to display minimum bactericidal concentrations ranging between 188/6 and 47/1.5 µg/ml of chlorhexidine/Tris-EDTA, which are well below the in-use concentrations of the two product's components.5 Treatment of larger numbers of dogs infected with *P. aeruginosa* is required to evaluate the *in vivo* efficacy of Otodine® against this pathogen.

Of the seven dogs not responding to treatment (n=5) or relapsing after treatment success (n=2), six were diagnosed with or under investigation for an underlying disorder. Their underlying disease did probably play a large part in why their infection could not be cleared, or relapsed within 2 to 4 weeks after the end of treatment. Among the nine dogs having an underlying disorder, only three cleared the infection and did not have a relapse.

Interestingly, three of the five cases that were regarded as treatment failures were subsequently treated with topical antibiotics without obtaining resolution of infection (data not shown). These findings indicate that neither Otodine® nor other antimicrobial product can be used as a sole treatment in these cases. Patients with predisposing or primary causes need to have these disorders investigated and addressed in addition to antimicrobial therapy.

The main limitation of this study is that no control group was given placebo or standard antibiotic treatment. It cannot be excluded that some of the cases would have progressed or even resolved without treatment with Otodine®. Furthermore, the design of the study does not allow comparison of the treatment outcome between the product tested and other possible antimicrobial formulations or treatment regimens. The outcome was recorded as success or failure one week after a standard treatment period of 10 days despite the fact that some cases could have required longer periods of treatment. Even though the scores reported in this study might not be useful if compared to those reported by other clinical trials, the differences observed over time within the same case should be considered as compelling as they were recorded by a single observer and supported by cytological examination, microbiological analysis and owner's reports.

The study shows that Otodine® can be used successfully as a first choice for treatment of OE without any additional antibacterial or antifungal therapy. The use of the product tested in this study or other products with similar formulation as the only form of antimicrobial treatment may contribute to cut down substantially antibiotic usage for treatment of OE.

The authors support the notion that this therapeutic approach may contribute to reduce the antibiotic selection pressure in favor of multi-resistant bacteria. As indicated by a previous study,5 ear cleansers based on chlorhexidine and EDTA are unlikely to co-select for the occurrence of MRSA and MRSP in the staphylococcal commensal flora of the dog since methicillin-resistant and susceptible *staphylococci* are equally susceptible to this antiseptic combination. This aspect is not of minor importance in consideration of the rapid spread of MRSP and MRSA observed in the dog population during the last years and the serious animal welfare problems and therapeutic challenges posed by the emergence of these bacteria in small animal medicine.

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Results

Table 1. Description of the study population and evaluation of treatment outcome in individual dogs. The outcome was assessed on the basis of the reduction of the sum of the clinical scores from day 1 to day 18 (one week after treatment), cytology and owner's perception of treatment outcome. Treatment was regarded as successful if the reduction of the clinical scores was at least 50%, cytology was normal (≤25 bacteria/HPF and ≤10 yeasts/HPF) and the owner perceived treatment as a success. Such dogs were followed up for a period of 4 weeks to study the possible occurrence of relapse.

Dog	Name	Breed	Age	Episodes of otitis during the last year	Last recorded antibiotic treatment	Otitis	Additional therapy	Underlying disorders	Evaluation of treatment outcome on day 18			
									Reduction of clinical score	Cytology	Owner's perception	Relapse
1	Lizz	Dachshound	16 y	0	1 y	Unilateral	Carboprofene	None	83% (6 to 1)	Normal	Success	No
2	Zoe	Flatcoated Retriever	6 y	2	1 m	Unilateral	None	None	100% (4 to 0)	Normal	Success	No
3	Rufssen	Mixed large breed	4 y	0	None	Unilateral	None	None	100% (3 to 0)	Normal	Success	No
4	Sigge	Griffon Belge	2 y	1	None	Unilateral	None	None	67% (9 to 3)	Abnormal	Failure	No
5	Mårten	Labrador Retriever	3 y	0	2 m	Unilateral	None	None	67% (6 to 2)	Normal	Success	No
6	Jackie	Jack Russel Terrier	2 y	0	6 m	Unilateral	Carboprofene	None	60% (5 to 2)	Normal	Success	No
7	Frank	Clumber Spaniel	3 y	2	1 y	Unilateral	None	Atopy	75% (4 to 1)	Normal	Success	No
8	Nanna	French bulldog	1 y	0	None	Unilateral	None	None	100% (6 to 0)	Normal	Success	No
9	Caiser	Wire Vorster	8 y	>3	1 w	Unilateral	Prednisolone	Investigated	None (7 to 7)	Abnormal	Failure	-
10	Tindra	Cocker Spaniel	6 y	10	4 m	Unilateral	None	Double Zepp	14% (7 to 6)	Abnormal	Failure	-
11	Gordon	French Bulldog	4 m	0	None	Bilateral	None	Food allergy	67% (6 to 2)	Normal	Success	No
12	Alice	French Bulldog	2 y	1	9 m	Unilateral	Malaseb	Allergy	89% (9 to 1)	Normal	Success	Yes
13	Theo	Shih Tzu	3 y	0	None	Unilateral	Prednisolone	Stenotic ears	88% (8 to 1)	Normal	Failure	-
14	Love	Cocker Spaniel	3 y	9	8 m	Bilateral	Carboprofene	Investigated	67% (6 to 2)	Normal	Success	Yes
15	Nellie	Portuguese Waterdog	1 y	1	5 m	Unilateral	Prednisolone	Allergy	50% (8 to 4)	Normal	Success	No
16	Selma	Cocker Spaniel/Poodle	1 y	1	2 m	Unilateral	Prednisolone	None	100% (4 to 0)	Normal	Success	No
17	Molly	Cocker Spaniel	8 y	5	1 m	Unilateral	Prednisolone	Stenotic ears	38% (8 to 5)	Abnormal	Failure	-

Table 2. Clinical scores (inflamation-exudation-discomfort), microbiology results and treatment outcomes in 19 dog ears affected by otitis externa and treated with the ear cleanser Otodine® as the sole form of antimicrobial treatment. Day 1, start of treatment; day 11, end of treatment; day 18, one week after treatment.

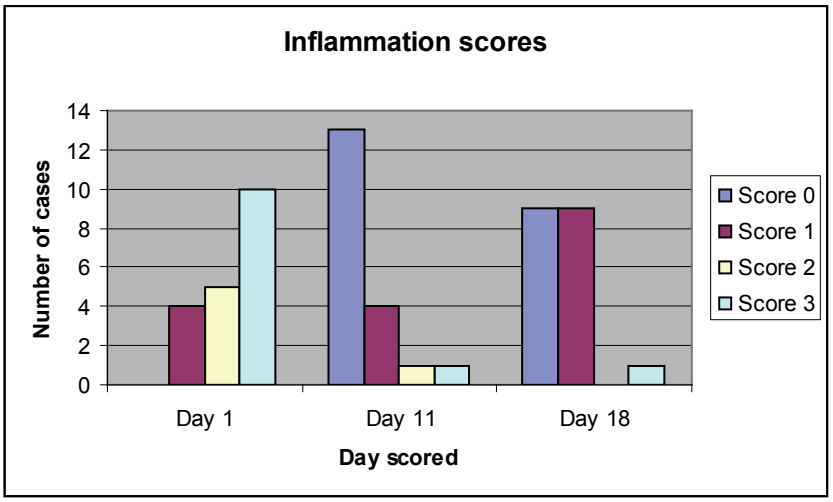
Dog ear	DAY 1			DAY 11			Day 18		
	Score	Cytology[a]	Microbiology	Score	Cytology	Microbiology	Score	Cytylogy	Microbiology
Lizz	1-2-3	10 C, 5 M	*Malassezia*	0-0-0	normC/<1M	Sterile	0-1-0	Norm C/<1 M	*Bacillus*
Zoe	3-1-0	Norm C	Sterile	0-1-0	0	Sterile	0-0-0	0	sterile
Ruffsen	2-0-1	15 C, 100 M	*S. pseudintermedius*, *Malassezia*	0-0-0	Norm C	CoNS[b], *P. aeruginosa*	0-0-0	0	sterile
Sigge	3-3-3	>100C, L	*S. pseudintermedius*, *Bacillus*	0-1-0	Norm C	*S. pseudintermedius*[c]	1-2-0	30C	*S. pseudintermedius*
Mårten	3-2-1	15 M	*Malassezia*	1-1-0	Norm C, 5 M	Sterile	1-1-0	Norm C/ 3 M	*C. auriscanis*[c]
Jackie	2-3-0	7 M	*Malassezia*	0-2-0	5M	*Acinetobacter*[c]	0-2-0	0	Sterile
Frank	2-2-0	5 M	*Bacillus*[c]	0-1-0	0	Sterile	1-0-0	0	Sterile
Nanna	2-3-1	30 C	*Malassezia*	0-1-0	0	Sterile	0-0-0	0	*Acinetobacter*
Cayser	1-3-3	30 C	*S. pseudintermedius*	1-3-3	L	Sterile	1-3-3	30 C/neutrofil	*S. pseudintermedius*
Tinda	3-2-2	30 C, 15M, L	*S. pseudintermedius, Malassezia*	2-1-1	L	Sterile	3-1-2	30C/5M	*S. pseudintermedius, Malassezia*
Gordon R	1-2-3	Norm C/4 M	Mix culture dominated by *C. auriscanis*	0-1-0	Norm C, 3 M	Sterile	0-1-0	Norm C	*S. pseudintermedius*[c]
Gordon L	1-2-3	Norm C/4 M	*S. pseudintermedius*	0-1-0	Norm C	Sterile	0-1-0	Norm C	*S. pseudintermedius*
Alice	3-3-3	100 C	*S. pseudintermedius, Malassezia*	3-1-0	Norm C	*S. pseudintermedius*	0-1-0	Norm C	Sterile
Theo	3-3-2	> 500 C/R	*C. auriscanis*, Enterobactericeae	0-1-0	Norm C	Sterile	1-0-0	Norm C	*S. pseudintermedius*
Love R	3-2-1	>100 C/R	*C. auriscanis*, CoNS[b]	0-2-0	Norm C	Sterile	1-1-0	Norm C	Sterile
Love L	2-3-1	>100 C/R	*C. auriscanis*, CoNS[b]	0-2-0	5 C, <1M	*Branhamella*	1-1-0	Norm C	Sterile
Nellie	3-2-3	15 C, 10 M	*S. pseudintermedius, Malassezia*	1-0-2	3 M	Sterile	1-1-2	2 M	*Branhamella*
Selma	3-1-0	30 M	*S. pseudintermedius*	0-0-0	10 M	*S. pseudintermedius*	0-0-0	8 M	Sterile
Molly	3-3-2	>500 R	*P. aeruginosa*, CoNS[b]	1-1-1	0	*P. aeruginosa*	1-2-2	>100 rods	*P. aeruginosa*, *S. canis*, CoNS[b]

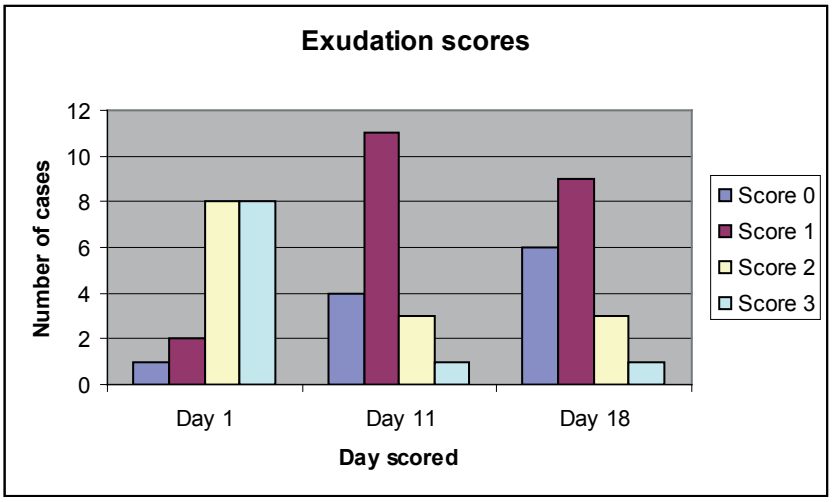
[a] C, number of cocci per HPF: M, number of Malassezia per HPFo; R, number of rods per HPF, L, presence of leukocytes ; Norm C, small clusters of cocci located on the top of the keratocytes only

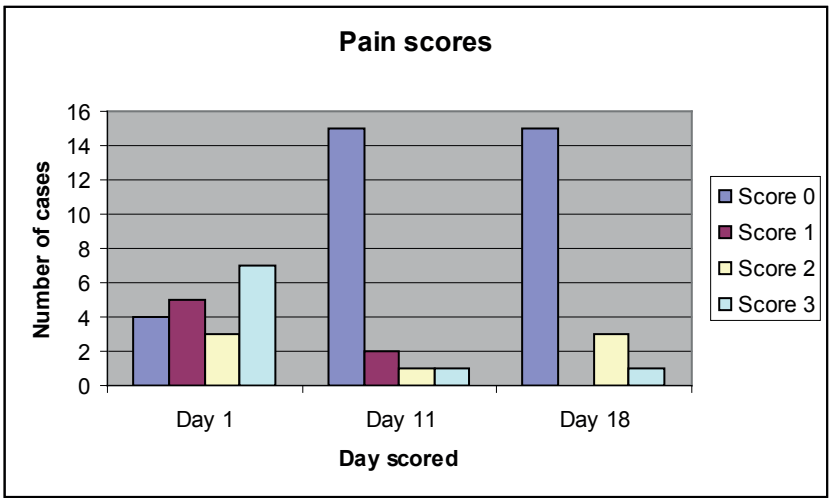
[b] CoNS, coagulase -negative staphylococci

[c] ≤ 10 CFU detected on blood agar

Figure 1. Distribution of clinical scores for inflammation, exudation and discomfort on days 1, 11 and 18. For all three clinical signs, the scores were higher before treatment (day 1) than immediately after treatment (day 11) and one week after cessation of treatment (day 18).







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