BACTERIOLOGICAL AND CLINICAL STUDIES ON PERIANAL FISTULA IN DOGS

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The present study was conducted to investigate bacteriological inhabitants, their antibiotic sensitivity and management protocols in clinical cases of perianal fistula in dogs. The condition was more prevalent (50%) in German shepherd dogs as compared to other breeds of dog and male animals were more predisposed to the condition as compARED TO females. Al the cases were positive for bacterial infection, however, 75% of the healthy animals also had the presence of bacterial micro-organisms in their perineal region. Proteus species was the most commonly isolated organism from the lesions of the affected animals followed by *S. pyogenes.* Cefotaxime was found to be the most effective antibiotics against the isolated organisms. Treatment with specific antibiotics resulted in good outcome, however, cryosurgery and administration of cyclosporine led to recovery in complicated cases.

Key words: Antibiotic sensitivity, Bacterial isolation, Dogs, Furunculosis, Perianal fistula

Introduction

The Perianal fistula sometimes described as anal furunculosis is characterized by chronic inflammation, ulceration and sinus formation in perianal and perirectal tissues (Griffiths *et al.,* 1999). Clinical signs of perianal fistulas include tenesmus, purulent discharge, self mutation, dyschezia, constipation, weight loss, lethargy, faecal incontinence, pain on examination of tail and perianal region and perianal itching (Mathews and Sukhiani, 1997; Ellison, 1995; Houlton, 1980). Typical lesions comprise multiple ulcerated, arborising sinuses (Matushek and Rosin, 1991). Although any breed of the dog may be affected, German Shepherd dogs are most predisposed to perianal fistual (Day and Weaver, 1992). The pathogenesis of perianal fistula remains poorly understood although there is increasing evidence suggesting an immune-mediated aetiology. Histopathological and bacteriological studies indicated that the inflammatory process is initially sterile (Killingworth *et al.,* 1988) and characterised by infiltration of lymphoid cells, plasma cells and eosinophils, consistent with immunological activation (Day and Weaver, 1992). Killingworth *et al.* (1988) found that there was 2:1 male to female ratio in dogs affected with perianal fistula. They isolated 11 types of bacteria from deep perianal tissues of whcih *E. coli*, *Staphylococcus aureus*, beta-hemolytic *Streptotocci* and *Proteus mirabilis* were the most common. The condition is frustrating to the animal owners and the veterinarians both as it is difficult to manage. Griffiths *et al.* (1999) advocated use of Cyclosporine as the sole treatment for perianal fistual. Surgical excision of residual fistulas and sac remnants, combined with immunosuppressive and antimicrobial therapy followed by surgery minimised potential morbidity associated with aggressive use of either medical or surgical treatment alone. In addition to antimicrobials the use of chemical cauterization, deroofing and pulguration, cryosurgery, tail amputation, surgical excision of all diseased tissue and laser excision have also been recommended for the treatment of perianal fistula (Ellison, 1995). The present study was undertaken to find out the bacterial contaminants of the perianl fitulous lesions, their antibiotic sensitivity and management of perianal fistula in dogs.

Materials and Methods

The study was conducted on eight clinical cases of perianal fistula presented to IVRI, polyclinic. The history regarding the age, sex, breed, onset and duration of the condition was recorded in all the cases. All the animals were subjected to thorough clinical examination, which included recording of heart rate, rectal temperature, respiratory rate, colour of mucous membrane, and condition and extent of the lesion. The Swab samples were collected from the lesion site of each animal. Similar numbers of samples were also collected from the perianal

area of the healthy animals to compare the bacteriological inhabitants between the affected and healthy animals. The bacterial isolates were subjected to antibiotic sensitivity testing to find most suitable antibiotics for each animal. The animals were then treated using the suitable medical/ surgical protocol. However, 2 animals did not present for post operative follow up after 7 days and hence had to be excluded from the study. Thus for the evaluation of treatment only six animals were included but anamnesis and initial clinical data is presented about all the animals.

Microbiological study

Swab samples were collected using sterilized hiculture collection device (VS) PW044 (Himedia Laboratories Pvt. Limited, Mumbai) which were stored immediately in sterile test tubes. In all the cases of perianal fistula the samples were collected on day 0 (before commencement of treatment), and then on days 3, 7 and 15 after the initiation of the treatment. The control samples for these cases were taken only once. These swabs were moistened in sterile nutrient broth in sterile test tubes and later on were inocualted onto blood/nutrient agar and MacConkey's agar plates and were incubated at 37oC till the growth appeared. Further processing for purification and typing of bacteria was done as per standard procedures (Carter, 1995).

Antibiotic sensitivity test was done in order to study the sensitivity pattern of the microorganisms isolated from the samples routienly used antibiotics. A recommended agar diffusion procedure called as Kirby Bauer method was used for testing cultures for antibiotic sensitivity (Bauer, 1966). Discs of those antimicrobial agents for which preparations are commonly avaialble in the market were preferred.

Treatment

Hairs from the perianal region were clipped and cleansing of the perianal region with 5% soution of povidone iodine was done twice daily to reduce suppurative inflammation. The systemic antibiotic (Chosen after the 0'day culture examination and its antibiotic sensitivity test) was started so as to reduce the effects of secondary bacterial infection. Meloxicam was orally given @0.2 mg/kg BID for five days.

In one case, which had developed very deep fistulous tract and did not show the signs of adequate healing, cryo-surgery of the fistulous tract was also performed using a cryoprobe on day 15 of treatment. In another case, having perirectal fibrosis cyclosporine tab (2.0 mg/kg body weight OD, daily) was given in addition to treatment given in other animals.

Results and Discussion

Although condition is reported in other breeds also, German Shephard dogs are reported to being predisposed to the condition (Harvey, 1972; Houlton, 1980). In the present study also 50% cases were GSD and rest were non descript. Increased density of apocrine sweat glands in the cutaneous zone of these anal canal of German Shepherd dogs have been implicated in the development of fistulous tracts (Patricelli *et al.*, 2002). GSD breed is also predisposed to other multiple immune mediated disease due to increased concentration of IL-2, IL-5 (Interleukin-5), IL-12 p40, TNF-□ , TNF-□ in intestinal tissues. Further research has indicated that CD3+ T lymphocyte and IgA and IgG secreting B lymphocytes to be prominent in inflammation of perianal fistula (Hanauer and Smith, 1993).

The age of the affected animals ranged between 7 and 10 years. Male dogs (75%) were affected more frequently than female dogs (25%). The results conformed to the observations of Killingworth *et al.* (1988) who reported more than 60 % males in the affected cases. In 50% dogs the circumference of the perianal region involved was between 180-360^{o} and in remainig 50% dogs it was less than 180°. Many cases (33.33%) were also infested with maggots. A dog exhibiting the signs of constipation had developed anal fibrosis in the perirectal region, which was evident on per rectal examination. Some of the animal owners had started the treatment by their own using turpentine oil, savlon solution, mustard oil etc. However, all the cases were brought to the polyclinic in bad shape.

The isolation of organisms from perianal fistula and control specimen at different intervals is shown in Table 1. Control swabs were positive for microbial culture in 75.00% of the specimens and rests of 25% specimens were free from any

bacterial growth. The micro-organisms isolated from control swabs were *S. pyogenes* (37.5% cases), *S. epidermidis* (25%) and *P. morganii* (12.5% cases). All the organisms isolated were in pure cultures. Among the dogs with perianal fistula, all the samples were found positive for bacterial infection. *Proteus spp.* was very predominant and was isolated from 50% cases of perianal fistula

Fig. 1: A case of perianal fistula in a dog

Fig. 2: Almost complete healing of the case after 15 days of treatment

It was followed by *S. pyogenes* (25.00%). *P. aeruginosa* and *S. aureus* were also cultured from perianal fistula (12.5% each). No multiple organisms were cultured from perianal fistula. The species of *Proteus* bacteria isolated from the cases of perianal fistula was *P. mirabilis* (50%) and *P. morganii* (50%).

Table 1: Isolation of different microorganisms at various intervals from cases of perianal fistuals in dogs

Organism Isolated	**Control**	**No. of isolates on day 0**	**No. of isolates on 3rd day**	**No. of isolates on 7th day**	**No. of isolates on 15th day**
	N=8	**N=8**	**N=7**	**N=6**	**N=6**
***1.Staphylococcus* spp.**	2	1	0	0	0
S. epidermidis	2	0	0	0	0
S. aureus	0	1	0	0	0
2. *Streptococcus pyogenes*	3	2	2	1	1
3. *Proteus* spp.	1	4	2	2	-
P. mirabilis	0	2	2	2	0
P. morganii	1	2	0	0	0
4. *Pseudomonas aeruginosa*	0	1	1	0	0
5. No growth	2	0	2	3	5

The pathogenic organisms were isolated from a very high (75%) number of samples taken around the perianal region even in normal healthy dogs. The most frequently isolated organisms from the anal region of the healthy dogs were *S. pyogenes* and *S. epidermidis*. Devriese and De Pelsmaecker (1987) also reported *S. intermedius* to be

frequently present in anal region. The presence of heavy microbial load in the perineal region may be attributable to the likelyhood of this area to get soiled with faeces and urine. In our study *S. epidermidis* was present in 25% of normal samples. Presence of heavy bacterial load in this region may also predispose the animals to opportunistic infection when there is some decrease in animal's immunity level. In affected animals *S. pyogenes* was isolated in 25% cases and *Proteus* spp. in 50% cases. Similar organisms were isolated by the Killingworth *et al* (1988). The isolation of more number of *Coliform* organisms (*Proteus* spp) than the other organisms, suggested the unhygenic condition and soiling of the wound with faecal material and urine.

Cefotaxime was found to be most effective antibiotics against the isolated bacteria, which was followed by ciprofloxacin, oflxacin and enrofloxacin. Ampicillin and oxytetracycline were least effective. Most of the Staphylococcus organisms were sensitive to cefotaxime followed by ofloxacin. *Proteus* spp were most sensitive to cephotaxime followed by ciprofloxacin. *S. pyogenes* organism was most sensitive to ciprofloxacin followed by cefotaxime. *P. aeruginosa* was most sensitive to ciprofloxacin followed by cefotaxime.

The microbial load started to decrease after initiation of the treatment with specific antibiotics and povidone-iodine. Only 5 out of 7 samples were positive for bacterial contamination on day 3. At this interval *S. pyogenes* and *P. mirabilis* were isolated from 2 samples each and *P. aeruginosa* was isolated from one sample. Infection reduced further by day 7 and only 3/6 samples were positive for isoaltion of *P. mirabilis* in 2 cases and *S. pyogenes* in 1 case. By day 15, the infection had almost completely subsided and only 1/6 sample was positive for *S. pyogenes.* The presence of *Streptococcus pyogenes* in one culture even on day 15 could not be considered as threat of infection since it was also isolated from 3/8 samples taken from the normal healthy dogs.

The mean rectal temperature on day 0 was 102.967±0.433oF, which was within the normal temperature range in dogs and remained near the base value until the end. The mean heart rate was 89.833±6.483 on day 0. It did not change much during the treatment period. The Mean RR on day 0 was 23.1667±1.225, which was within normal range. During posttreatment period RR fluctuated slightly below the base line values. The colour of mucous membrane was normal in all the cases and general condition of each case was good.

Type of exudate on day 0 ranged from serous to purulent. The type of exudate was between fibrinous and serous on day 3 and 7. Type of exudation changed further and the type of exudation varied between none and serous on day 15. The quantity of exudate ranged between slight and moderate on day 0. The quantity of exudate decreased gradually and had almost ceased in all the cases except in one case where slight exudation was still present on 15th day. A gradual decrease in the swelling was recorded at all the post-treatment intervals. No peripheral swelling was observed on day 7 and 15. By the day 15 the perianal fistula disappeared almost completely in 4 dogs. Cryosurgery was applied in one dog. It resulted in sloughing of the skin and healing by 2nd intension. The case in which cyclosporine drug was started also showed good response to treatment by day 30 but anal fibrosis was still evident. A residual lesion may still be present even after long term treatment with cyclosporine (Hardie *et al.*, 2005).

Gradual decrease in the type of exudate, quantity of exudate, swelling and depth of the perianal fistulas at different intervals reflected the progressive remission of the disease. A satisfactory recovery from the disease was in agreement with the results of antibiotic therapy used by the Mathews and Sukhiani (1997). Cyclosporine was used for treatment in one case which was non-responsive to antibiotic therapy. Cyclosporine is a T cell specific immunosuppressive agent, which is thought to act by reversibly inhibiting the synthesis and/or release of IL-2 or other cytokines by T-helper cell. Its use in combination with antibiotics in one case showed good results. However, Griffiths *et al*. (1999) feared that the use of antibiotics with cyclosporine may result in incompatible drug interactions. Cryosurgery used in one case after 15 days was aimed at removal of dead tissue and initiation of new tissue growth. It led to sloughing of the tissue and healing took place by 2nd intension.

It was concluded that Proteus species is the most common inhabitant of prianal fistula lesions. Treatment with specific antibiotics against the bacterial infection results in good outcome. However, cryosurgery and administration of cyclosporine may be useful in complicated cases.

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