

## Chronic episodic diarrhoea associated with apparent intestinal colonisation by the yeasts *Saccharomyces cerevisiae* and *Candida famata* in a German shepherd dog

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### ABSTRACT

A 3-year-old German shepherd dog was presented with a history of lifelong episodic diarrhoea. An adverse reaction to food was considered the most likely cause of the diarrhoea. The dog had received prolonged antibiotic therapy for most of its life as well as receiving probiotics containing the yeast *Saccharomyces cerevisiae* (syn. *S. boulardi*) for a year before referral. The probiotic was discontinued 2 months before to referral. Examination and culture of faecal samples identified yeast-like organisms, *S. cerevisiae* and *Candida famata*. *S. cerevisiae* has been isolated from humans in association with predisposing conditions such as prolonged sojourns in hospital, immunosuppression, broad-spectrum antibiotic therapy and prosthetic devices, but is regarded as non-pathogenic in humans and is rarely associated with disease in animals. *C. famata* has been isolated from animals, humans and the environment, but is regarded as a very rare pathogen. No evidence of immunosuppression was found in the dog. The presence of yeasts in the faecal isolates and the history of prolonged use of antibiotics and probiotics with a concurrent adverse reaction to food, suggest that conditions may have occurred within the bowel that made it possible for the yeasts to colonise parts of it. This has apparently not been reported before.

**Key words:** *Candida*, canine, diarrhoea, *Saccharomyces*, yeast.

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### INTRODUCTION

Both *Saccharomyces cerevisiae* (syn. *S. boulardi*) and *Candida famata* are yeasts that are widespread in the environment. *S. cerevisiae* and *S. boulardi* are generally regarded as the same organism but in a recent report they are treated as separate organisms<sup>9</sup>. *S. cerevisiae*, also known as brewer's or baker's yeast, is found on plants, fruits and in soil<sup>2</sup> and is widely used in fermentation in wine and beer production and bakeries, as a nutritional supplement and in humans as a probiotic adjunct to treat or prevent intestinal disorders such as antibiotic-associated diarrhoea and acute infantile diarrhoeal diseases<sup>3,6</sup>. It is also used as a supplement in the treatment of animals suffering from intestinal disorders (RJM). The terms

'probiotic' or 'biotherapeutic agent' have been used in the literature to describe microorganisms that have antagonistic activity towards pathogens *in vivo*<sup>3</sup>.

On account of its long association with humans, *S. cerevisiae* is generally considered to be non-pathogenic, but it has recently been found in a number of cases of sepsis in humans apparently predisposed by factors such as prolonged periods in hospital, immunosuppression, broad-spectrum antibiotic therapy and foreign devices such as central venous catheters and prosthetic cardiac valves<sup>1</sup>. A case of fungaemia in a 1-year-old girl undergoing prolonged treatment with a probiotic containing *S. boulardi* has recently been reported (Pletinex M, Legein J and Van den Plas Y, 1995 as quoted by Elmer *et al.*<sup>3</sup>). As far as we could ascertain, *S. cerevisiae* is an extremely rare pathogen in animals. It has been isolated from milk of a mastitic cow on one occasion<sup>7</sup>.

To determine the potential pathogenicity of certain strains of *S. cerevisiae*, several

studies have been carried out<sup>1,2,8</sup>. Clemens *et al.*<sup>2</sup> compared the pathogenesis of isolates of *S. cerevisiae* associated with human disease with isolates recovered from sources such as natural and industrial fermentation. They defined the virulence of the organism following inoculation into immune-incompetent complement deficient -1 (CD-1) mice as the capacity to show modest proliferation in the brain and prolonged persistence in the tissues of the mice. Their results indicated that the pathogenicity of *S. cerevisiae* is represented by a continuum without a clear distinction between virulence or avirulence. The majority of the clinical isolates were better able to persist *in vivo* than isolates from non-clinical sources and were assigned to groups considered to be virulent or of intermediate virulence. This was in contrast to the non-clinical isolates, where less than half the number tested were assigned to the intermediate virulence group and the rest were considered avirulent. This study was further refined by Byron *et al.*<sup>1</sup> using complement factor-5-deficient (CCD-5) mice. Twelve *S. cerevisiae* isolates, of which 8 were from clinical sources, 2 were genetically defined laboratory strains, 1 was a segregant and 1 was from a non-clinical source, were variously categorised as being highly virulent, *i.e.* causing death of more than 80% of the infected mice, of intermediate virulence, causing death of less than 80% of the infected mice, of low virulence in those cases in which the organism proliferated in high numbers in the brains of infected mice that did not die; and avirulent on account of the low numbers of yeast cells recovered from the organs of euthanased mice 14 days after infection. The isolate from the non-clinical source was placed in the intermediate virulence category.

These studies did not show whether clinical isolates of *S. cerevisiae* themselves possessed unusual properties that might differentiate them from avirulent isolates. McCusker *et al.*<sup>8</sup> demonstrated that significant differences in the ability to

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grow at 39 °C and 42 °C were observed when virulent and intermediate groups were compared with the avirulent group. In addition, significant differences in the ability to form pseudohyphae were seen when the avirulent group was compared with the other groups.

*C. famata*, while frequently isolated from air, soil, water, plant material and animals, and human and animal faeces, has been found to be a very rare aetiological agent in disease processes in animals and humans<sup>10</sup>. In animals it has been isolated from the udder of cows with mastitis, genital secretions of ruminants, the mouth of vitamin A-deficient pigs, and a fungal arthritis of the fetlock in a horse<sup>10</sup>.

A case of lifelong episodic diarrhoea in a dog that might have been aggravated by colonisation of the intestinal mucosa by *S. cerevisiae* and *C. famata* is reported here.

### CASE HISTORY

During January 1996 a male German shepherd dog aged 3 years and 4 months, weighing 31.5 kg was presented to the Onderstepoort Veterinary Academic Hospital (OVAH), Faculty of Veterinary Science, University of Pretoria, for examination. It had a history of lifelong episodic diarrhoea and poor appetite. The dog had been examined and treated for gastroenteritis by veterinarians on numerous occasions, as each bout of diarrhoea would not resolve without treatment. During one particular bout of diarrhoea when the dog was about 5 months old, the consulting veterinarian had resorted to a radiographic examination of the abdomen, an exploratory laparotomy and serum biochemistry, but nothing of diagnostic significance was revealed by any of these investigations. On another occasion, when the dog was 9 months old, a faecal examination had revealed the presence of a *Salmonella* sp. During his lifetime he had occasionally been treated for intestinal helminths. The drugs used for treating the diarrhoea varied to some extent but generally included an antibiotic, such as a lincomycin-spectinomycin combination, or a chloramphenicol-oxytetracycline combination, as well as a *per os* formulation containing streptomycin, neomycin, phthalylsulphthiazole, aminopentamide sulphate, kaolin, pectin and electrolytes (Enteritis Suspension, Centaur [Sanvet]). The latter was the most frequently prescribed therapy. The last course of treatment that he had received from a veterinarian was 2.5 months before his presentation to OVAH and consisted of a course of Buscopan Compositum (Janssen Animal Health) and Enteritis Suspension (Centaur [Sanvet]), and an

Table 1: Serum immunoglobulin profile.

Immunoglobulin	Test result	Normal range <sup>4</sup>
IgG (mg/dl)	720	1000–2000
IgA (mg/dl)	47	40–160
IgM (mg/dl)	200	100–200

injection of long-acting penicillin.

The 1st sign that a bout of diarrhoea was imminent was that a soft stool was passed. The stool rapidly became slimy and watery and at times contained blood clots, and the frequency of defecation was considerably increased. Tenesmus generally accompanied defecation. During the episodes occasional vomiting occurred, and the patient's appetite decreased rapidly until he became anorexic. The attacks of diarrhoea were usually triggered by feeding uncooked marrow bones, or food such as small pieces of fatty meat or fowl livers given to him as tidbits.

During his entire life he had been fed various commercial dog foods and for a certain period a spoonful of mincemeat was given each day. He also received half a cup of milk every evening. In order to restore his intestinal flora after bouts of diarrhoea during which antibiotic therapy had been administered, attempts were made to feed him yoghurt. These were, however, fruitless, and as a result, courses (2 capsules twice a day before meals for 5 days) of the probiotic Interflora (Restan Laboratories), which is a preparation of the yeast *Saccharomyces boulardi*, were administered frequently. During the year preceding January 1996 he had received such a course 2 or 3 times a month until its administration was stopped 2 months before his presentation. In addition, he was occasionally given a preparation containing *inter alia*, multivitamins or brewer's yeast (*S. boulardi*). The owner was of the opinion that initially the Interflora had helped to control the diarrhoea and that it even seemed to have a prophylactic effect in that it appeared to prevent an attack of diarrhoea. It had, however, ceased to have this apparent effect, and the bouts of diarrhoea recurred, so administration was curtailed 2 months before presentation to OVAH. During the 2.5 months before presentation to OVAH the patient had lost 3 kg in body weight.

### MATERIALS AND METHODS

After initial clinical examination, the dog was admitted to OVAH. The initial tests comprised full haematology, serum biochemistry, urine and faecal analysis. Culture of faecal material followed standard laboratory practice for aerobic and

anaerobic bacterial and fungal culture. Identification of faecal isolated yeasts was performed using API 20c AUX ver. 2.2 (bioMérieux, France). In addition, trypsin-like-immunoassay (Double antibody canine TLI, Diagnostic Products, Randburg) was performed to determine possible exocrine pancreatic insufficiencies. Abdominal radiographic and ultrasound examination were also performed. Since selective IgA deficiency does occur in German shepherd dogs<sup>4</sup> and the early onset of gastrointestinal signs may have indicated the presence of this condition, serum immunoglobulin levels A, G and M were also determined (VMRD canine radioimmuno diffusion, AEC/Amersham).

### RESULTS

Clinical examination of the dog did not reveal significant abnormalities, although on palpation the abdomen was somewhat tense and superficial lymph nodes were slightly enlarged. His habitus was good. He did pass loose stools with a light pasty appearance, but with normal frequency, on several occasions while in hospital. Apart from mildly raised concentrations of urea, creatinine and albumin, no noteworthy changes were evident in the serum biochemistry blood test. The haematological examination did not reveal any changes considered to be of significance and the TLI failed to show exocrine pancreatic insufficiency. With the exception of a lower than normal value for IgG, his serum gamma globulin values were within the normal range (Table 1).

As the radiographic and ultrasound examination of the abdomen revealed the presence of enlarged cranial mesenteric lymph nodes and liver, a liver aspirate smear was made and examined. This showed that hepatic haemosiderosis was present but there was no cellular reaction associated with it. Faecal flotation examinations, however, proved of interest. These showed the presence of numerous round structures (6–9 µm in diameter) that at first were incorrectly identified as protozoa. These organisms were present during the entire period in hospital. In view of this, the dog was placed on metronidazole 600 mg twice a day p.o. for 7 days until more thorough investigations

of the faeces were carried out. Further examination revealed that these organisms were yeasts, present in large numbers, the majority degenerate and apparently dead. None of the yeasts observed showed budding, which in a viable organism occurs approximately once every 20 minutes. Fungal cultures of the faeces and isolations revealed the presence of *S. cerevisiae* and *C. famata*. Once the yeasts were identified, the dog was given a full course of nystatin (100 000 IU twice a day for 10 days). Repeated faecal analysis and culture did not, however, reveal a reduction in the number of these organisms in the faeces. Owing to the history of adverse reaction to food, the owner was requested to place the patient on a bland diet consisting of rice and mutton for a 10-week trial period. On re-examination 14 days later the diarrhoea had ceased and the yeasts had disappeared from the faeces. A follow-up faecal culture for the presence of yeasts 3 months after discharge from hospital was negative. The serum urea and creatinine were within normal bounds when retested several months later.

## DISCUSSION

In view of the apparent sensitivity of the dog to certain foodstuffs, particularly those containing fat, either an adverse reaction to food together with inflammatory bowel disease (IBD), or an exocrine pancreatic disorder were initially considered as possible primary conditions. Unfortunately the presence of IBD could not be verified, as the owner refused permission for biopsy of the bowel. However, once the animal was placed on a mutton and rice diet as part of an elimination-challenge trial, the diarrhoea ceased and *S. cerevisiae* and *C. famata* were no longer seen in the faeces or on isolation. Re-exposure of the dog after 10 weeks on the elimination diet to previously-mentioned foods elicited the adverse response described in the case

history. This strengthened the contention that an adverse reaction to food was playing a role or was the primary cause<sup>5</sup>.

Following the identification of the very numerous *S. cerevisiae* and *C. famata* and the exclusion by radiographic and ultrasound examination of any gut-associated granulomatous lesion that could have been caused by a fungus, it was assumed that these organisms had colonised an irritated mucosa of the proximal intestine where they had multiplied profusely.

The yeasts *S. cerevisiae* and *C. famata* are both ubiquitous organisms. *C. famata* has, however, been more often associated with disease processes in animals than has *S. cerevisiae*. The pathogenicity of the latter organism isolated from the dog was not investigated but it would be of interest to do so, as well as to determine whether it possesses the properties associated with virulence, such as being able to grow at supra-optimal temperatures and to produce pseudohyphae<sup>8</sup>. As noted above, it has been shown that *S. cerevisiae* originating from non-clinical sources can be pathogenic, albeit in immune-incompetent CD-1 and CD-5 mice<sup>1,2</sup>. No obvious signs of immune incompetence were found in the dog. A possible source of *S. cerevisiae* was the probiotic (Interflora) and/or the brewer's yeast that were administered periodically over a long time. In humans this yeast does pose a potential problem in those with compromised immune function<sup>1</sup>, as well as those suffering from debilitating disease or receiving aggressive antibacterial therapy<sup>2</sup>. Since the dog had received multiple doses of various antibiotics for an extended period and had a history of adverse reaction to certain foods, it seems likely that with repeated administration of probiotics it is possible for these agents to have colonised parts of the bowel.

If the 2 species of yeast did colonise the intestinal mucosa and in fact played a role in aggravating the intestinal irritation, it is not known for how long their effects were

present. The dog's diarrhoea did worsen and he did lose several kg in body weight in the 2.5 months before his admission to hospital. This suggests that their presence might have played a pathogenic role.

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