Yeasts in the gastrointestinal tract of preweaned calves and possible involvement of *Candida glabrata* in neonatal calf diarrhea

D. Elad, J. Brenner, A. Markovics, B. Yakobson, S. Shlomovitz & J. Basan
The "Kimron" Veterinary Institute, P.O. Box 12, Beit Dagan, 50250 Israel

Abstract

To examine the possibility of a mycotic involvement in neonatal calf diarrhea (NCD) the presence of fungi was assessed in (a) the intestinal contents of dead calves and fecal samples submitted for routine laboratory examination, (b) fecal specimens, sampled once in winter and once in summer, of calves raised on 2 farms with different management systems, and (c) mucosal scrapings of various segments of the digestive tract of a diarrheic calf, massively shedding *Candida glabrata.*

C. glabrata was the most prevalent fungal species isolated from the routine samples. It was the only fungus which was shed by the calves on the 2 farms, for continuous, more or less prolonged periods, but exclusively in the winter months. Diarrhea and *C. glabrata* shedding seemed to be associated. *C. glabrata* colonized the abomasum (the functional equivalent of the monogastric stomach) but not the other segments of the digestive tract of the euthanized calf

Based on the findings of this study it seems that while some yeast species may be considered as commensals of the digestive tract of calves, and consequently their isolation from intestinal contents or fecal samples has no clinical significance, others, such as *C. glabrata* may be involved in enteric pathogenic processes. Moreover, characteristics of the culture, previous chemotherapeutic treatments, the animal's age and possibly climatic conditions should be taken into account before deciding on the fungal isolate's clinical relevance. Determination of mycotic involvement in NCD by routine mycological examination of intestinal contents and fecal samples of diarrheic calves may be useful to avoid unnecessary and potentially harmful antibacterial therapy.

Key words: Calves, *Candida glabrata*, diarrhea, yeast

Abbreviations: GIT: Gastrointestinal tract, NCD: Neonatal calf diarrhea

Introduction

Neonatal calf diarrhea (NCD) is arguably the most important pathological entity in causing economic losses due to morbidity and mortality. NCD may be caused by a highly virulent primary pathogen, such as enterotoxigenic *Escherichia coli*, or, more frequently, by mixed infections of various microorganisms [1]. While the role of viruses, bacteria and protozoa in causing NCD has been investigated [2], the possible role of fungi in enteric pathology has received only limited attention. This is probably the result of the assumption that fungi are part of the normal microbial flora of the digestive system [3] and consequently of no clinical relevance, unless they are isolated in conjunction with pathological changes. This assumption, until recently prevailing in human medicine, has been subject to reconsideration. A number of reports published during the last years [4–6] indicate the possibility of connection between fungi, especially yeasts, and cases of diarrhea in human patients, especially children, with no recognizable underlying conditions to justify infection by opportunistic pathogens. Association between yeasts and disorders of the digestive tract in these cases was made by exclusion (no other pathological microorganisms found), the presence of an elevated number of yeasts in the feces, or fol-

lowing failure of antibacterial therapy but successful treatment of the patient with antimycotic drugs.

This study was devised to appraise the possibility of a similar involvement of fungi in NCD.

Materials and methods

To assess the possibility of mycotic involvement in NCD a study consisting of 3 parts was initiated:

1. Assessment of the prevalence of fungi in the gastrointestinal tract (GIT) of calves: During a period of 30 months, the intestinal contents of 147 calves that died after showing clinical signs of NCD and 353 fecal samples of diarrheic calves, up to 1 month of age, which were submitted for routine microbiological examination, were examined mycologically.
2. Study of the clinical significance of fungi in the GIT:
   A field trial in which the presence of mycotic agents in feces of calves of various ages and under different management and climatic conditions was investigated.
3. Examination of the anatomic sites in the GIT to be colonized by fungi and the interaction between these microorganisms and the mucosa:
   One 10 day old diarrheic calf, massively shedding *C. glabrata*, was euthanized and mucosal scrapings of various segments of the digestive tract (abomasum, rumen, duodenum, jejunum, ileum, cecum, colon and rectum) and their contents were examined microscopically and microbiologically. Samples of the segments were examined histopathologically.

Laboratory examinations of samples

Mycology. Approximately 0.1 g of intestinal contents or fecal specimens material were suspended in 3 ml sterile saline. From this suspension, 0.1 ml was inoculated onto CHROMagar (CHROMagar, Paris, France) plates which were incubated at 37 °C [7]. Thus a semiquantitative indication of the number of yeasts present in the samples, as well as the ratio between different yeast species, in case of mixed growth, was obtained. Two yeast colonies (of each species in mixed culture) were subcultured on Sabouraud dextrose agar and identified by the ID 32C system (Bio-Meneux SA, Marcy l'Etoile, France). CHROMagar

plates were kept for 7 days before being discarded as negative. Feed was examined for the presence of mycotic contamination as previously described [8].

Bacteriology. Enterotoxigenic *Escherichia coli*: One loopfull of the above mentioned suspension was inoculated onto E-agar [9] plates, incubated overnight at 37 °C and the resulting cultures tested by the slide agglutination test with anti K-99 monoclonal antibodies (Central Veterinary Laboratory, Weybridge, UK). For salmonella enrichment, tetrathionate broth was added to the rest of the suspension, the tubes were incubated at 37 °C overnight, following which they were inoculated onto McConkey and Brilliant green agar plates. These plates were reincubated for 24 hours under the same conditions and colonies suspected to be *Salmonella* spp. were tested biochemically and serologically (Welcome Diagnostics, Dartford, England).

Virology. The fecal samples were examined for the presence of coronavirus and rotavirus (serogroup A) by ELISA with the Pathasure Bovine Enteritis system (Cambridge Veterinary Sciences Ltd., UK).

Parasitology. Fecal samples and intestinal contents were examined for the presence of cryptosporidia by sugar flotation and modified Ziehl–Neelsen staining [10], as previously described.

Field trial

Experimental design. To assess environmental influence on the epidemiology of yeasts in the digestive tract of calves, 2 dairy farms with different management systems were included in the survey. In addition, based on previous findings that NCD is influenced by seasonal factors [11], samples were taken during the hot summer months (July–August) and the cooler, rainy, winter months (December–January).

Animals and sampling. Twenty four female calves, divided into 4 groups were included in the survey. The groups consisted of:

(a) Six calves on farm A in the summer
(b) Six calves on farm A in the winter
(c) Six calves on farm B in the summer
(d) Six calves on farm B in the winter

Animals were chosen by sequential order of birth. Daily rectal fecal samples were taken during the first 4 days of life and once every 2 days up to 14 days

of age. After that age samples were taken as long as diarrhea and/or yeast shedding persisted. Shedding was assumed to have stopped after 2 consecutive fecal samples negative for yeasts. One week after the last sampling an additional fecal sample was taken to assess whether the absence of yeast from the feces was definitive or not. Specimens were processed within 30 minutes after the sampling.

Management. Feeding practices on both farms included in the survey were similar. Each calf was fed immediately after it's birth 2 liters of first colostrum, followed by additional 2 liters 12 hours later. The colostrum was obtained from the dam on farm A and from a colostrum pool on farm B. On days 2 and 3 the calves were fed second and third day colostrum, respectively, by the same scheme. From the fourth day on, calves were fed 2 liters of commercial milk substitutes (150 g/l), twice daily. Dry food, composed of corn and cotton seed, commercial concentrates, hay and dry milk substitute were available. Water was accessible *ad libitum.*

Farm A was located in an agricultural research facility. Each calf was housed in an individual pen, placed on the ground. Consequently no direct contact between the animals was possible. Highly experienced personnel were in charge of the calves and individual care was given to each calf. The dry food made available to the calves was not medicated.

Farm B was located in a large collective (kibbutz) holding. Calves were kept in raised individual pens up to the age of 3–5 days and then transferred to yards, where 8–10 animals of homologous age groups were reared. Personnel were not as experienced as on farm A (due to frequent substitutions) and individual supervision of each calf was limited. The commercial concentrate incorporated in the dry food, made available to the calves, was oxytetracycline medicated.

Samples of the dry food and milk replacer fed to the calves on each farm were examined for mycotic contamination as previously described [8].

Examination of euthanized calf

One calf, intensely shedding *C. glabrata* was euthanized. Slides of mucosal scrapings of the various segments of the euthanized calf's digestive system were Giemsa stained and examined microscopically. Scrapings as well as contents of each segment were examined microbiologically. PAS stained samples of each segment were examined histopathologically.

To assess the capability of the *C. glabrata* strain isolated from the euthanized calf's abomasum (the functional equivalent of the monogastric stomach) and 4 of the fecal isolates (2 from each farm) to grow under the highly acidic conditions present in this organ – pH 2–2.8 – [12], the strain was suspended in distilled water to a turbidity equaling 2 on the McFarland standard. Of this suspension, 0.1 ml was inoculated into Sabouraud dextrose broth, acidified with HCl to pH values as low as 1.4. The inoculated broth tubes were incubated at 37 °C for 4 days. Growth was examined daily.

Results

Routine examinations

Yeasts were isolated from 26.5% (39/147) of the intestinal contents of dead calves and 38.2% (135/353) of the fecal samples. Twenty six of the isolates (66.7%) from dead calves and 95 (70.4%) of the fecal isolates were identified as *Candida glabrata* (Fig. 1). Other fungal species were isolated on rare occasions: *C. catenulata* ($n = 14$), *C. rugosa* ($n = 11$), *C. krusei* ($n = 11$), *Geotrichium candidum* ($n = 6$), *Trichosporon cutaneum* ($n = 5$), *C. tropicalis* ($n = 3$), *C. kefyr* ($n = 2$), *C. lypolytica* ($n = 1$), *C. colliculosa* ($n = 1$) and *C. parapsilosis* ($n = 1$). Unlike *C. glabrata*, however, cultures of these species consisted of only a few (1–20) colonies. Twenty three (19.0%) of *C. glabrata* positive calves were in their first week of life, 86 (71.1%) in their second week and 12 (9.9%) were between 15 and 30 days old (Fig. 1).

Field trial

Summer months: On farm A, all the calves had diarrhea, starting at the end of their first week of life, for 6 to 10 days. On farm B, only one calf had diarrhea, on days 8 to 12. Microbial findings on both farms were similar: Bacterial pathogens and coronavirus were not found in the calves during this period, rotavirus and cryptosporidia, the latter in very low numbers, were found sporadically during the whole 2 week period but were not associated with the clinical signs. *Candida krusei, C. rugosa* and *Trichosporon cutaneum* were isolated from some fecal samples but neither the number of colonies (1–5) nor the duration of the yeasts' presence (1–2 days) indicated colonization by these microorganisms. A few colonies of *C. glabrata* were isolated from the feces of one calf from farm A on day
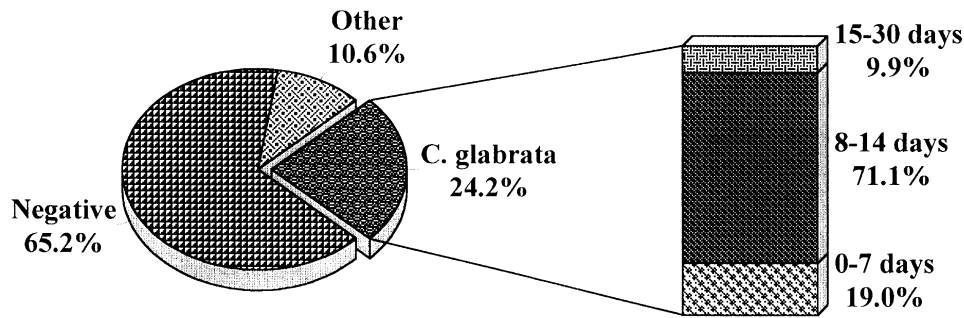
Figure 1. Epidemiology of *Candida glabrata* in calves: prevalence and age distribution.

12, following a week of diarrhea. Two days later this calf died of incarcerated diaphragmatic hernia and its intestinal contents yielded rich cultures of *C. glabrata*.

Winter months: The results of the survey during this period are more complex then those of the summer months and are presented in Fig. 2. All 6 calves on farm A had diarrhea which started between day 3 and 6 of their lives. *C. glabrata* was isolated from all but one of the calves. Shedding of this yeast started usually 3–5 days after the onset of diarrhea. The periods of *C. glabrata* shedding and intermittent diarrhea were generally overlapping. This phenomenon is especially evident in calf No. 010, which had diarrhea for an exceptionally long period (until 55 days of age) and shed *C. glabrata* for an equally prolonged time span (until 45 days of age). Based on the semi quantitative character of the culture method, an initial increase and a final decrease in *C. glabrata* colony counts was observed. During most of the shedding period, however, yeast cultures were extremely rich and consisted of confluent or semiconfluent growth of the *C. glabrata*. *C. krusei, C. catenulata*, rotavirus and cryptosporidia were found sporadically (Fig. 2) in low numbers and for not more than a few consecutive days. One exception was calf No. 009 (Fig. 2), from which mixed cultures of *C. glabrata, C. krusei* and *C. catenulata* were isolated (the latter two in numbers higher than in other cases) for 10 days, during which it was treated with antibiotics (penicillin/streptomycin) against a mouth infection caused by *Actinomyces pyogenes*.

On farm B, 5 out of the 6 calves had diarrhea but its duration was generally shorter than on farm A, and did not exceed the age of 22 days. The animals which had diarrhea were the same animals that shed *C. glabrata*. Unlike farm A, the shedding of the yeast started in 4 calves before or concomitantly with the diarrhea. A few colonies (1–5) of *T cutaneum, Geotrichium can-*

didum or *C. rugosa* were isolated once from 2 calves. Rotavirus was found sporadically but cryptosporidia were absent.

Enterotoxigenic *E. coli* and *Salmonella* spp. were not isolated during the survey. *C. glabrata* was not found in samples of the milk replacer or dry feed fed to the calves.

Examination of euthanized calf

No pathological changes were observed at the necropsy of the calf. Cultures of abomasal and intestinal contents (all the segments) yielded rich cultures of *C. glabrata*. A few colonies of *C. krusei* and *C. catenulata* were also present but *C. glabrata* was clearly the dominant yeast species. Microscopic examination of the mucosal scrapings of the various segments showed that the abomasal mucosa was heavily colonized by yeasts. No hyphae, pseudohyphae or elongated yeasts (typical of *C. krusei*) were observed. Only a few yeast cells, were present on the intestinal segments.

The histopathological examination of the various segments confirmed these findings: the abomasum was the only segment to be colonized by yeasts which seemed to interact with the mucosa (Fig. 3). No macroscopic or microscopic pathological changes of the GIT were observed.

All the *C. glabrata* strains examined for acid resistance grew at pH = 2.0 after overnight incubation, and at pH = 1.8 after 2 days.

Enterotoxigenic *E. coli* and *Salmonella* spp. were not isolated from the various segments, rotavirus was not found and cryptosporidia were found in very low numbers (13/cc).
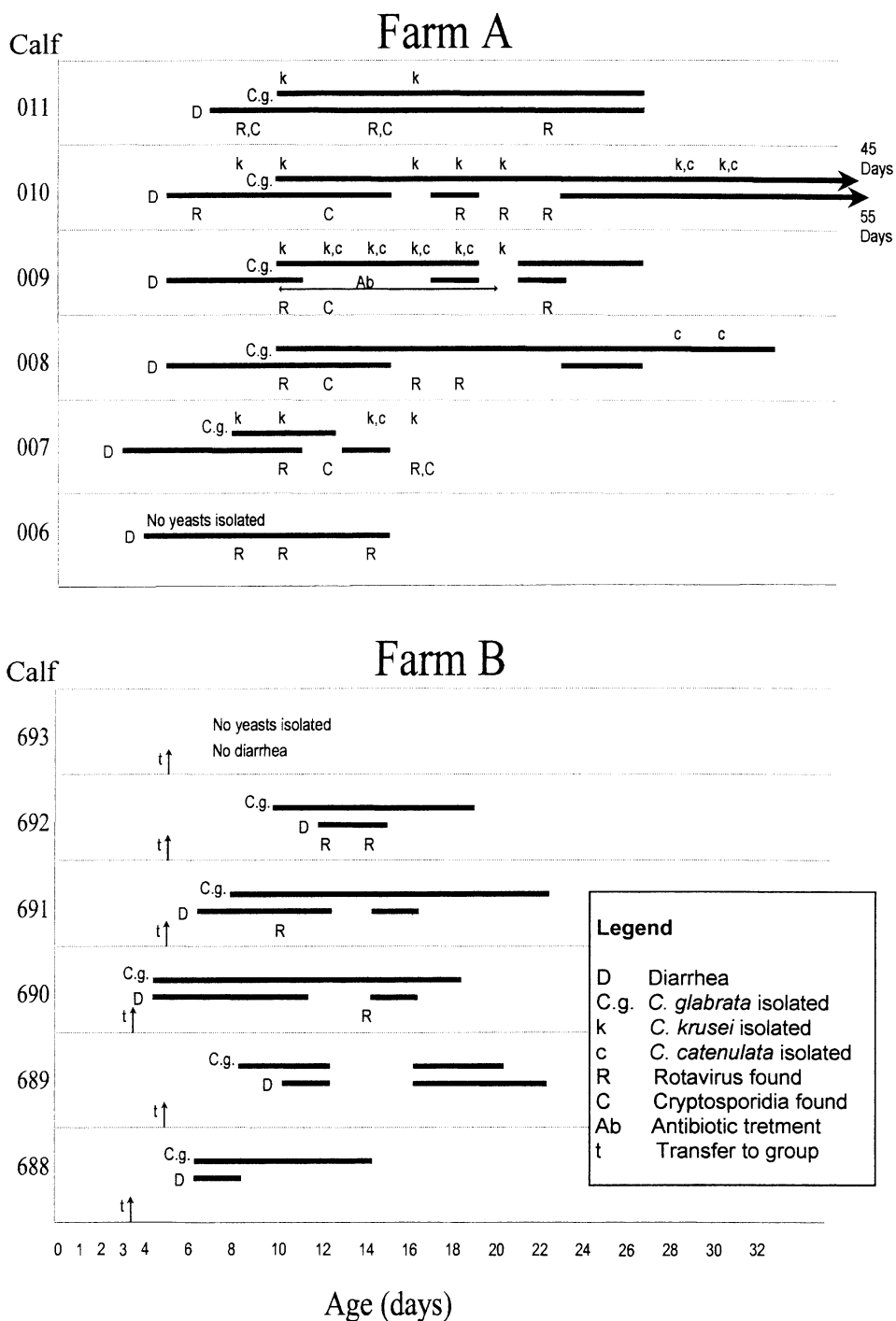
Calf

Farm A

011
k   k
C.g. ━━━━━━━━━━━━━━━━━
D ━━━━━━━━━━━━━━━━━
R,C   R,C   R

45 Days

010
k   k   k   k   k   k,c   k,c
C.g. ━━━━━━━━━━━━━━━━━━━━━━━━━━━━▶
D ━━━━━━━━   ━━━   ━━━━━━━━━━━▶
R   C   R   R   R

55 Days

009
k   k,c   k,c   k,c   k,c   k
C.g. ━━━━━━━━━━━━━━   ━━━━━
D ━━━━━━━
←━━ Ab ━━→
R   C   R

008
c   c
C.g. ━━━━━━━━━━━━━━━━━━━━━
D ━━━━━━   ━━━━
R   C   R   R

007
k   k   k,c   k
C.g. ━━━━━━━   ━━━
D ━━━
R   C   R,C

006
No yeasts isolated
D ━━━━━━━━━━━
R   R   R

Calf

Farm B

693
No yeasts isolated
No diarrhea
t↑

692
C.g. ━━━━━━━━
D ━━━
R   R

691
C.g. ━━━━━━━━━━━━━
D ━━━   ━━
t↑   R

690
C.g. ━━━━━━━━━━━
D ━━━━━   ━━
t↑   R

689
C.g. ━━━━   ━━━━
D ━━   ━━━━━
t↑

688
C.g. ━━━━━━━
D ━━
t↑

Legend

D   Diarrhea
C.g. C. glabrata isolated
k   C. krusei isolated
c   C. catenulata isolated
R   Rotavirus found
C   Cryptosporidia found
Ab   Antibiotic tretment
t   Transfer to group

0 1 2 3 4   6   8   10   12   14   16   18   20   22   24   26   28   30   32

Age (days)

Figure 2. Periods of diarrhea and shedding of *Candida Glabrata* by the calves on the two farms included in the field trial.
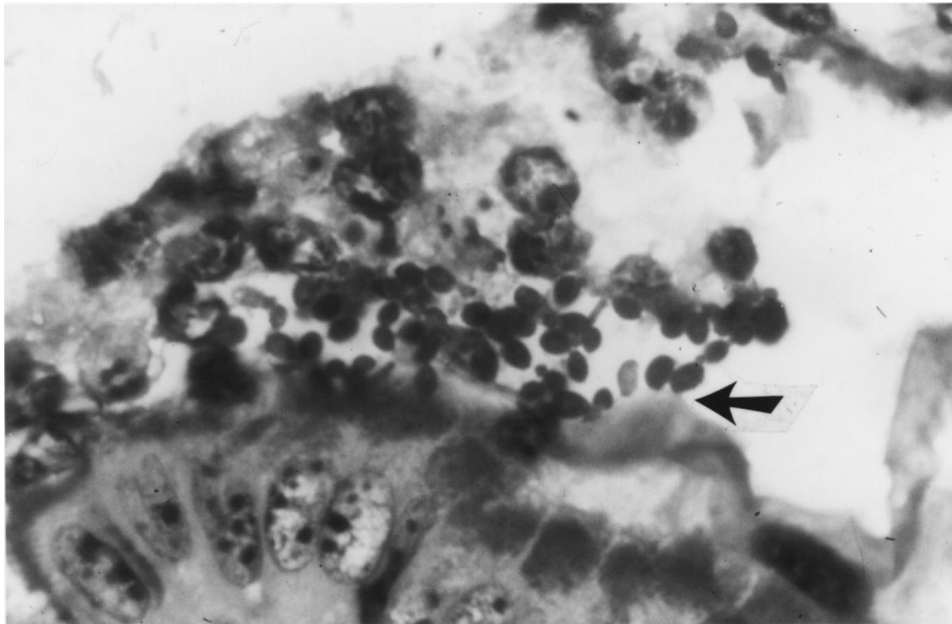
Figure 3. Yeasts colonizing the abomasal mucosa from which *Candida glabrata* was cultured. (PAS stain). Note possible interaction between yeast cells and mucosa (arrow).

Discussion

During the last years fungal diarrhea, especially in children, has received much attention [6]. *C. albicans, C. tropicalis, C. krusei, C. glabrata* and other yeast species have been associated with the syndrome [6]. Various diagnostic criteria have been suggested to differentiate between normal individuals shedding yeasts and patients afflicted with noninvasive fungal diarrhea. The presence of hyphae in the stools [13] is not sufficient as cases of diarrhea in which only budding yeast cells were observed in the feces and which were successfully treated with antimycotic drugs [14] have been reported. Moreover, one of the salient characteristics (and the cause of many taxonomic controversies) of *C. glabrata* is that it does not form either pseudo- or true hyphae. Heavy to confluent growth, such as cultured from the calves in this study, may be considered an indication of non-invasive fungal diarrhea [6].

In our epidemiologic study, yeast were found in the GIT of 34.8% of the examined samples, with *C. glabrata* emerging as the most prevalent species (69.5%) in preweaned calves suffering from NCD. Although it was not possible to obtain sufficient data regarding antibacterial treatment of the dead and diarrheic calves which were examined in the first part of the study, it may be, however, safely assumed that most (if not all) of the former and many of the latter

received such drugs. *C. glabrata* has been reported to be the most prevalent yeast species in young calves, especially if fed with milk replacers, but was not found to be connected with diarrhea [15]. In addition, *C. glabrata* is one of the most prevalent species isolated from human sources, often second only to *C. albicans* [16].

The field trial confirmed the findings of the epidemiologic study as to the prevalence of *C. glabrata*. In addition, it showed that *C. glabrata* was the only yeast present in very high numbers in the feces of young calves for periods longer than 7 days. Other species were present only sporadically and for brief periods. This indicates that the ability of *C. glabrata* to colonize the GIT of young calves under the conditions prevailing during the trial exceed that of other yeast species.

The comparison between diarrhea and presence of *C. glabrata* in the feces of young calves during the winter months indicates a connection between these two observations (Fig. 2). This connection is underscored by the case of calf 010 on farm A, in which both diarrhea and excretion of the fungus were exceptionally prolonged. The nature of this connection is not clear and several possibilities must be considered:

(a) Colonization by *C. glabrata* causes the diarrhea.
   Although no pathological changes were present in

the euthanized calf, other mechanisms, such as fungal metabolites, might be involved. This mode of action would be analogous to the one observed in enterotoxemic colibacillosis, where no or only mild pathologic changes are observed in the GIT during diarrhea [3].

(b) Conditions produced in the digestive tract by the diarrhea favor the proliferation of the yeast. The appearance of *C. glabrata* in the feces of the calf 2 days before its death following an incarcerated diaphragmatic hernia seems to support this possibility.

(c) A common underlying condition, such as interference with the process of establishing a natural microflora, causes both diarrhea and colonization by *C. glabrata*.

Further studies are necessary to clarify the nature of this connection.

The examination of the euthanized calf showed that the abomasum was the only segment of the GIT in which an interaction between the mucosa and the yeasts was observed. The clear predominance of *C. glabrata* in cultures made from these segments indicates that the yeasts observed microscopically are *C. glabrata* as well. This assumption is further substantiated by the morphology (absence of hyphae and pseudohyphae) of the yeast cells. Colonization of the human stomach by yeasts, including *C. glabrata*, has been reported [17, 18], often without the induction of pathological changes [17]. Due to the low pH, the only yeast species able to grow in this organ have to be able to do so under stringent acidic conditions. The *C. glabrata* isolates examined in this study were found comparable to human isolates [17,19], to be highly acid resistant, and thus capable of growth in the abomasum. Higher pH values and a competitive intestinal microflora, established earlier than in the abomasum, are likely causes for the limited colonization of the intestine by *C. glabrata*.

A number of reports dealing with fungal infections of the digestive tract of preweaned ruminants have been published, most indicating the involvement of various yeast species in cases of necrotic abomasitis [20–22]. A common factor to these cases was the treatment of the animals with antibacterial drugs, considered predisposing to fungal infections. Animals included in the field trial, (with the exception of calf 009 on farm A), were not subjected to antibacterial treatment. The medicated dry food made available to the calves on farm B is unlikely to have been ingested in

relevant quantities given the young age of the animals. It is noteworthy that the only period during which yeast species other than *C. glabrata* (*C. krusei* and *C. catenulata*) were isolated for 10 consecutive days was parallel to the period of antibacterial treatment of calf 009 on farm A. This observation, in addition to the self-limiting characteristic of *C. glabrata* colonization in untreated calves seems to support other reports [20–23] which indicate that antibacterial treatment may induce pathological processes in which yeasts are involved.

The source of the *C. glabrata* strains isolated during our study is not clear. It has not been found in the feed samples and it does not seem to remain in the digestive tract of calves for more than a few weeks. The examination of additional potential environmental sources might clarify this point. Oro-fecal transmission of *C. glabrata* between calves is likely. This hypothesis is supported by the earlier onset of *C. glabrata* shedding on farm B than on farm A (see in next paragraph).

Based on the findings of this study it seems that the assumption that yeasts in general are part of the normal flora of the digestive tract of ruminants, and consequently that their isolation from intestinal contents or fecal samples has no clinical significance, should be reconsidered. Some species, such as *C. catenulata, C. rugosa* or *G. candidum*, appeared in our study to be harmless, others, such as *C. glabrata* may be involved in enteric pathogenic processes. Consequently, the yeast species, qualitative (pureness) and quantitative (CFU number) characteristics of the culture, previous chemotherapeutic treatments, the animal's age and possibly climatic conditions should be taken into account before deciding on the fungal isolate's clinical relevance.

We suggest that at least some yeast species should be considered with other potentially pathogenic microorganisms which may cause diarrhea under certain conditions but are not considered primary enteric pathogens. Diarrhea in which yeasts are involved is mild and self limiting. Antibacterial treatment of such cases, might be not only unnecessary but counterproductive, resulting in the intensification of the microbial imbalance in favor of the mycotic flora in the GIT. Massive necrosis of the forestomach mucosa caused by *C. glabrata* infection exacerbated by antibacterial treatment has been reported [22]. Determination of mycotic involvement in NCD by routine mycological examination of intestinal contents and fecal samples of

diarrheic calves may be useful to avoid inappropriate antibacterial therapy.

References

1. Brenner J, Elad D, Markovics A, Grinberg A, Trainin Z. Epidemiological study of neonatal calf diarrhoea in Israel – A one year survey of faecal samples. Isr J Vet Med 1993; 48: 113–116.

2. Brenner J, Elad D, Van Ham M, Markovics A, Perl S. Microbiological and pathological findings in young calves suffering from neonatal diseases. Isr J Vet Med 1995; 50: 21–24.

3. Barker IK, VanDreumel AA, Palmer N. The Alimentary System. In: Jubb KVF, Kennedy PC, Palmer N, eds. Pathology of Domestic Animals, 4th ed., Vol 2, Chapter I, San Diego: Academic Press Inc, 1993

4. Enveani IB, Obi CL, Jokpeyibo M. Prevalence of *Candida* species in Nigerian children with diarrhoea. J Diarrhoeal Dis Res 1994; 12: 133–135.

5. Levine J, Dykoski K. *Candida* associated diarrhea: a syndrome in search of credibility. Clin Inf Dis 1995; 21: 881–886.

6. Talwar P, Chakrabarti A, Chawla A, Mehta S, Walia BNS, Lumar L, Chugj KS. Fungal diarrhoea: association of different fungi and seasonal variation in their incidence. Mycopathologia 1990; 110: 101–105.

7. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J Clin Microbiol 1996; 32: 1923–1929.

8. Elad D, Shpigel NY, Winkler M, Klinger I, Fuchs V, Saran A, Faingold D. Feed contamination with *Candida krusei* as a probable source of mycotic mastitis in dairy cows. J Am Vet Med Assoc 1995; 207: 620–622.

9. Francis DH, Remmers GA, DeZeeuw PS. Production of K88, K99, and 987P antigens by *Escherichia coli* cultured on synthetic and complex media. J Clin Microbiol 1982; 15: 181–183.

10. Markovics A, Elad D, Pipano E. The prevalence of Cryptosporidium in dairy calves in Israel. Isr J Vet Med 1984; 41: 134–139.

11. Brenner J, Orgad U, Burd P, Gat Z. The influence of climate on the mortality of young calves in two different farming systems in Israel. Isr J Vet Med 1989; 45: 32–42.

12. Roy JHB. The Calf 4th ed. London/Boston: Butterworth, 1980: 204.

13. Kozinn PJ, Taschdjian CL. 1962. Enteric candidiasis. Diagnosis and clinical considerations. Pediatrics 1962; 30: 71–85.

14. Kane JG, Chretien JH, Caragusi VF. Diarrhoea caused by *Candida*. Lancet 1976; 1: 335–336.

15. Gedek B. Besiedlung des Verdaungstraktes vom Kalb mit Hefen. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt I Orig 1969; 211: 94–101.

16. Kwon-Chung K.J, Bennett JE. Medical Mycology., Philadelphia: Lea & Febiger, 1993: 282–285.

17. Bernhardt H. Zum Vorkommen von Hefepilzen im Magen des Menschen. Mycosen 1968; 11: 799–806.

18. Staib F. Resistenz von Hefen und hefeartige Pilzen gegen Schwefelsaure. Z Hyg 1958; 145: 26–33.

19. Schonebeck J. Incidence of yeast-like fungi in gastric juice under normal and pathologic conditions. Scand J Gastroent 1968; 3: 351–354.

20. Mills JHL, Hirth RS. Systemic candidiasis in calves on prolonged antibiotic therapy. J Am Vet Med Assoc 1967; 150: 862–870.

21. Neitzke JP, Schiefer B. Incidence of mycotic gastritis in calves up to 30 days of age. Can Vet J 1974; 15: 139–143.

22. Wada Y, Nakaoka Y, Matsui T, Ikeda T. Candidiasis caused by *Candida glabrata* in the forestomachs of a calf J Com Pathol 1994; 111: 315–319.

23. Cross RF, Moorhead PD, Jones JE. *Candida albicans* infection of the forestomachs of a calf J Am Vet Med Assoc 1970; 157: 1325–1330.

Address for correspondence: Daniel Elad, D.V.M., Ph.D. Kimron Veterinary Institute, P.O. Box 12, 50250 Bet-Dagan, Israel
E-mail: elad@agri.huji.ac.il; Fax: (972) 39681753