*Opis przypadku* *Case report*

# Zygomycosis of the abomasum in neonatal calves during treatment of diarrhea caused by Escherichia coli: a case report

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Summary

**An outbreak of neonatal diarrhea occurred at a high producing dairy farm consisting of 460 holstein-friesian cows. Severe diarrhea appeared mostly between 3-5 days of life and despite of intensive treatment, lasted about 7 days and in 5 cases was fatal. Through the previous four months dry cows were fed by hay silage markedly overgrown with mould. From rectal swabs of eight examined calves Escherichia coli strains of different antibiotic susceptibility were isolated. Postmortem examination of two calves showed marked dehydration, enteritis and abomasitis with ulceration. Bacteriological aerobic culture of inner organ samples showed abundant growth of Escherichia coli and from abomasum intensive growth of Candida sp. Histological examination revealed Zygomycetes mycosis of the abomasum wall. Failure/partial failure of passive transfer was found in two other examined calves. A multifactor origin of the diarrhea outbreak was diagnosed. Antibiotic therapy based on susceptibility of isolated E. coli strains and appropriate fluid therapy was conducted. Farm staff was trained to proper colostrum feeding the calves. Additionally, the mould contaminated hay silage was discarded and vaccination of dry cows by E.coli/rotavirus/coronavirus commercial vaccine was performed. The health normalization of the calves was achieved after the next two months of the improved animals' management.**

**Keywords: diarrhoea, abomasum mycosis, Escherichia coli, failure of passive transfer**

Enterotoxic *Escherichia coli* strains are a common cause of neonatal calf diarrhea and result in significant economic loss (1, 19, 24). Antibiotic resistance of diarrheic bacterial strains is an increasing problem in dairy farms caused by repeated, frequently uncontrolled, excessive use of antibiotics. The pathological course may be complicated by fungal infections. In cattle, skin mycosis as well as alimentary tract and lung mycosis occur the most frequently, while mycoses of other organs are rarely diagnosed (2-4, 12, 17, 20, 23). Alimentary tract mycosis is most commonly associated with *Zygomycotina*, such as *Mucor*, *Rhizopus*, and *Absidia* and less frequently with *Candida* and *Aspergillus* spp. In postmortem examination of cattle older than 6 months, systemic mycosis was found in 6.5% of cases (3). The majority of the cases (84.4%) were alimentary tract infections. Pathological changes in the alimentary tract were observed mostly in the rumen (73.7%), omasum (71.1%), abomasum (34.2%), reticulum (21.1%), intestine (15.8%), omaso-abomasal orifice (7.9%), and tongue (2.6%) (3). Simultaneous infection of the forestomach and abomasum occurred in 31.6% of cases. Mucormycosis was found in 94.7%, and aspergillosis in 31.6%. Complicated infections of *Mucorales* and *Aspergillus* spp. were found in 26.3% of cases (10 animals), but 21% (8 animals) showed mixed changes typical of mucormycosis and aspergillosis. In all cases gross postmortem findings were foci of hemorrhagic necrosis. Microscopically, thrombosis, coagulative necrosis and hyphae typical of *Mucorales* or *Aspergillus* spp. were seen. In necropsy of calves under 6 months of age, systemic mycoses were diagnosed in 4.7% of cases (2), of which the majority (63.2%) constituted alimentary tract mycosis. The

highest percentages of the alimentary tract mycoses were related to mucormycosis (91.7%), aspergillosis (41.7%), and candidiasis (9.3%). Similarly to older animals, the pathological changes in mucormycosis and aspergillosis were characterized by focal hemorrhagic necrosis, with a proliferation of hyphae and emboli in the mucosal membrane and muscular layers of the forestomach, abomasum, and small intestine. In the case of omasum candidiasis, mucosal membrane hyperkeratosis with pseudohyphae and microconidia were observed (2). In 33.3% of calves, mixed infection by *Mucorales* and *Aspergillus* spp. was detected. In calves below 30 days of age with alimentary tract lesions at necropsy, 3.8% were mycotic abomasitis and forestomach inflammation (21) and Zygomycota were isolated from the abomasum. Typical mucosal membrane lesions are roundish, red-black foci deeply penetrating all layers of the stomach wall, frequently associated with an extension of the inflammatory process to the omentum and peritoneum. Jensen et al. (12) observed ulcerative mycotic lesions of the largest omasal lamellae. Their location and histopathological pattern suggested that reflux of acidic abomasum content influenced the pathogenesis. Participation of *Aspergillus* spp. and *Zygomycetes* was determined histopathologically. Their differentiation was enabled based on morphological features with conventional and immunofluorescence staining (12). Long term antibiotic treatment in the course of diarrhea, iatrogenic immunosuppression, acidosis, and early weaning are the most important factors predisposing to alimentary tract mycosis in calves (2, 21). Multidirectional pathogenic influence of mycotoxins was found in most species of farm animals. Among the effects of mycotoxins are as follows: immunosuppression associated with inhibition of T- and B-lymphocyte activity, antibody production, disturbances of effector macrophage/neutrophil functions. Consequently, the susceptibility to infection increases (22). The susceptibility of calves to infection also depends on the transfer of passive immunity. Failure of passive transfer increases the risk of diarrhea and respiratory tract infections (5, 6, 8).

The aim of this study was to explain the causes of a severe diarrhea outbreak in neonatal calves in a high-producing dairy farm.

## Case description

In calves born in a dairy farm with 460 high-yielding cows (10 400 kg milk per 305 day lactation), an outbreak of severe diarrhea occurred starting from the end of January to the middle of March, 2007. During the disease outbreak, all newborn calves showed diarrhea. Diarrhea appeared mostly between 3-5 days of life and lasted for about 7 days despite treatment. Large volumes of watery, yellow-gray feces and rapid dehydration of the calves were observed. According to the severity of dehydration, appetite loss, depression and tachypnoe were observed. Inner body temperature was normal. Five cases had a fatal course despite intensive treatment with antibiotics and oral and intravenous rehydration. After several days of treatment, signs of bronchopneumonia appeared in some cases.

**Treatment.** At the start of the diarrhea outbreak the farm veterinarian used the following antimicrobials administered for 7 days with the highest doses recommended by the producer: ampicillin, florfenicol, enrofloxacin, trimethoprim + sulfadoxine, amoxycillin and cefquinom. The antimicrobials were selected for the treatment of different calves, but no significant improvement was achieved. Despite the use of different antimicrobials in combination with intravenous rehydration using Duphalyte (6 ml/kg b.w.), multielectrolyte solution (1000 ml containing: 141.51 mmol $Na^+$, 5.1 mmol $K^+$, 3.60 mmol $Ca^{2+}$, 1.97 mmol $Mg^{2+}$, 109.05 mmol $Cl^-$, 33.95 mmol $CH_3COO^-$, 3.06 mmol $C_6H_5O_7^{3-}$/calf), and 5% glucose (10 ml/kg b.w.) as well as oral rehydration using Boviform, Rehydral, Rehyvet, and Effydral, in some cases, diarrhea did not disappear or in others returned within few days.

Unsuccessful treatment inclined the farm veterinarian to consult the case with staff of the Department of Immunology and Veterinary Preventive Medicine.

**Newborn calves' care.** The newborn calves were moved to individual straw-bedded boxes in a separate room immediately after birth. During the first 7 days of life they were fed three times a day from the bucket with a nipple with their dams' colostrum and milk, and on the respective days twice a day with milk replacer. The cows that calved at night were milked automatically first at 4:00 A.M. Therefore the time of the first calves' colostrum feeding varied between 1-9 hours after birth. Moreover, the calves had free access to water, hay, and a concentrate with a 50% addition of whole corn grains.

**Cows.** For four months before the onset of diarrhea, dry cows had been fed with hay-silage that was markedly overgrown with mould. The owner did not give permission to examine the hay-silage for mycotoxins. In multiparous cows an elevated ratio of paresis puerperalis (> 5% of calvings) and markedly liver steatosis was observed in two necropsied cows that suddenly died. During the two months before the diarrhea outbreak 81 calves were born. Seven stillbirths in primiparous cows were found, but none of the calves died.

**Diagnostic procedures.** Bacteriological culture was performed on 8 rectal swabs from diarrheic calves (Epivet, Faculty Laboratory) according to the procedure PB-09 using propagating and selective media, and antibiotic susceptibility was estimated.

A necropsy was performed on the two calves which died (No. 9-10). Bacteriological culture and histopathological examination of inner organ sections was conducted. Sections of the abomasal wall were fixed in 8% neutral formalin and paraffin slabs were stained by the Hematoxilin & Eosin and Periodic Acid-Schiff methods.

Jugular vein blood samples were taken from seven calves aged 24-60 hours (2 at first visit and 5 one month later) and two from their dams. Within 4 hours after blood sampling blood serum was centrifugated (2,000 g for 10 min at room temperature) and serum was stored until use at –20°C. The presence of elevated haptoglobin (Hp) concentration was estimated by the Spooner method (25). Total serum protein

(Biuret method) and gammaglobulin (paper electrophoresis) were estimated in calves and cows according to Furman et al. (8). Additionally, in the cows' serum asparagine transferase, gamma-glutamyl transferase, non-esterified fatty acids, and betahydroxybutyrate levels were measured using Erba XL-300 analyzer (laboratory Vetlab Wrocław, enzymes' activity and BHB were measured by kinetic reactions; NEFA using ACS-ACOD-MEHA method).

## Results and discussion

Signs of severe dehydration (deeply sunken eyeballs) and emaciation were observed in the two necropsied calves, with the skin of the para-anal region coated with feces. In both cases the abomasum was filled with a large volume of fluid (4-5 L). The mucosal membrane was strongly congested, exfoliated and covered with an increased amount of mucus. Mucosal folds were significantly swollen. On the mucosa surface numerous hemorrhagic foci of different dimensions and areas of cream foci of necrosis surrounded by zones of congestion were seen (Fig. 1). The small intestine was atonic and flaccid and with a watery gray-yellow content. Segments of the mucosal membrane were strongly congested. The mesenteric lymph nodes were enlarged. The liver was regular size, diffuse, irregularly supplied with blood, pale, and of fragile consistency. Other inner organs showed no significant lesions.

Bacteriological aerobic culture of inner organ samples showed abundant growth of *Escherichia coli*. Culture from the abomasal mucosal membrane showed abundant growth of *Candida sp*.

Under microscopic examination, the abomasal mucosal membrane and, to a greater extent, the submucosal membrane were edematous with a diffuse infiltration of neutrophils and eosinophils. Hyphae were growing in numerous places on the surface of the mucosal membrane and penetrated the abomasal wall up to the lamina muscularis mucosae. This induced damage to the glandular structure and necrosis of the mucosal membrane. The regressive changes in the tissue structure were accompanied by massive inflammatory infiltration with a dominance of neutrophils. The blood vessels of the mucosal and submucosal membranes were obstructed by thrombi and in some cases with hyphae (Fig. 2).

Proliferating mycelial hyphae showed features characteristic of *Zygomycetes*: they were wide, of unequal thickness, branched at right angles, and not possessing the transverse septums typical of *Aspergillus* (Fig. 3).

The bovine alimentary tract, and especially the rumen and abomasum, constitute a frequent site of mycotic infection. Mycelial hyphae that proliferate within the stomach wall penetrate the blood vessels, causing microthrombi and microinfarcts. They are visible macroscopically as hemorrhagic-necrotic foci and may be spread to other organs, e.g. liver, lungs, and kidneys. Peyer's patches are the gateway of the fungi's

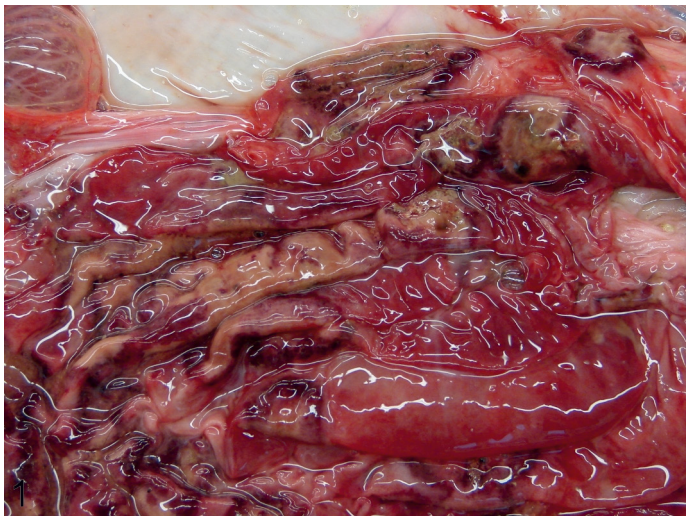

**Fig. 1. Abomasal mucosal membrane. Calf No. 9. Numerous cream foci of superficial necrosis of the mucosal membrane surrounded by zones of congestion**

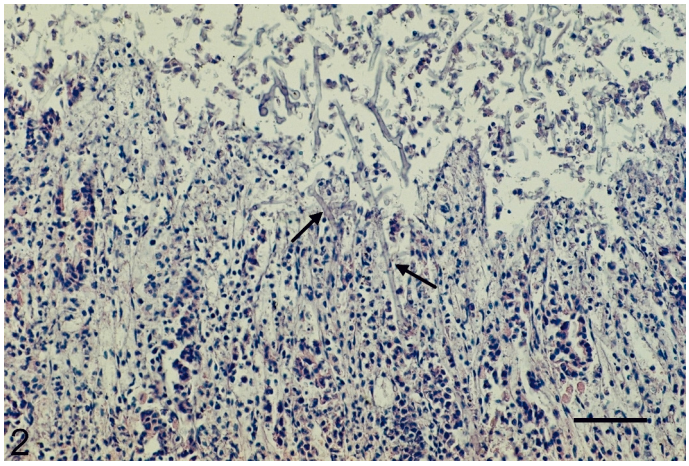

**Fig. 2. Abomasal mucosal membrane. Calf No. 9. Proliferation of mycelial hyphae (arrows) on the surface and deep penetration of the mucosal membrane causing necrosis and massive inflammatory infiltrations. HE. Bar = 50 µm**

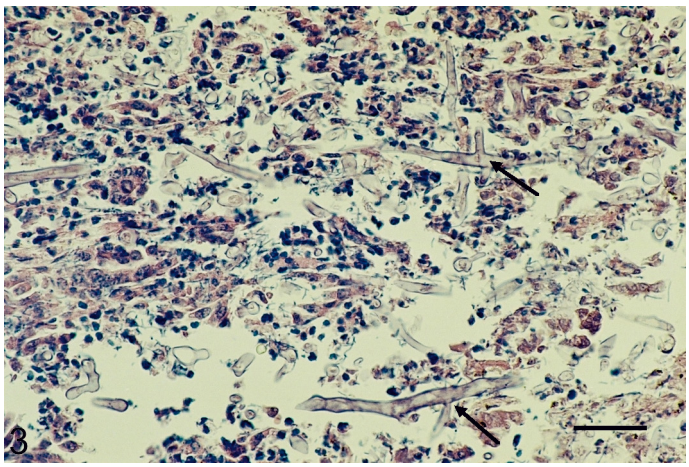

**Fig. 3. Abomasal mucosal membrane. Calf No. 9. Mycelia proliferating within the abomasal mucosal membrane. Characteristic of Zygomycetes broad hyphae of unequal thickness, branching at right angles (arrows), without transverse septums typical of *Aspergillus*. HE. Bar = 25 µm**

penetration into lymphatic vessels (15). The presence of fungi was indicated in 1.9% of abnormal lymph nodes (mainly mesenteric lymph nodes) in slaughtered cattle (16). The limited pathogenicity of fungi needs the cooperation of additional factors enabling invasion. Most commonly small necrotic foci appear in mucosal membrane as a consequence of thrombi that arise during bacterial endotoxemia. They may also be small erosions occurring in the rumen during infectious bovine rhinotracheitis. Other promoting factors are: achlorhydria of the abomasum, immunodeficiency, chronic steroid therapy (15), dysbacteriosis caused by chronic antibiotic therapy, early weaning (2), as well as rumen acidosis, mastitis, puerperium, anemia, emaciation (4), stasis of content in the forestomach, metabolic disturbances, and stress (13).

Mildly elevated aspartate aminotransferase levels (Tab. 2) might resulted from peripartum muscle injuries but significantly elevated gamma-glutamyl transferase levels in both examined dams indicated the hepatocellular injury (18). It may be a late consequence of the suspected exposure of dry cows to mycotoxins (7) due to feeding the dry cows with mould overgrown silage. It was probably not associated with excessive lipomobilization, because of normal non-esterified fatty acids and betahydroxybutyrate levels. It might had a negative influence on the health of fetuses. In experimental exposure of Simmental dairy cows to desoxynivalenon and zearelenon normal gamma-glutamyl transferase and mildly elevated aspartate aminotransferase were observed (10). Elevated Hp concentrations in both dams may result from postparturient inflammation (11). Elevated Hp was also found in serum of 30 hours old calf showing diarrhea, and this protein is not detected earlier than 12-24 hours of acute inflammation course. It shows that acute inflammatory response started at least shortly after birth. The situation was changed in serum samples from 5 calves at the same age taken one month after first visit. Mean gamma-globulin concentration exceeded 14 g/l and may be evaluated as satisfactory (8). Moreover in all 5 calves no elevated Hp was found. It indicates the proper health status.

**Tab. 1. Antibiotic susceptibility of *Escherichia coli* strains* isolated from calves' rectal swabs (14-16.02.2007)**

| Antibiotic | Calves No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Colistin | +++ | +++ | ++ | +++ | + | +++ | +++ | + |
| Lincospectin | ++ | ++ | ++ | ++ | +++ | +++ | +++ | +++ |
| Amoxiklav | + | + | ++ | + | ++ | + | + | + |
| Cefalexin | + | ++ | ++ | ++ | +++ | + | ++ | ++ |
| Cefuroxim | + | ++ | ++ | + | +++ | + | ++ | ++ |
| Gentamycin | | | | | | ++ | | |

Explanation: *Strains were resistant to: amikacin, amoxycillin, ampicillin, chloramphenicol, ciprofloxacin, clindamycin, enrofloxacin, erythromycin, florfenicol, muciprocin, neomycin, norfloxacin, oxytetracycline, penicillin, polymyxin B, rifampicin, streptomycin, sulfonamides, trimethoprim

The presented case is an example of a sequence of events leading to iatrogenic mycosis. The pathological process was initiated by the poor immune status of the calves (the low serum immunoglobulin concentration) and an exposure of the pregnant cows to mycotoxins present in the hay-silage fed during the dry period. The presence of pathogenic *Escherichia coli* strains on the farm induced the outbreak of alimentary tract infection in all the newborn calves. Detected very low γ-globulin concentrations in both calves examined at the age of 24-30 hours (Tab. 2) indicated the significant hazard of a failure of passive transfer (FPT) within this herd (8). This caused high susceptibility to infection.

Antimicrobials administered in the first phase of treatment caused disequilibrium of the alimentary tract's microflora ecosystem in course of *E. coli* infection. This disequilibrium allowed the opportunistic fungal infection. The diagnosis of abomasal mycosis caused by *Zygomycetes* was based in both necropsied calves on histopathological examination. The probability of detecting mycosis caused by *Zygomycetes* by routine histopathological examination is slightly lower than using indirect immunofluorescence, but at least twofold greater than using culture methods (12, 14, 17). In the presented case only *Candida* sp. was

**Tab. 2. Blood serum parameters in calves and cows examined at the first visit**

| Parameter (normal range) | No. 11* | No. 12** | No. 5 (about 24 h old) | No. 7 (about 30 h old) | Nos. 13-17*** |
|---|---|---|---|---|---|
| AST IU/L (58-100) | 105 | 102 | Nd | Nd | Nd |
| GGT IU/L (3.55-30.67) | 73.2 | 44.6 | Nd | Nd | Nd |
| NEFA mmol/L (< 0.6) | 0.54 | 0.53 | Nd | Nd | Nd |
| BHB mmol/L (< 1.4 mmol/L) | 0.25 | 0.92 | Nd | Nd | Nd |
| Haptoglobin g/L | > 0.2 | > 0.2 | Negative | > 0.2 | Negative |
| TSP g/L (in cows 67-75; in 2 days old calves 64 ± 7) | 78.7 | 74.5 | 43.6 | 55.3 | 58.1-65.4 (mean 62.8) |
| γ-glob. g/L (in cows 16.9-22.3; in 2 days old calves > 15) | 16.23 | 8.73 | 1.58 | 4.12 | 11.7-18.2 (mean 14.2) |

Explanations: AST – asparagine transferase; GGT – gamma-glutamyl transferase; NEFA – non-esterified fatty acids; BHB – betahydroxybutyrate; TSP – total serum protein; γ-glob. – serum gammaglobulins; Nd – not determined; *Cow No 11 (mother of the calf No 5); **Cow No 12 (mother of the calf No 7); ***The range of TSP and γ-glob concentrations estimated one month later in five calves aged 24-60 hours

isolated using mycological culture. It is a normal and common representative of the microbials of the calf alimentary tract mucosa; it grows well and is easy to isolate from the mucosal surface. *Zygomycetes* grow in the deeper parts of the mucosal membrane and are usually not isolated by mycological culture (9, 21). Histopathological examination of affected tissues has a high diagnostic impact because *Zygomycetes* morphology differs significantly from those of *Candida* spp. and *Aspergillus* spp.

On account of the multifactorial pathogenesis of the diarrhea outbreak, the management was focused on overcoming the pathogenic bacteria, improving passive transfer and the specific antibody activity of colostrum, and intensive rehydration. Antibiotic susceptibility differed among the *Escherichia coli* isolates (Tab. 1). Therefore the calves were treated with colistin + linkomycin + spectinomycin simultaneously for 7 days with doses recommended by producers. Moreover, as oral and intravenous rehydration was continued, each calf additionally obtained 30-50 ml of Boviglobin® (pooled gamma-globulin preparation from slaughtered cattle, Biowet Drwalew S.A.) subcutaneously. The staff was trained in calf care and the first colostrum quality monitoring was started up. Additionally, intensive newborn calves colostrum feeding and estimation of the immunoglobulin concentration in the calves' sera at the age of 24-48 hours, using the Zinc Sulfate Turbidity Test (ZSTT, Pro Animali, Wroclaw) were recommended. A program of vaccinating pregnant cows with Trivacton 6 (Merial) was applied. These procedures significantly moderated the course of diarrhea and decreased its frequency. However, during two-months of observation about 40% of the calves below 2 weeks of age came down with diarrhea.

The primary cause of the described case was probably enterotoxic diarrhea and endotoxemia caused by *E. coli* followed by alimentary tract disequilibrium caused by antibiotic therapy. In the calves with FPT, the concurrent *Candida* sp. and *Zygomycetes* infection caused the abomasitis. Both calves died before the 10$^{th}$ day of life. Neitzke and Schiefer (21) presented a similar observation, indicating the predisposition of calves below one month of life to fungal abomasal infections. Because abomasal mycosis is usually not a direct cause of calf death, its importance is underestimated on large farms.

In conclusion the simultaneous antibiotic therapy based on antibiotic sensitivity, vaccination of cows with enterotoxic *E. coli* strain antigens allowed the control of pathogenic strains. The first colostrum quality testing and a colostrum feeding regime improved the passive transfer in calves. The above mentioned changes in calves' management, as well as elimination of mould-overgrown silage from pregnant cows' feed enabled the stabilization of the farm's situation. Within two months of the improved health management about 40% of calves still developed mild diarrhea that disappeared shortly after starting the treatment. The calves' health normalization was achieved after two months, when calves from vaccinated dams not exposed to mycotoxins in late gestation were born.

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