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# Clinical and pathological findings of an outbreak of Tyzzer's disease in a rabbit colony in Croatia

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**ABSTRACT**

Clinical and pathological findings found during an outbreak of Tyzzer's disease in a rabbit colony in Croatia are described in this paper. The disease occurred in young rabbits, approximately 50 days old. Over a period of six months, 148 weaned rabbits from a total group of 1753 weaned animals died with symptoms of severe gastrointestinal disease. The main clinical signs observed were diarrhea, abdominal distension, anorexia, weight loss and apathy. Five weaned rabbits were examined at the Department of Pathology of Veterinary Faculty. In all necropsied animals severe entero-hepatic lesions were seen characterized by disseminated hepatic focal necroses and transmural necrotic typhlitis. Only one animal had necrotic myocarditis. For histopathology the tissue blocks were sectioned in 5μm thick sections and stained using HE, PAS, GMS and the Giemsa method. Immunohistochemistry using anti-*Clostridium piliforme* RT and MSK strains reacted positively within the hepatocytes and intestine. Polymerase chain reaction (PCR) assay (liver and caecum) amplified the 196-bp DNA fragment specific to 16S ribosomal RNA of *C. piliforme*. This is the first confirmed and described case of Tyzzer's disease in rabbits in Croatia.

**Key words:** Tyzzer's disease, *Clostridium piliforme*, rabbits, polymerase chain reaction, immunohistochemistry

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**Introduction**

Tyzzer's disease is an enterohepatic infection caused by *Clostridium piliforme*, (formerly *Bacillus piliformis*) a large, intracellular, filamentous, spore forming, anaerobic, gram-variable rod-shaped bacterium (DUNCAN et al., 1993; FRANKLIN et al., 1994; HIRSCH and BIBERSTEIN, 2004; BROOKS et al., 2006). It has been reported in various laboratory, domestic and non-domestic animals (FUJIWARA, 1978; POONACHA, 1997; IKEGAMI et al., 1999a and 1999b; MANDOKI and VETESI, 2000; LANGAN et al., 2000; RAYMOND et al., 2001; FOSGATE et al., 2002). The disease was reported in an HIV-1 infected human patient (SMITH et al., 1996) and non human primates (WANER et al., 2005; SASSEVILLE et al., 2007). Antigenic differences have been demonstrated in strains of this bacteria isolated from different species. Young animals are most frequently affected in outbreaks of *C. piliforme* (ST DENIS et al., 2000; PERCY and BARTHOLD, 2001; BROWN et al., 2007). They often die without clinical symptoms (FRANKLIN et al., 1994). The most common clinical signs, when present, include diarrhea, abdominal distension, anorexia, weight loss and apathy (FRANKLIN et al., 1994; FOSGATE et al., 2002; WANER et al., 2005; BROOKS et al., 2006). During epizootics in domestic rabbits all ages may be affected, but weaned young are most frequently affected. The disease is predisposed by immunosuppression, poor husbandry, overcrowding, high temperatures, and incorrect diet (HARCOURT-BROWN, 2002; IKEGAMI et al., 1999b). Animals usually become infected by ingestion of spores from contaminated material. There has been speculation that intrauterine transmission may occur in rabbits (PERCY and BARTHOLD, 2001). Following oral exposure, bacteria initially pass through the epithelium of the intestine (the most frequent ileum, caecum and colon). The lesion may be limited to the intestinal tract, but after multiplication of *C. piliforme* and colonization of the gastrointestinal tract with tissue damage, the bacilli penetrate into the portal circulation (STALKER and HAYES, 2007). After that, they disseminate throughout the body, especially to the liver and myocardium where they cause acute to subacute necrotic lesions (PERCY and BARTHOLD, 2001; HARCOURT-BROWN, 2002; BROWN et al., 2007). The target organs of *C. piliforme* vary among affected animals. In rabbits the most frequent target organs are the intestines, liver and myocardium. The bacillus causes necrotizing and inflammatory lesions (HILLYER and QUESENBERRY, 1997; GELBERG, 2007; McCULLEN, 2007). Morbidity varies from 10% to over 50% and mortality in affected animals is high (PERCY and BARTHOLD, 2001). The diagnosis of Tyzzer's disease is based on clinical or gross postmortem findings, pathohistological findings by staining HE (Hematoxylin and eosin) and silver method stains (Warthin-Starry, GMS), Giemsa methods for visualization of bacilli, immunohistochemistry and PCR (FURUKAWA et al., 2002; BORCHERS et al., 2008).

The purpose of this paper is to describe the first confirmed case of Tyzzer disease in Croatia.

**Materials and methods**

During a period of six months (from December 2000 to June 2001) 148 weaned rabbits from a total group of 1753 weaned animals, approximately 50 days old, died with symptoms of severe gastrointestinal disease in a rabbit colony (experimental animal breeding facility) in Croatia. Usually, diseased animals died 4-5 days after the onset of the symptoms. Five weaned rabbits were examined at the Department of General Pathology and Pathological Morphology of the Veterinary Faculty. Tissue sections of liver, caecum, colon and heart were taken for histopathological examination. Tissue sections were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with HE and eosin (HE). Modified Gomori's methenamine silver (GMS) stain, Periodic Acid Schiff (PAS) and Giemsa method were used for visualization of bacteria in tissue sections. For immunohistochemistry, sections were stained using mouse antiserum to *C. piliforme* RT and MSK strains (Dr. S. Kawamura, Laboratory of Biomedical science, Department of Veterinary Medical Science, University of Tokyo, Japan). The reaction was visualized with peroxidase-conjugated streptavidin (Histofine SAB-PO Kit, Nichirei Corp., Tokyo, Japan) using diaminobenzidine as a substrate. Polymerase Chain Reaction (PCR) (liver and caecum) using the 196-bp DNA fragment specific to 16S ribosomal RNA of *C. piliforme* was performed using a previously published protocol (IKEGAMI et al., 1999b).

**Results**

In all five necropsied animals, mild dehydration, abdominal distension, fecal staining in the perineal region and severe entero-hepatic lesions were seen. The caecum and colon contained watery, partly gelatinous content. The wall of the affected areas of the intestine, particularly the caecum, was edematous and markedly thickened, while microscopically transmural necrotic typhlitis with submucosal oedema, leucocytic infiltration, bacterial coccobacillary colonization and hemorrhages were seen (Fig. 2). On the serosal surface of the caecum and colon, fibrinous exudate was noted (Fig. 1a).

On the liver disseminated pale miliary foci up to 2-3 mm in diameter were found macroscopically (Fig. 1b). Histologically, the liver lesions were characterized by multifocal coagulative necrosis with neutrophilic and mononuclear-cell infiltration (Fig. 3). On the periphery of the necrosis, numerous filamentous bacilli arranged in a criss-cross pattern were detected in the hepatocyte cytoplasm (Giemsa and GMS stain) (Fig. 4).

In one animal focal degeneration and necrotic cardiac myofibrils with small numbers of macrophages and lymphocytes were detected.

Immunohistochemistry revealed that filamentous bacilli in the liver and caecum reacted positively with anti-RT and anti-MSK strains antisera of the *C. piliforme* (Figs. 5 and 6).

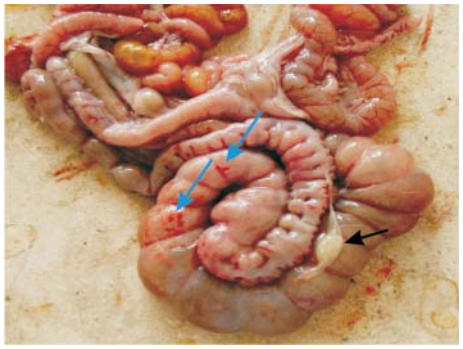

Fig. 1a. Hemorrhages (blue arrows) and fibrinous exudates on the serosal surface of the caecum and colon (black arrow)

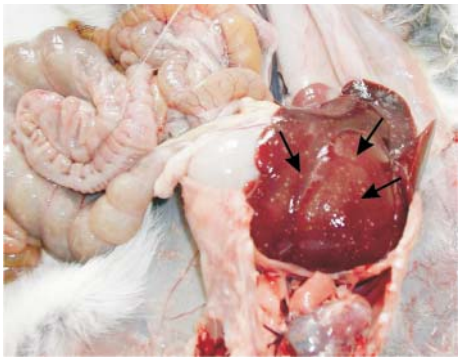

Fig. 1b. Macroscopy finding on the liver. Disseminated pale miliary foci up to 2-3 mm in diameter (arrows)

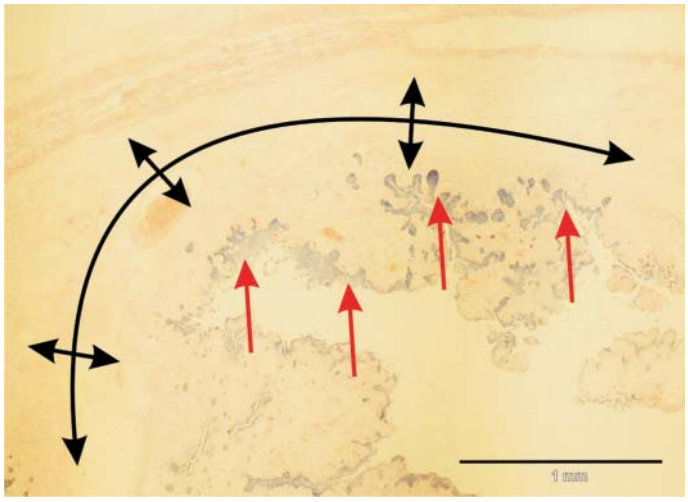

Fig. 2. Caecum. Transmural necrotic typhlitis (black arrows) and bacterial cocobacillary colonization (red arrows). H&E.

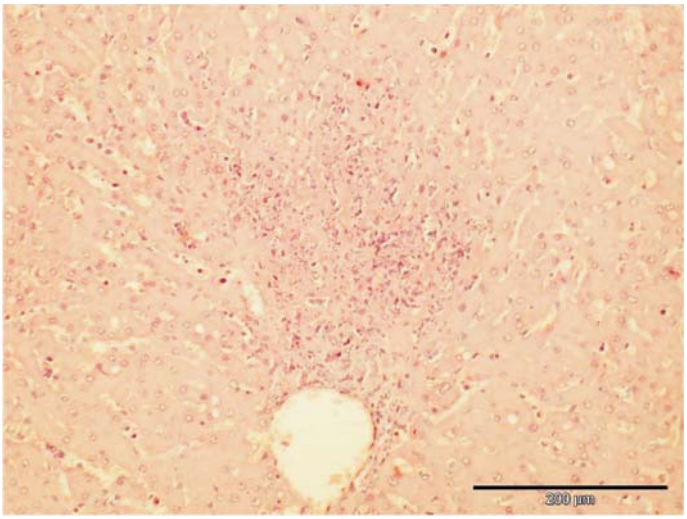


Fig. 3. Liver. Coagulative focal necrosis with neutrophilic and mononuclear cell infiltration. H&E.

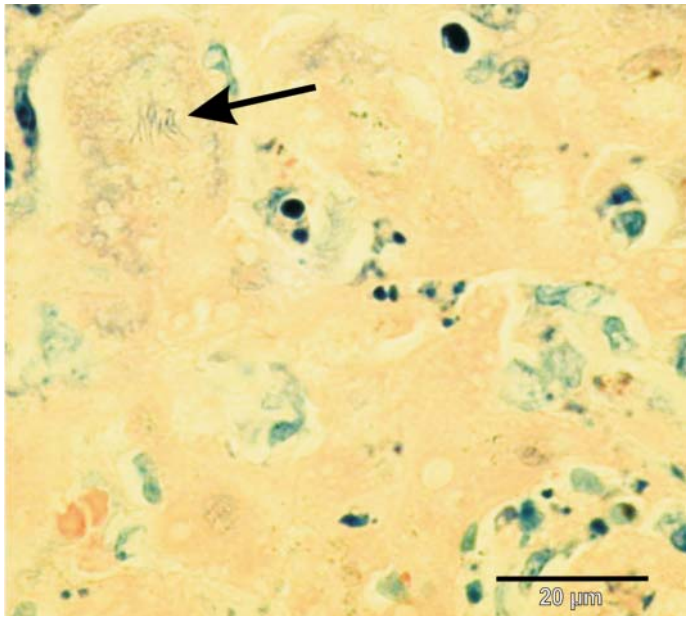


Fig. 4. Liver. In the hepatocyte cytoplasm (on the periphery of the necrosis) there are numerous filamentous bacilli arranged in a criss-cross pattern (arrow). Giemsa stain.

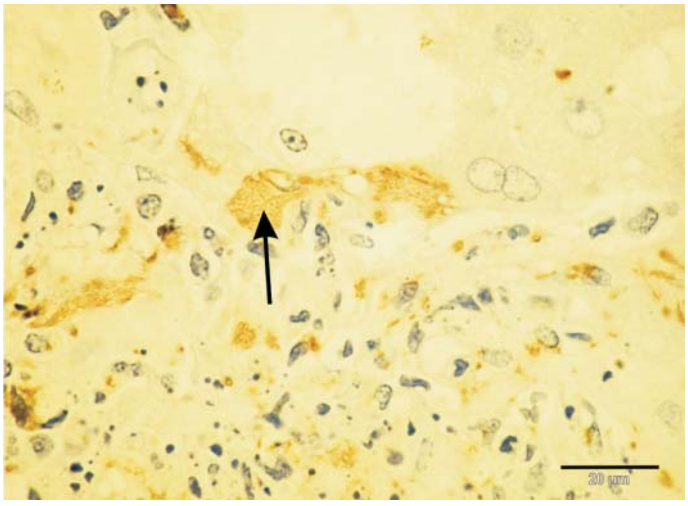

Fig. 5. Liver. Filamentous bacilli in the liver reacted positively with anti RT and anti MSK strains antiserum of the *C. piliforme* (arrow). Immunohistochemistry.

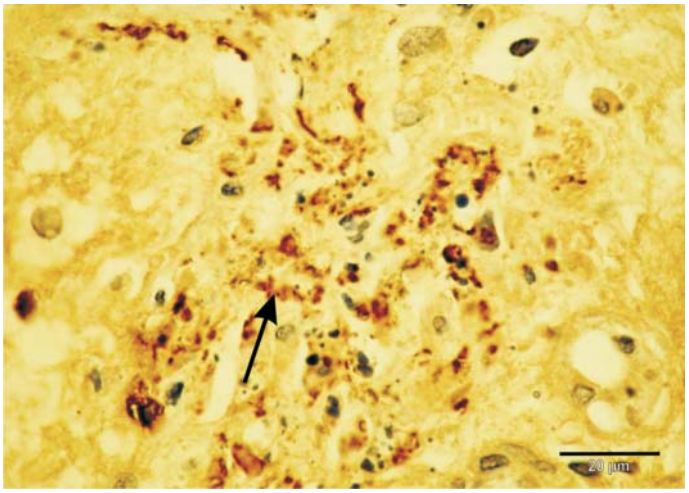

Fig. 6. Caecum. Positive reaction in the caecum with anti RT and anti MSK strains antisera of the *C. piliforme* (arrow). Immunohistochemistry; ×100

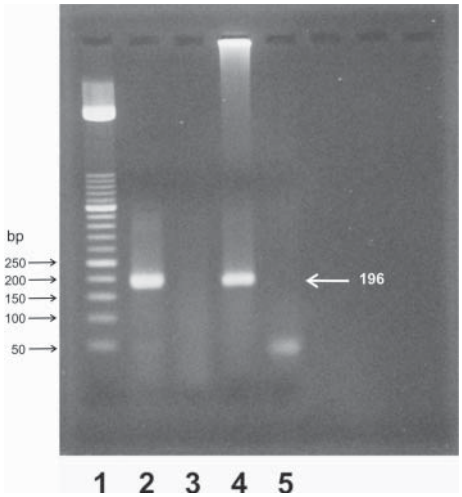


Fig. 7. PCR. 1. Marker. 2. Liver (positive: 196bp). 3. Stomach (negative). 4. Caecum (positive: 196bp) 5.(negative control). The 196 bp DNA fragment specific to 16S ribosomal RNA of *C. piliforme* was amplified from the liver and caecum.

Likewise, using PCR, the 196-bp DNA fragment specific to 16S ribosomal RNA of *C. piliforme* was amplified from the liver and caecum (Fig. 7).

**Discussion**

Tyzzer's disease is a severe infectious disease caused by *Clostridium piliforme* which has been reported in various animal species (laboratory, domestic and wild animals) and immunodepressed men. Although the disease has been known for a long time (TYZZER, 1917) our paper presents the first clinical and pathological findings in an outbreak of this disease in rabbits described in Croatia. In our case young animals were affected, which is in accordance with studies conducted by other authors (ST DENIS et al., 2000; PERCY and BARTHOLD, 2001; BROWN et al., 2007). In investigated rabbits in our study diarrhea, abdominal distension, anorexia, weight loss and apathy were detected as the major clinical signs. That is in agreement with clinical symptoms described in numerous other studies (FRANKLIN et al., 1994; FOSGATE et al., 2002; WANER et al., 2005; BROOKS et al., 2006). As was mentioned earlier, the most frequent target organs in rabbits are the intestine, liver and myocardium in which this bacteria causes necrotizing and inflammatory lesions (HILLYER and QUESENBERRY, 1997; GELBERG, 2007; McCULLEN, 2007). Our histopathological and gross findings were similar to those seen in rabbits and other species of animals with

Tyzzer's disease (FUJIWARA, 1978; POONACHA, 1997; IKEGAMI et al., 1999a and 1999b; MANDOKI and VETESI, 2000; LANGAN et al., 2000; RAYMOND et al., 2001; FOSGATE et al., 2002; GELBERG, 2007; McCULLEN, 2007; BROWN et al., 2007). In our case animals died after weaning which is a predisposing factor for the development of the disease. Usually, animals become infected following the ingestion of spores from contaminated material (YOUNG et al., 1995; RAYMOND et al., 2001; WANER et al., 2005; McCULLEN 2007). In our case the disease was probably transmitted by wild rodents. The diagnosis of Tyzzer's disease is based on clinical or gross postmortem findings, pathohistological lesions with visualization of the bacilli, immunohistochemistry and PCR (FURUKAWA et al., 2002; BORCHERS et al., 2008; HEADLEY et al., 2009). In our case the disease was confirmed using all above mentioned methods. In conclusion, we have described the first confirmed case of Tyzzer's disease in Croatia.

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**SAŽETAK**

U radu su opisani klinički i patološki nalazi Tyzzerove bolesti u jednom uzgoju kunića u Hrvatskoj. Bolest se pojavila u mladih kunića prosječne dobi od 50 dana. U razdoblju od 6 mjeseci uginulo je 148 odbijenih kunića od ukupno 1753 životinje sa znakovima jake gastrointestinalne bolesti. Glavni su klinički znaci bili proljev, proširenje trbuha, anoreksija, gubitak težine i apatija. U Zavodu za opću patologiju i patološku morfologiju Veterinarskoga fakulteta bilo je pregledano pet životinja. Na razudbi su u svih životinja utvrđene jake enterohepatične lezije karakterizirane diseminiranim fokalnim nekrozama po jetrima i nekrotičnom upalom cijele stijenke slijepoga crijeva. U jedne životinje je utvrđen i nekrotični miokarditis. Za patohistološku pretragu uklopljena tkiva rezana su u rezove debljine 5 µm i bojena HE, PAS, GMS i Giemsa metodom. Imunohistokemijska reakcija pri upotrebi anti-RT i anti-MSK sojeva antiseruma protiv *Clostridium piliforme* bila je pozitivna unutar hepatocita i crijeva (Dr. S. Kawamura, Laboratory of Biomedical Science, Dept. of Veterinary Medical Science, University of Tokyo). PCR je pokazao amplifikaciju 196-bp DNA fragmenta specifičnoga za 16S ribosomsku RNA bakterije *C. piliforme.* Ovo je prvi potvrđeni i opisani slučaj Tyzzerove bolesti u kunića u Hrvatskoj.

**Ključne riječi**: Tyzzerova bolest, *Clostridium piliforme*, kunić, lančana reakcija polimerazom, imunohistokemija