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YERSINIA RUCKERI SEPTICAEMIA IN EXPERIMENTALLY INFECTED CARP (*CYPRINUS CARPIO* L.) FINGERLINGS

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The presence of *Yersinia ruckeri*, the causal agent of enteric redmouth disease (ERM) in salmonids and a few other freshwater fish, has so far been reported from a variety of sources including the intestine of healthy carp. Since there are no data on the pathogenicity of this bacterium for carp, 15 fingerlings were experimentally infected by intraperitoneal injection of about 5×10^5 cells. Thirteen injected fish were moribund or died within 4 days with septicaemic lesions. Two survivors were sampled on Day 28 after infection. *Yersinia ruckeri* was reisolated from the internal organs of all experimental fish. By histopathological examination moribund fish had generalised bacteriaemia with inflammation, degeneration and necrotic foci in kidney, liver and spleen, corresponding to findings described previously in ERM of rainbow trout. Survivors of challenge on Day 28 had a chronic disease characterised by prominent peritonitis and enteritis, exhaustion of the erythroid, granuloid and lymphoid components in haematopoietic kidney tissue as well as focal degeneration and necrosis in organs. These data indicate a high sensitivity of carp to intraperitoneal infection with a relatively low dose of *Y. ruckeri*.

Key words: *Yersinia ruckeri*, enteric redmouth disease (ERM), common carp (*Cyprinus carpio*), histopathology

Yersinia ruckeri is the causal agent of the acute to chronic enteric redmouth disease (ERM) or yersiniosis of salmonid fish. The disease and the organism were first described in the United States (Ross et al., 1966; Rucker, 1966) and thereafter in several European countries and Australia (Austin and Austin, 1987). In Croatia, Oraić (1993) reported ERM in rainbow trout (*Oncorhynchus mykiss*) caused by serovar I.

Although *Y. ruckeri* most commonly affects the rainbow trout, its host range probably includes all salmonids (McDaniel, 1979). It was also isolated from seven diseased and six apparently healthy non-salmonid species, a human

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clinical specimen and a variety of other sources, including river water (data from Stevenson et al., 1993). The bacterium can survive starvation in freshwater for at least 4 months (Thorsen et al., 1992). Isolation from healthy common carp (*Cyprinus carpio* L.) was reported by Fuhrmann et al. (1984), Schlotfeldt et al. (1985), Austin and Austin (1987) and Enriquez and Zamora (1987), but there are no data as to whether the bacterium can cause disease in this species. We tested the sensitivity of carp to intraperitoneal infection with *Y. ruckeri* and registered its high susceptibility.

Materials and methods

Yersinia ruckeri for inoculation of carp belonged to serovar I (reference isolate RD-16 was kindly provided by R. L. Davies). It was inoculated onto tryptose soy agar (TSA, Oxoid) and incubated at 22 °C for 48 h. Colonies were washed off with sterile 0.65% NaCl solution (further on: SS), suspended by mixing and sedimented by centrifugation. The sediment was resuspended in SS and serial tenfold dilutions in SS were prepared for determination of bacterial cell concentration using McFarland's turbidimetric method. Carp were inoculated intraperitoneally with about 5×10^5 cells in 0.1 ml of SS.

Carp for the experiment were brought to the laboratory from a fish farm free of major diseases. Before the experiment, five of them were dissected and examined by bacteriological and virological methods. After seven days, 30 carp of about 15 g (approx. 85 mm) were randomly divided into two groups. Each fish in Group 1 was inoculated with *Y. ruckeri* suspension. Carp in Group 2 were injected with 0.1 ml of SS. Groups were placed into two glass aquaria (100 litres) provided with a constant slow flow of dechlorinated tap water (14–18 °C) and aeration. The experiment lasted for 28 days.

Sampling

Moribund carp as well as survivors in Groups 1 and 2 on Day 28 were anaesthetised with MS-222 and bled by cutting the gill arches. All fish were examined for external lesions and by dissection. The parenchymatous organs of all fish were sampled for bacteriological and virological examination and those of moribund ones and of survivors from both groups also for histology.

Bacteriological examination

Materials from kidneys and liver were inoculated onto TSA plates and incubated for 48 h at 22 °C. The latter temperature was used for incubation of all subsequent inoculations of media and tests. Selected colonies were subcultured on TSA plates. The 24-h growth was used for preparation of Gram-stained smears,

inoculation of Shott-Waltman's agar (further on: Sw) (Waltman et al., 1984), and of tryptone soy broth for determination of motility. Media for conventional biochemical tests, including the test for oxidation or fermentation of glucose (Hugh and Leifson, 1953), were inoculated with 48-h-old growth on TSA subculture. Motility was determined by the "hanging drop" method after 24 h. Other results were recorded after 48 or 72 h except for the methyl red and Voges-Proskauer (VP) reactions which were read 5 days after incubation. The production of diffusible pigments was determined according to King et al. (1954). Isolates were identified according to Krieg and Holt (1984) as well as Amos (1985).

Virological examination

Kidney, spleen and liver samples were examined for presence of virus by inoculation of EPC cells (Fijan et al., 1983) and by standard isolation technique at 20 °C.

Histological examination

Samples of the heart, kidney, spleen, liver and intestine were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin (Romeis, 1968) and examined by light microscopy.

Results

Control carp (Group B) had no clinical or pathoanatomical signs of disease. All of them were alive on Day 28. The results of bacteriological and virological examinations were negative. The organs were histologically normal.

The course of mortality and results of bacteriological examination of carp in Group 1 are presented in Table 1. Virological examinations were negative. On Day 2 the dead and moribund carp had exophthalmos, dark skin coloration, petechial bleeding and suffusion on the gill arches, belly, bases of pectoral and pelvic fins, operculi, lateral line area and at the injection site. The gills were pale. All internal organs were oedematous. Petechial bleeding was pronounced in the liver. The peritoneal cavity contained a small amount of a pale red and slightly turbid fluid. On Days 3 and 4 the dead and moribund carp had the same symptoms. One of them had petechial bleeding in the oral cavity.

On Day 28, two survivors in Group 1 had pale gills and a yellow fluid in the second half of the intestine.

Gram- and cytochrome oxidase negative, motile rods were isolated from the organs of all fish in Group 1. They were found in pure culture in 14 fish. This isolate inoculated onto SW agar showed a green colony with a zone of hydrolysis. A second, cytochrome oxidase positive, Gram-negative rod was iso-

lated from one carp which died on day 3. After inoculation onto two King's selective plates it was tentatively identified as *Pseudomonas* sp. The basic biochemical characteristics of the isolates are presented in Table 2.

Table 1

Mortality and results of bacteriological examination of carp infected experimentally with *Yersinia ruckeri* (Group 1)

Day	Dead + moribund sampled carp	Survivors (%)	Bacteriological findings
2	5 + 3	7 (53.33)	*Y. ruckeri*
3	1 + 2	4 (20.00)	*Y. ruckeri* (+ *P. fluorescens* in 1 fish)
4	+ 2	2 (13.33)	*Y. ruckeri*
28		2 (13.33)	*Y. ruckeri*

Table 2

Some characteristics of *Yersinia ruckeri* and of *Pseudomonas* sp. isolated from carp injected with *Y. ruckeri* strain RD-16

Characteristic	Reaction*	
	Y. ruckeri	*Pseudomonas* sp.
Gram	–	–
Motility at 22 °C	+	+
Oxidase	–	+
Indole	–	–
Urease	–	–
O/F test	+	–
Catalase	+	+
Voges-Proskauer	–	–
Methyl red	+	–
Simmons citrate	+	+
Gelatinase	+	+
Fermentation of:		
Glucose	+	+
Sucrose	–	–
SW agar	+	/
King A	/	+
King B	/	–

*+ = positive; – = negative; / = strain was not inoculated onto this medium

Histological examination of five moribund fish collected near the water surface on Days 2 and 3 revealed oedema, degeneration, necrosis and perivas-

cular bleeding in all organs. The necrotic gut epithelium was sloughed off and the submucosa was inflamed. Peritonitis with phagocytosis of bacterial cells was also pronounced. Clumps of bacteria were found in kidneys, liver and spleen. The adventitia of blood vessels contained groups of phagocytic cells surrounded by rodlet cells. Two moribund fish collected on Day 4 exhibited the same type of lesions and peritonitis.

The ventricular muscle of two carp from Group 1 sampled on Day 28 had groups of rodlet cells and leukocyte accumulation, a few foci of degeneration (Figs 1 and 2) and areas with pigment cells (Fig. 2).

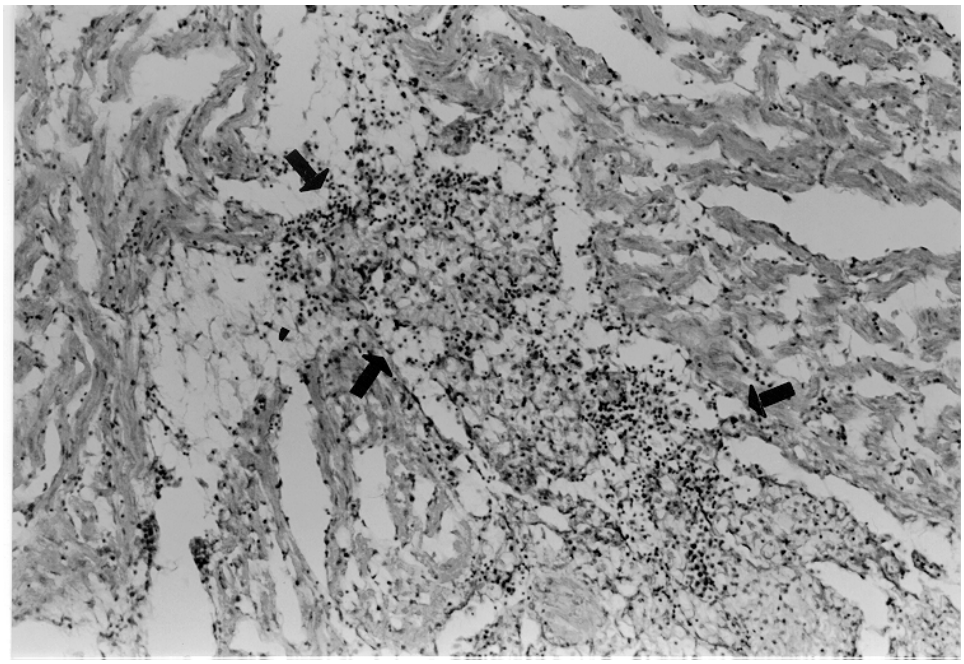

Fig. 1. Accumulation of rodlet cells and leukocytes (delineated by 3 arrows) and myodegeneration in carp with chronic yersiniosis, sampled on Day 28 after intraperitoneal injection of *Yersinia ruckeri*. Haematoxylin and eosin (HE), × 174

Local foci of chronic pericarditis were also present. The liver serosa was thickened due to chronic peritonitis and the underlying parenchymatous tissue layer was infiltrated, degenerated and occasionally necrotic (Fig. 3). The epithelium of the bile ducts was degenerated or necrotic and often sloughed off (Fig. 4). The pancreatic tissue was normal. The peritoneum covering the lobes of trunk kidney was rather thick and contained capillaries and pigment cells (Fig. 5). Some of the tubules had degenerated and necrotic cells (Figs 5 and 5a).

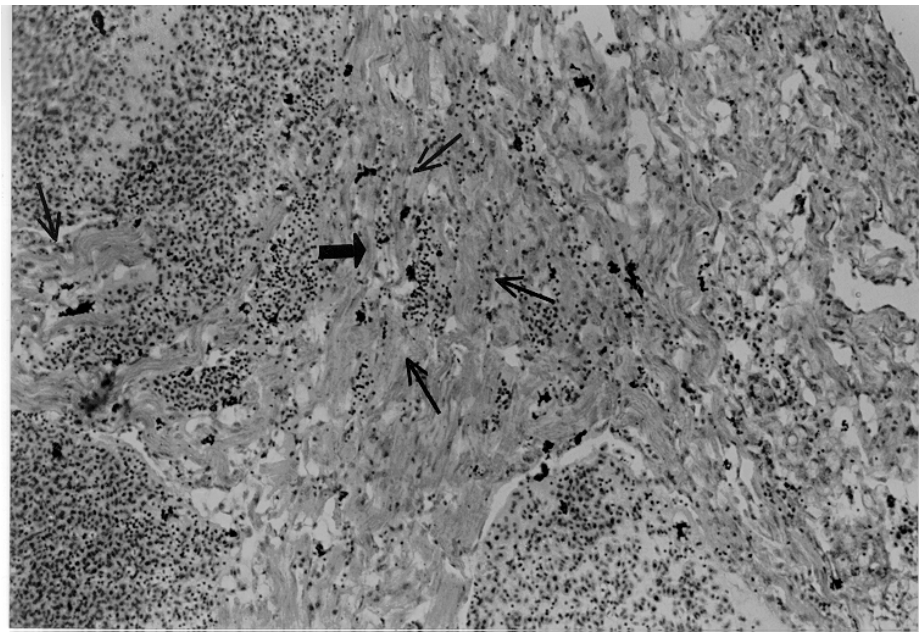

Fig. 2. Widened capillaries, pigment cells (arrow) and occasional myodegeneration in chronic yersiniosis. HE, × 166

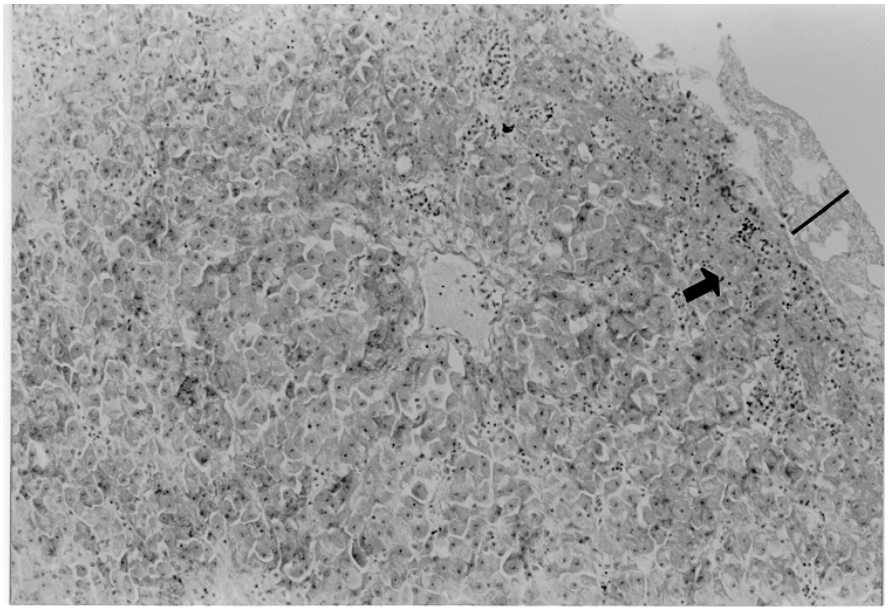

Fig. 3. Widened serosa (bar), with degeneration, subserosal necrosis and infiltration (arrow) in the liver. HE, × 160

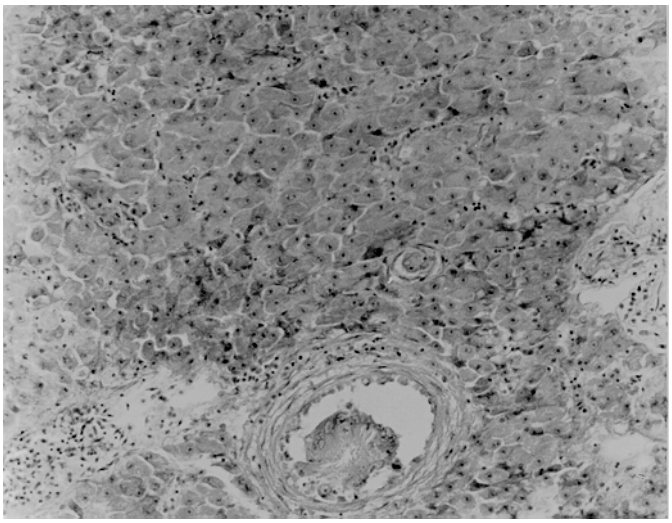

Fig. 4. Partial desquamation of epithelium in large and small bile ducts in chronic yersiniosis. HE, × 175

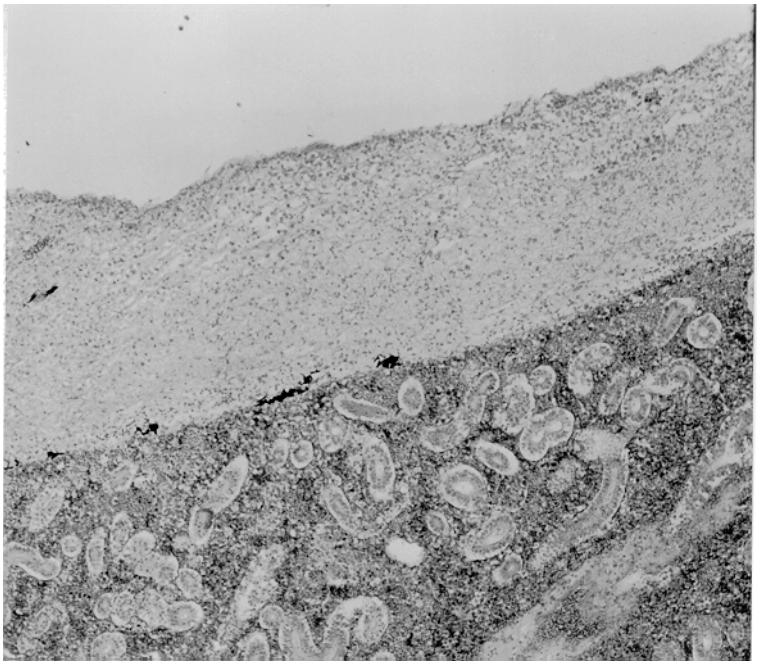

Fig. 5. Enormously widened serosa with vascularization and pigment cells on the surface of the kidney lobe in chronic yersiniosis. HE, × 90

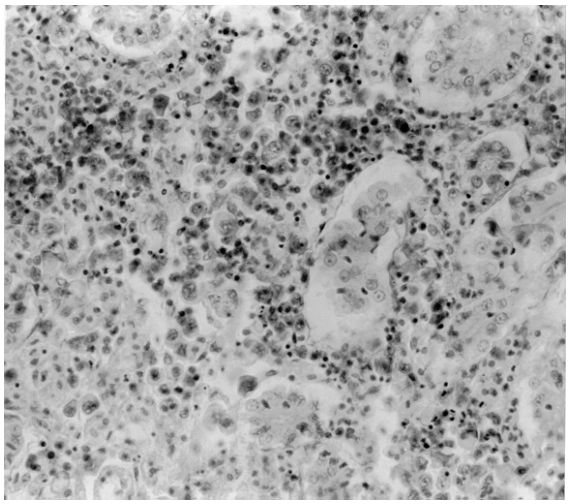

Fig. 5a. Necrotic tubules in kidneys and depletion of lymphoid, erythroid and granuloid cells in chronic yersiniosis. HE, × 265

The walls of some blood vessels were widened. The haematopoietic tissue consisted mainly of large mononuclear cells with a lack of normal amount of lymphoid, erythroid and granuloid cells (Fig. 5a). The serosa covering the spleen and the underlying tissue were mostly intact but the part exposed to the parietal peritoneum (not covered by liver or intestine) was slightly thickened and a narrow subserous zone was necrotic (Fig. 6).

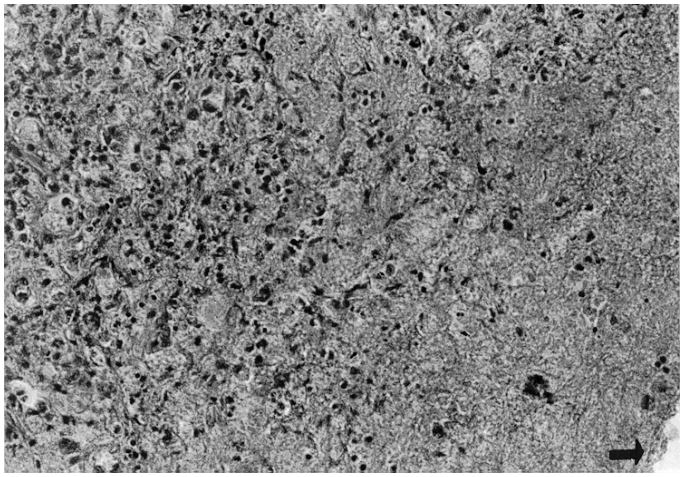

Fig. 6. Necrotic subserous zone in spleen on the part exposed to the parietal peritoneum (arrow). HE, × 124

The intestinal epithelium of the hindgut was degenerated, necrotic and sloughed off, and the submucosa was inflamed (Fig. 7).

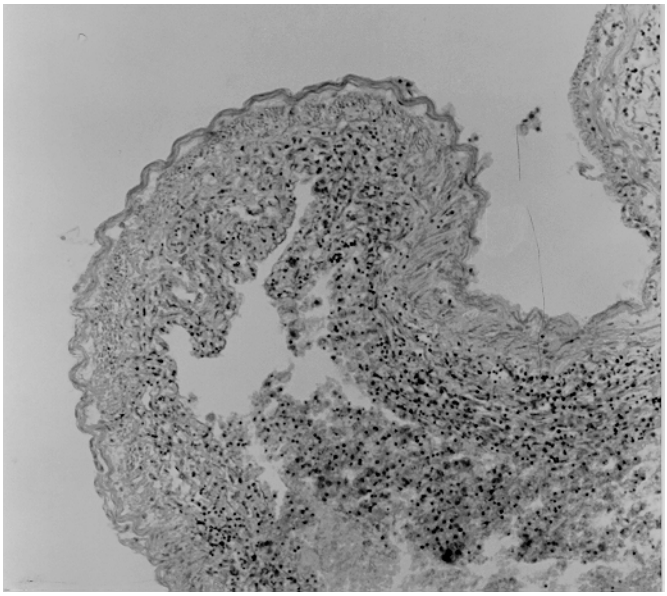

Fig. 7. Inflamed submucosa, necrotic and sloughed epithelium of the intestine. HE, × 90

Discussion

The characteristics of the cytochrome oxidase negative isolates from carp in Group 1, presented in Table 2, correspond to those of *Y. ruckeri*. More detailed tests and serotyping were not carried out because the presence of a strain other than the inoculated one was not expected in septicaemia after intraperitoneal injection. The attributes of *Pseudomonas* sp. studied (Table 2) place it close to *P. fluorescens.*

Our results provide the first information on the pathogenicity of *Y. ruckeri* for the common carp under experimental conditions. The mortality rate of over 85% within 4 days with pathoanatomical and histological lesions characteristic of septicaemia as well as the reisolation of *Y. ruckeri* from internal organs of all experimental fish and its presence in pure culture in 14 out of 15 carp indicate the high susceptibility of this species to intraperitoneal infection with a relatively low dose. Comparison of our results with data reported by Stevenson et al. (1993) on virulence tests with serovar I and II strains for salmonids by intraperitoneal injection suggests a higher or equal sensitivity of experimental carp to this route of

challenge. Two survivors on Day 28 had signs of chronic yersiniosis with prominent peritonitis, enteritis and anaemia. Factors of possible significance for the high susceptibility of experimental carp such as the temperature during the experiment, the physiological status including stress, as well as the relationship between fish size and dose of bacteria remain to be investigated.

The majority of septicaemic changes seen in the experimental carp were not very specific but still corresponded to those of ERM in salmonids (Rucker, 1966; Stevenson et al., 1993). However, enlargement and dark colour of the spleen (Rucker, 1966) were not found in carp. As in cases of *Y. ruckeri* infection in salmonids described by Frerichs et al. (1985) and Sparboe et al. (1986), most carp did not have reddened areas around the mouth. Findings corresponding to chronic proliferative peritonitis and to the depletion of haematopoietic kidney tissue in its major components in the two survivors sampled on Day 28 have not been reported so far. Peritonitis seems to be the consequence of long-lasting inflammation in the infected peritoneal cavity. From the peritoneal cavity the infection slightly progressed into organs (*per continuitatem*) as can be seen in Figs 3 and 6. The scarcity of erythroid, lymphoid and granuloid cells in the haematopoietic tissue should be viewed in the light of findings by Quentel and Aldrin (1986) and Lehmann et al. (1989) about decreased red and white blood cell counts in ERM-infected rainbow trout. Such blood cell loss was probably occurring in the two carp throughout the postinoculation period and could be responsible for the depletion of haematopoietic tissue seen 28 days after infection.

Our results raise the question whether carp yersiniosis can occur under natural circumstances. The hitherto reported incidence of *Y. ruckeri* in the intestine of healthy carp was low (Fuhrmann et al., 1984; Enriquez and Zamora, 1987) and the probability of a secondary infection thus seems to be remote. However, the relatively common and widespread occurrence of *Y. ruckeri* in the aquatic environment suggests that it may be involved in secondary septicaemic conditions in the same way as ubiquitous motile aeromonads (Roberts, 1993) and pseudomonads (Inglis and Hendrie, 1993).

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