**Yersinia ruckeri** infection in cultured Nile tilapia, *Oreochromis niloticus*, at a semi-intensive fish farm in lower Egypt

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A *Yersinia ruckeri* infection was recorded in 2007 among a group of Nile tilapia, *Oreochromis niloticus*, from an earthen pond at a semi-intensive fish farm in Sharkiya Province, lower Egypt. The outbreak took place during the late winter-spring transition period, effecting a total of 130 out of 150 fish, with a prevalence of 66.6%. The affected fish presented typical signs of yersiniosis, including extensive lip, mouth, fin and skin hemorrhages and hemorrhagic gastroenteritis, together with congestion of the internal organs. Tentative confirmation of the *Yersinia ruckeri* isolates was achieved using polymerase chain reaction (PCR), conventional biochemical assays and the API 20 E system. Mortalities were the result of a sharp fluctuation in water temperature, predation due to migratory birds that ‘stopover’ at this site, and to overstocking of ponds.

**Keywords:** earthen ponds, fish diseases, infection prevalence, yersiniosis

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**Introduction**

Enteric Redmouth Disease (ERM) is a septicemic disease caused by *Yersinia ruckeri*, a gram negative ‘rod’ species of the family Enterobacteriaceae. However, the disease is better known as ‘yersiniosis’ or ‘*Yersinia* septicemia’, because the classic red mouth condition is not necessarily seen. Internationally, the disease was first described in rainbow trout, *Oncorhyncus mykiss*, in 1958 in the United States, and has become endemic in North America (Bullock et al. 1977). Outbreaks of yersiniosis in Europe occurred in 1981 in France, Germany and the United Kingdom and it later appeared in most European countries. In Egypt, *Yersinia ruckeri* was first isolated from both apparently healthy and moribund Nile tilapia from the Nile River in Giza Province (Hussein et al. 1997). Subsequently, it was isolated in the Nile Delta region from apparently healthy and diseased Nile tilapia, African catfish and common carp, with relatively low prevalences (12.5, 8 and 5% respectively) (Abd-El Latief 2001).

Clinically, yersiniosis is characterised by the development of chronic or acute entero-septicemia, with the presence of congestive or hemorrhagic zones on the skin, gills, mouth and intestine, and exophthalmia is also frequently evident (Fuhrmann et al. 1983, Rigos and Stevenson 2001). The disease usually occurs as an acute condition with high morbidity and mortality rates, necessitating the search for rapid and accurate methods for the specific detection of *Y. ruckeri*.

The host range of the disease includes salmonids such as rainbow trout, Atlantic salmon and Pacific salmon, and non-salmonids such as emerald shiners *Notropis atherinoides*, fathead minnows *Pimephales promelas* (Michel et al. 1986), goldfish *Carassius auratus* (McArdle and Dooley-Martyn 1985) and farmed whitefish *Coregonus* spp. (Rintamaki et al. 1986). In addition, *Y. ruckeri* infections have occurred in several farmed marine species such as turbbot *Scophthalmus maximus*, seabass *Dicentrarchus labrax* and seabream *Sparus auratus* (Bullock and Cipriano 1990). Experimentally, *Y. ruckeri* infection has been established in common carp (*Cyprinus carpio*) fingerlings (Berc et al. 1999). *Y. ruckeri* type I (Hagerman strain) infection has been reported in sharptooth catfish *Olarias ganeipinus*, and common carp *Cyprinus carpio* (Abd-El Latief 2001).

The disease is most contagious at water temperatures of 15–20 °C and the incubation period of infection is 5–10 days (Fuhrmann et al. 1983, Roberts 1983). Transmission is primarily horizontal from fish to fish through the water. *Yersinia ruckeri* was isolated from both pond algae and sediment in an earthen fish pond in France, suggesting that these micro-habitats may be the natural infection source areas (Coquet et al. 2002). The infection is generally seen in per-acute or acute form, especially in young fish, when there is an abrupt rise in water temperature in spring, whereas chronic infections usually occur in yearling fish in early winter (Danley et al. 1999, Rigos and Stevenson 2001). With respect to the most recent diagnostic methods for ERM, polymerase chain reaction (PCR) represents a widely-used alternative to traditional identification methods. Although pathogenicity and virulence genes have been used as target regions, the rRNA molecule is now accepted as a
tool for identification purposes (Altinok et al. 2001). Recent phylogenetic studies of the genus *Yersinia*, based on 16S rRNA sequencing, have shown that this genus represents a tight cluster within the family Enterobacteriaceae, with *Y. ruckeri* forming a separate subline within the *Yersinia* five-subline cluster (Ibrahim et al. 1993, Altinok et al. 2001). This suggests that, by taking advantage of the differences at the level of 16S rRNA sequences, specific oligonucleotides that can be used in a PCR assay for diagnostic purposes can be designed (Gibello et al. 1999).

The current study presents an epidemiological and diagnostic pathway of natural infections of *Y. ruckeri* in cultured tilapia that may better explain mortalities that occur during sudden temperature fluctuations during the winter-spring period.

**Materials and methods**

**Fish and sample collection**

In the late spring of 2007 sudden mortalities occurred during the production stage of Nile tilapia in earthen ponds at a semi-intensive fish farm in Sharkiya Province, lower Egypt, as a result of abrupt changes in water temperature: first an increase from 16 to 24 °C followed by a sharp decrease to 14 °C over three successive days. A total of 150 live fish were collected and brought to the Fish Disease and Management Laboratory (FDML) at Cairo University. The fish were kept in well-aerated glass aquaria at 25 °C and examined for any abnormal behavioral changes, external signs, and gross lesions.

**Bacteriological examination**

A total of 130 of the 150 fish, with typical lesions and other symptoms of yersiniosis, were euthanised and dissected under aseptic conditions using the three line technique. Bacteriological swabs were taken from kidneys and intestinal contents and spread onto blood agar (BA) and Shotts–Waltman agar (SWA) (Waltman and Shotts 1984). Inoculated plates were incubated at 25 °C for 24–48 hours.

Bacterial isolates were presumptively identified using conventional biochemical tests including catalase with 3% hydrogen peroxide solution, cytochrome oxidase with oxidase strips (Remel), motility in a motility test medium (BD Biosciences, MA), citrate utilisation using Simmons citrate (Remel), sugar utilisation using triple sugar iron (TSI, Remel), oxidation/fermentation of glucose using OF basal media with glucose as the sole carbohydrate source (BD Biosciences), and esculin hydrolysis using bile esculin agar (Remel). Further biochemical testing was performed using API20E and 20NE tests (Bio-Mérieux, NC), with isolates incubated at 25 °C. Results were interpreted at 24–48 hours according to the manufacturer’s instructions. Isolates were presumptively identified as *Yersinia ruckeri* according to the standard criteria of Ewing et al. (1978).

**Molecular identification**

Chromosomal DNA was extracted from 100 μl of the bacterial suspension (a single colony of each of the isolated bacteria suspended in 100 μl of sterile saline) using DNeasy tissue extraction kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions. The extracted DNA was amplified using oligonucleotide primer set specifically for *Yersinia ruckeria* (Gibello et al. 1999). Two forward primers — YR1 (59-AGCAGGAGGAGGTATAAGTG-39) and YR2 (59-CACTTACACCTCCTCTCCGACG-39), and two reverse primers, YR3 (59-GAAGGGCAAAGGCATCTCTG-39) and YR4 (59-AGTAATGTCGGGATARCTGCC-39) — were used.

The controls consisted of a PCR mixture without DNA template (negative control) and with DNA extracted from known *Yersinia ruckeri* (ATCC 29473 positive control). Thermal cycling was done in a thermal cycler (Bio-Rad, Hercules, CA) using 10 ng of template DNA. 0.2 mM each of dNTP, 16 pmol each of primer, 5 ml of 10 × reaction buffer, and 1.25 units of Taq DNA polymerase (Invitrogen, Carlsbad, CA) in a volume of 50 ml of reaction mix, 25 cycles of denaturation for one minute at 92 °C, annealing at the selected temperature for one minute, and extension for one minute at 72 °C, followed by a final extension step of 72 °C for five minutes. Negative (no template DNA) and positive (50 ng of purified DNA from *Y. ruckeri*) controls were included in each batch of PCR mixtures. The PCR products were eletrophoresed in 2% agarose gel (Invitrogen), stained with ethidium bromide, visualised with ultraviolet light, and photographed using a Kodak EDAS system (Eastman Kodak, Rochester, NY). Isolates were considered positive when a 575 bp band was detected.

**Results**

External clinical examination revealed the presence of marked congestion to extensive hemorrhages along all fins, the dorsal musculature, and lips (Figures 1 and 2). Internal lesions included severe congestion of the spleen, kidneys, and intestine (hemorrhagic gastroenteritis), with subcutaneous and muscular hemorrhages (Figure 3). A total of 130 out of 150 fish were clinically affected (87% prevalence), but *Yersinia ruckeria* was retrieved from only 100 fish (relative prevalence 76.9%, actual prevalence 66.6%).

Bacterial isolates were presumptively identified as *Y. ruckeri* using the following biochemical profile: gram negative short bacilli, weakly motile, catalase positive, cytochrome oxidase negative, Esculin negative, nitrate reduction positive, citrate utilisation positive, glucose utilised oxidatively and fermentatively (O/F +/+), TSI- K/A (alkaline slant/acid butt) and presence of minute yellow to slight green colonies with an
opaque halo on SWA media. Identifications using BioMérieux API20E and 20NE rapid test strips were used (Table 1). Isolates were confirmed to be \textit{Y. ruckeri} type I after proving negative to a sorbitol utilisation test. Consistent with \textit{Yersinia ruckeri} standard ATCC strain, all isolates produced a 575 bp band when tested by means of PCR (Figure 4).

**Discussion**

Nile tilapia are the foundation of the pisciculture industry in Egypt. Several diseases pose a critical threat to tilapia culture in the Egyptian Nile Delta, among which yersiniosis, motile \textit{Aeromonas} septicemia and streptococcosis are the predominant threats. The association of ERM with abrupt fluctuations in water temperature (Danley \textit{et al.} 1999; Rigos and Stevenson 2001), overcrowding and harvest stress, makes this pre-eminent.

Despite the fact that \textit{Y. ruckeri} has been described primarily as a salmonid pathogen, there are increasing numbers of reports of its isolation from diseased non-salmonid species (Bullock and Cipriano 1990, McArdle and Dooley-Martyn 1985, Michel \textit{et al.} 1986, Hussein \textit{et al.} 1997, Berc \textit{et al.} 1999, Abd-El Latief 2001). Thus, the current study adds a new report of yersiniosis to the non salmonid host range, and a first report of a natural outbreak of yersiniosis among Nile tilapia in a semi-intensive fish pond in Lower Egypt.

The clinical indications in Nile tilapia were closely similar to those reported for the same disease in salmonids and some non-salmonid species (Fuhrmann \textit{et al.} 1983, Rigos and Stevenson 2001). The 66.6% overall prevalence of yersiniosis in the affected tilapia exceeds similar clinical infections among farmed trout and salmon in Turkey, Canada, Croatia, USA and Spain (Tobback \textit{et al.} 2007). This could be due to the use of agricultural drainage water in aquaculture, fluctuating climatic changes, and faulty management practices associated with tilapia culture in semi-intensive earthen pond systems. In addition, the affected fish farm is a transient stop for some migratory birds crossing northern Egypt and this might further facilitate the spread of this disease to other ponds.

The extent of the disease among the entire pond population, with mortalities approaching 87%, as well as the severity of the lesions on individual fish, suggests the high virulence of the \textit{Y. ruckeri} isolates retrieved from the clinical cases as well as the possible suppression of the fish’s resistance. The present outbreak was associated with sharp temperature changes within three successive days, jeopardising the fish’s immune system and initiating rapid invasion by \textit{Y. ruckeri} that were already present in the pond water and sediment (Coquet \textit{et al.} 2002).

This study reports on the critical role of environmental and managerial stresses in initiating outbreaks of yersiniosis

![Figure 2: Yersiniosis-infected \textit{Oreochromis niloticus} showing characteristic external signs of red mouth disease](image1)

![Figure 3: Yersiniosis-infected \textit{Oreochromis niloticus} with congested spleen, liver and intestine, hemorrhagic kidney and subcutaneous hemorrhages](image2)

**Table 1:** Biochemical profiles of isolates, based on conventional tests, API 20E and API 20 NE profiles

<table>
<thead>
<tr>
<th>Test</th>
<th>Retrieved isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase</td>
<td>–</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>+</td>
</tr>
<tr>
<td>Vogus–Proskauer</td>
<td>+</td>
</tr>
<tr>
<td>(\text{H}_2\text{S})</td>
<td>–</td>
</tr>
<tr>
<td>Tryptophan for deaminase</td>
<td>–</td>
</tr>
<tr>
<td>Tryptophan for indole production</td>
<td>–</td>
</tr>
<tr>
<td>Beta galactosidase</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dehydrodase</td>
<td>+</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
</tr>
<tr>
<td>Glucose for O/F</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>–</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
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<tr>
<td>Salicin</td>
<td>–</td>
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<tr>
<td>Melibiose</td>
<td>–</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
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<tr>
<td>Arabinose</td>
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in cultured tilapia and emphasises the urgent need for a practical method to combat infectious diseases that threaten tilapia culture in Egypt.

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References

ABD-EL LATIEF JI (2001) Yersinia microorganisms as the causative agent of enteric redmouth disease in delta Nile fishes. MSc thesis, Faculty of Veterinary Medicine, Cairo University


MCARDLE JF and DOOLEYMARTYN C (1985) Isolation of Yersinia ruckeri type I (Hagerman strain) from goldfish, Carassius auratus (L.). Bulletin of the European Association of Fish Pathology 5: 10–11
