

# Difficulties in diagnosing terminal ileitis due to *Yersinia pseudotuberculosis*

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**Abstract** We report three patients with terminal ileitis and positive fecal cultures with *Yersinia pseudotuberculosis*. From one patient, a virulence plasmid (pYV)-negative *Y. pseudotuberculosis* was isolated, which represents the second finding of a pYV-negative isolate associated with human disease. All patients were treated with ciprofloxacin and fully recovered. Since conventional culture methods for yersiniosis are gradually replaced with molecular tests not recognizing *Y. pseudotuberculosis*, we recommend to include a specific culture medium or to apply a specific polymerase chain reaction (PCR) assay on fecal samples from patients suspected of terminal ileitis.

## Introduction

The genus *Yersinia* belongs to the family of Enterobacteriaceae and is composed of 11 species, of which only *Y. pestis*, *Y. pseudotuberculosis*, and some *Y. enterocolitica* serotypes are

human pathogens [1–3]. While *Y. pestis* is known as the causative agent of the plague and causes no gastrointestinal disease, *Y. enterocolitica* and *Y. pseudotuberculosis* are known as causative agents of gastrointestinal disease [1–4].

*Y. pseudotuberculosis* is a zoonotic pathogen transmitted by the fecal–oral route that has been recovered from various rodents, rabbits, deer, bats, farm animals, and wild and domestic birds [2, 3, 5, 6]. In 1883, Knapp and Masshoff were the first to isolate *Y. pseudotuberculosis* from a mesenteric lymph node of a patient with a clinical syndrome of acute appendicitis [7]. After an incubation time of 3–7 days, *Y. pseudotuberculosis* may cause mucosal ulceration in the terminal ileum and, sometimes, the adjacent colon, lesions in Peyer's patches, and enlargement of mesenteric lymph nodes [8, 9]. In severe cases, septicemia, thrombosis of mesenteric vessels, focal abscesses, intestinal necrosis, and hemorrhage may occur. Predisposing host factors for a severe course are diabetes mellitus, liver cirrhosis, and hemochromatosis [10, 11]. Post-infectious complications such as reactive (poly)arthritis and erythema nodosum can result from an immunological reaction within several weeks [2].

*Y. pseudotuberculosis* is a pleomorphic, facultative anaerobic, non-spore-forming, Gram-negative rod that grows at temperatures ranging from 4 to 43 °C, with the optimal temperature being between 25 and 28 °C. *Y. pseudotuberculosis* grows on most routine media, including MacConkey, blood, and chocolate agar, incubated at 35 °C in ambient air, but may be overgrown by other enteric bacteria in clinical samples. Therefore, a selective agar, cefsulodin–irgasan–novobiocin (CIN) agar, incubated at 25–28 °C, is used for the isolation of *Y. pseudotuberculosis* from fecal samples [12–14]. CIN agar contains D-mannitol and fermentation of this carbohydrate in the presence of neutral red produces characteristic red colonies. Other bacteria, such as *Plesiomonas* and *Aeromonas* species, also grow on CIN agar, but these can be differentiated from *Yersinia* species by their positive oxidase test. *Y. pseudotuberculosis* can be serotyped based on O-

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somatic antigen, which is the most distal component of the lipopolysaccharide membrane. Twenty-one serotypes have been identified, with O Group I (O:1) accounting for most human cases and sometimes O:2 or O:3 [2, 15]. The virulence of *Y. pseudotuberculosis* is associated with the presence of multiple virulence markers, including the plasmid of *Yersinia* virulence (pYV), the invasion adhesion molecule (*invA*) gene, the attachment invasion locus (*ail*) gene, and the high pathogenicity island (HPI) [1–3, 16–19].

We report three patients with terminal ileitis due to *Y. pseudotuberculosis* diagnosed by conventional culture.

## Case reports

### Case 1

A previously healthy 24-year-old female was admitted to a local hospital with progressive right lower quadrant abdominal pain, suspected of appendicitis. There was no diarrhea and temperature was 37.7 °C. On physical examination, there was pain on percussion and rebound tenderness of the right lower abdominal quadrant. Laboratory examinations showed a leukocytosis (total white cell count  $12.1 \times 10^9/l$ ), an elevated C-reactive protein level (49 mg/l), and no other abnormalities. A pregnancy test was negative and urine sediment was normal. A computed tomography (CT) scan showed a normal appendix, significant thickening of the terminal ileum wall over a length of 7 cm, thickening of the adjacent cecum wall, enlarged mesenteric lymph nodes, and no free intraperitoneal fluid.

Morbus Crohn was suspected, and prednisone and azathioprine were started. A colonoscopy was performed and revealed a swollen mucous membrane with little ulceration of the terminal ileum and adjacent cecum. Biopsies were performed and pathological examination showed no signs of M. Crohn or idiopathic colitis. To exclude an infectious origin, a fecal culture was performed. The fecal culture revealed growth of *Y. pseudotuberculosis* on CIN medium. Prednisone and azathioprine were stopped and the patient was treated with ciprofloxacin 500 mg twice daily for 5 days. At follow up 2 months later, there were no complaints, laboratory examinations were normal, and a magnetic resonance imaging (MRI) enterography showed no abnormalities.

### Case 2

A previously healthy 17-year-old female presented to the emergency department with progressive right lower quadrant abdominal pain since 5 days, suspected of appendicitis. There was no diarrhea and temperature was 38.3 °C. On physical examination, there was rebound tenderness of the right lower abdominal quadrant. Laboratory examinations showed a leukocytosis (total white cell count  $14 \times 10^9/l$ ), an elevated C-reactive

protein level (54 mg/l), an elevated erythrocyte sedimentation rate (29 mm/h), and no other abnormalities. A pregnancy test was negative and urine sediment was normal. An abdominal ultrasonography could not visualize the appendix, but showed enlarged mesenteric lymph nodes. A CT scan showed a normal appendix, significant thickening of the terminal ileum wall over a length of 10 cm, enlarged mesenteric lymph nodes, and no free intraperitoneal fluid (Fig. 1).

A terminal ileitis was suspected, with a differential diagnosis of M. Crohn and an infectious ileitis. Two fecal cultures were performed and both grew *Y. pseudotuberculosis* on CIN medium. The patient was treated with ciprofloxacin 500 mg twice daily for 5 days. At follow up 2 months later, there were no complaints, laboratory examinations were normal, and abdominal ultrasonography showed no abnormalities.

### Case 3

A previously healthy 33-year-old female presented to the emergency department of a local hospital with progressive right lower quadrant abdominal pain since 7 days, suspected of appendicitis. There was no diarrhea and temperature was 37.3 °C. On physical examination, there was rebound tenderness of the right lower abdominal quadrant. Laboratory examinations showed normal leukocytes (total white cell count  $7.9 \times 10^9/l$ ), and an elevated C-reactive protein level (31 mg/l). Urine sediment was normal. An abdominal ultrasonography showed thickening of the terminal ileum atypical for M. Crohn and enlarged mesenteric lymph nodes. No CT scan was performed.

An infectious terminal ileitis was suspected and a fecal culture was performed. Prior to final diagnosis, treatment was started with ciprofloxacin 500 mg twice daily for 5 days. The fecal culture revealed growth of *Y. pseudotuberculosis* on CIN medium. At follow up 1 month later, there were no complaints



**Fig. 1** Computed tomography (CT) scan of case 2, showing an enlarged mesenteric lymph node (arrow)

and laboratory examinations were normal. No control ultrasound or CT scan was performed.

## Results

Because of the clinical suspicion of an infectious terminal ileitis in all three cases, fecal samples were incubated on CIN agar at 25–28 °C, for the isolation of *Yersinia* species. The cultured isolates were then identified as *Y. pseudotuberculosis* by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (Bruker Daltonik GmbH, Bremen, Germany) [20, 21]. Further characterization of the isolated *Y. pseudotuberculosis* strains was performed by serotyping and molecular techniques. Two isolates belonged to serotype O1, the most frequently found type in human infections and one isolate was not serotyped [2, 3]. *Y. enterocolitica* 16S rRNA polymerase chain reaction (PCR), performed at the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands) and the Robert Koch institute (RKI, Wernigerode, Germany), was negative in two isolates and was not performed for one isolate. *Y. enterocolitica* ail gene PCR, performed at the RKI, was negative in all three isolates. No diagnostics were performed for the *Y. pseudotuberculosis* chromosomal *invA* and *ail* gene or the HPI, because these were not available at the RIVM and RKI. Two isolates were positive in the *Y. pseudotuberculosis* pYV PCR, performed at the RKI, and one isolate was negative. Susceptibility of the *Y. pseudotuberculosis* was determined with Etest (AB Biodisk, Solna, Sweden) and performed for amoxicillin [minimum inhibitory concentration (MIC) 0.25–0.5 µg/ml], cefuroxime (MIC 0.5–0.75 µg/ml), chloramphenicol (MIC 1.5–3 µg/ml), ciprofloxacin (MIC 0.023–0.047 µg/ml), and tetracycline (MIC 0.75–1.5 µg/ml).

## Discussion

In 2012, three unrelated patients were diagnosed with *Y. pseudotuberculosis*-associated terminal ileitis in different hospitals in the Netherlands. The patients were female, between 17 and 33 years of age, and presented with progressive right lower abdominal pain and fever. None of the patients had underlying disease. Remarkably, radiological results were the first indication of terminal ileitis in all patients. All patients fully recovered after treatment with ciprofloxacin without any complication.

The virulence of *Y. pseudotuberculosis* is associated with the presence of a genetically conserved virulence plasmid, termed pYV. It is thought that loss of pYV leads to avirulence [1, 2, 22]. pYV carries components of a type three secretion system, which forms a needle structure on the surface of *Y. pseudotuberculosis* that interacts with target cells and enables the

injection of at least six different *Yersinia* outer membrane proteins (Yops) [1, 2, 22, 23]. Yops disrupt the primary immune response and adaptive inflammatory cascade, and, in this way, prevent phagocytosis [2, 3, 22, 23]. Besides plasmid-encoded genes, *Y. pseudotuberculosis* also have chromosomal-encoded virulence markers, like the *invA* and *ail* genes, and some strains harbor the HPI. The *invA* gene encodes for an epithelial cell adhesion molecule, which facilitates efficient binding to intestinal mucosal cells and translocation from the lumen to Peyer's patches. The *ail* gene encodes for an outer membrane protein that mediates complement resistance; its nucleotides composition differs slightly between *Y. enterocolitica* and *Y. pseudotuberculosis* [18, 19, 24, 25]. The HPI encodes an iron uptake system for the siderophore yersiniabactin and its presence makes a strain highly virulent. HPI plays a key role in the systemic spread of *Yersinia*, by which infection of the spleen and liver can occur [3, 16].

The absence of pYV in an isolate causing terminal ileitis (case 1) is in contradiction with the common hypothesis that the absence of pYV leads to avirulence of the bacteria [1, 2, 22]. However, it is reported in the literature that *Y. pseudotuberculosis* can lose pYV after subculture and storage, in particular, at 37 °C [18]. Consequently, the pYV is not an ideal DNA target for the detection of pathogenic *Yersinia* strains, and it is better to combine it with the detection of a chromosomal virulence gene [17]. Animal infection studies showed that pYV-negative *Y. pseudotuberculosis* strains are able to establish intestinal infections and even colonize the Peyer's patches and mesenteric lymph nodes, showing that the presence of pYV is not required for the entry of *Yersinia* into host tissues [3, 26]. For instance, Fukushima et al. reported a case of a Japanese girl with a mesenteric lymphadenitis caused by a pYV-negative *Y. pseudotuberculosis* that was positive for the chromosomal virulence markers [26]. So, although the loss of pYV leads to a significant decrease in the level of virulence, it seems likely that, also, pYV-negative strains can cause human infection [3, 26].

Although the reported incidence of human infections due to *Y. pseudotuberculosis* is low, this may be due to the fact that CIN agar is not systematically used for the culture of *Yersinia* spp. in diarrheal patients. Moreover, most of the molecular diagnostic techniques use the *Y. enterocolitica* ail gene as the target, which is not present in *Y. pseudotuberculosis* [24, 27, 28]. This means that selective culture techniques or the performance of a specific *Y. pseudotuberculosis* PCR are warranted when *Y. pseudotuberculosis* is suspected, and also when diarrhea is not present. We also want to encourage the development of *Y. pseudotuberculosis*-specific PCRs for the combined detection of pYV and chromosomal-encoded genes in the light of the pYV-negative cultured *Y. pseudotuberculosis* isolate.

**Conflict of interest** The authors declare that they have no conflict of interest.

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