Research Note

Bioserotypes and Virulence Markers of *Yersinia enterocolitica* Strains Isolated from Mallards (*Anas platyrhynchos*) and Pheasants (*Phasianus colchicus*)

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ABSTRACT

*Yersinia enterocolitica* is the causative agent of yersiniosis in different animal species and in humans. Food contaminated with *Y. enterocolitica* is the main source of infection for humans, and swine plays a major role in the transmission of the disease. There are a limited number of reports of the prevalence of *Y. enterocolitica* in wild animals and birds. This study characterized virulence markers associated with *Y. enterocolitica* isolates recovered from mallards and pheasants. *Y. enterocolitica* strains were isolated from 5 (11.11%) of 45 mallards originating from a cold culture (peptone, sorbitol, and bile salts medium) belonging to biotype 1A. Serotyping showed that three of these five serotypes represented serotype O:8, one belonged to serotype O:5, and one did not agglutinate with any of the sera and was classified as nonidentified. Molecular analysis for virulence markers detected the ystB gene, which encodes an enterotoxin, in five isolates. *Y. enterocolitica* was not detected in any of the 16 examined pheasants.

Research has shown that mallards can carry and shed *Yersinia enterocolitica* and are a potential source of *Y. enterocolitica* infections. The majority of the isolated strains belonged to bioserotype 1A/O:8; this serotype, rarely detected anywhere in the world, included amplicons of the ystB gene that might present risks to public health.

*Y. enterocolitica* is the causative agent of yersiniosis, a disease reported in animal species and in humans. In animals, the infection is usually asymptomatic or is accompanied by short-term diarrhea, whereas in humans, particularly in small children, the disease may be fatal in the most severe cases (2). It is thought that food contaminated with *Y. enterocolitica* is the main source of infection for humans; this is reflected in the most recent reports of the European Food Safety Authority (EFSA), which recognizes yersiniosis as one of the three most important foodborne zoonoses in the European Union (3, 4, 5).

From among the six known biotypes of *Y. enterocolitica*, the strains 1B, 2, 3, 4, and 5 are considered pathogenic for animals and people; these strains have the pYV virulence plasmid and the *ail* and *inv* chromosomal genes, whose products are responsible for the processes of invasion and adhesion to the intestinal epithelial cells, as well as the ystA gene, which codes for the production of the YstA enterotoxin. Biotype 1A without the classical virulence markers was, until recently, regarded as nonpathogenic (16, 21, 23). Lately, however, there have been reports of clinical cases of yersiniosis caused by biotype 1A; thus strains of this particular biotype should not be excluded in advance from being considered potentially pathogenic (16). Generally, *Y. enterocolitica* biotype 1A strains possess the ystB gene responsible for the production of the YstB enterotoxin; this most likely causes the diarrhea that occurs in the course of the yersiniosis caused by this biotype.

Pigs are thought to be the most important factor in the transmission of *Y. enterocolitica* infections because of the high level of pork consumption in Europe and the phylogenetic similarity between *Y. enterocolitica* isolated from pigs and the strains isolated from clinical cases in humans (8, 10, 12). Although consumption of wild game may have increased due to its perceived superior nutritional value or other health benefits associated with it, there are a limited number of reports on the prevalence of *Y. enterocolitica* in wild animals and birds.

The search for potentially pathogenic *Y. enterocolitica* strains in wild birds is particularly interesting since avian migration may play an important epidemiological role. In addition to meat contaminated with *Y. enterocolitica*, consumption of water from natural sources is a significant factor in the transmission of the disease; therefore, an attempt to determine the prevalence of this pathogen is needed. Moreover, the use of birdshot in bird shooting may contaminate carcasses with *Y. enterocolitica* from the digestive tract damaged by birdshot. Therefore, the authors examined cloacal swabs from mallards (*Anas platyrhynchos*).
for potentially pathogenic _Y. enterocolitica_ strains, to characterize the strains and to determine the presence of virulence markers in strains potentially pathogenic for humans. For comparative purposes, the experiment was also carried out on pheasants (_Phasianus colchicus_) from an enclosed habitat.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** The materials for the study consisted of 90 cloacal swabs from 45 mallards and 32 cloacal swabs from 16 pheasants obtained in Poland. Two samples were taken from each bird to determine the ability of _Y. enterocolitica_ to grow under low temperature conditions. One swab from each bird was then placed in a test tube with 9 ml of irgasan, ticarcillin, and potassium chloride (Biocorp Ltd, Warsaw, Poland) medium (warm culture, prepared according to PN-EN ISO 10273); simultaneously, the other swab from each bird was placed in a test tube with 9 ml of peptone, sorbitol, and bile salts (PSB) medium (cold culture, prepared according to PN-EN ISO 10273). The irgasan, ticarcillin, and potassium chloride medium was incubated at 25°C for 48 h, and the PSB medium was incubated at 4°C for 3 weeks. Next, 0.5 ml of culture was transferred into 4.5 ml of 0.5% KOH in 0.5% NaCl for 20 s, after which a loopful was streaked onto a cefsulodin, irgasan, and novobiocin (Yersinia selective agar base according to Schiemann; Yersinia selective supplement, Merck KGaA, Darmstadt, Germany) plate and incubated at 30°C for 48 h. Further biochemical identification of one to five typical colonies from each cefsulodin, irgasan, and novobiocin plate was carried out according to the PN-EN ISO 10273 standard, to make a preliminary selection of potentially pathogenic _Y. enterocolitica_ strains.

**Serotype and biotype determination.** The determination of the serologic group of the examined strains was performed using the slide agglutination test. Live bacterial cells from the 24-h blood agar culture (Grasso Biotech, Starogard Gdanski, Poland) were used as an antigen, and the sera for the somatic antigens O:3, O:5, O:8, and O:9 came from ITEST company (Hradec Kralove, Czech Republic). The cells of the tested strain were suspended in a drop of 0.85% NaCl placed on a glass slide and then connected with a drop of serum placed nearby and mixed with the bacteriological oese. After shaking for 1 min, agglutination with one of the four sera used was considered a positive result. If there was no agglutination with any serum, the strain was regarded as nonidentified. Biotype determination of the examined strains was made in accordance with the PN-EN ISO 10273 standard.

**DNA isolation.** Genomic DNA isolation was performed with the Genomic Mini kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer’s instructions and was stored at −20°C for further analyses.

**Primers and triplex PCR conditions.** Multiplex PCR included the amplification of three genes: _ail_, _ystA_, and _ystB_. The sequences of the primers, synthesized in the DNA Sequencing Laboratory of the Biochemistry and Biophysics Institute of the Polish Academy of Sciences, Oligo, Warsaw, were published by Harnett et al. (6) (_ail_), and Platt-Samoraj et al. (17) (_ystA_, _ystB_). Multiplex PCR was carried out using HotStarTaq Plus DNA polymerase (Qiagen GmbH, Hilden, Germany) and HotStarTaq Plus Master Mix Kit (Qiagen). The 20-μl reaction mixture contained about 120 ng of isolated DNA (from 1 to 3 μl), 10 μl of HotStarTaq Plus Master Mix 2×, 2 μl of CoralLoad

![FIGURE 1. Electrophoretic separation of _ail_, _ystA_, and _ystB_ gene amplification products from _Y. enterocolitica_ strains in triplex PCR. M, size marker GeneRuler 100-bp DNA Ladder Plus (Fermentas); lane 1, positive control, contains DNA isolated from a reference strain of _Y. enterocolitica_ O:8 (ACTT 23715); lane 2, positive control, contains DNA isolated from a reference strain of _Y. enterocolitica_ O:5; lane 3, zero control, does not contain bacterial DNA, contains ail, ystA, and ystB gene primers; and lanes 4 to 8, products of amplification of _ystB_ gene fragments from _Y. enterocolitica_ strains isolated from mallards.](image-url)
biotyping and serotyping and triplex PCR did not confirm that this isolate belonged to *Y. enterocolitica*; therefore, it is assumed that a different *Yersinia* species was isolated.

**DISCUSSION**

The results of these studies have expanded the knowledge of the prevalence of *Y. enterocolitica* in wild birds, along with some interesting observations. The percentage of isolated *Y. enterocolitica* strains for the number of tested mallards is significantly higher than in previous reports (15, 18). In our studies, *Y. enterocolitica* was detected in 5 (11.11%) of 45 mallards, whereas Shayegani et al. (18) confirmed the presence of this microorganism in only 1 (4.2%) of 24 tested mallards. In a study on the prevalence of *Y. enterocolitica* in migrating birds in Sweden, Niskanen et al. (15) examined only one mallard and did not detect this bacterium. As for pheasants, our studies did not confirm the carrier state of *Y. enterocolitica* in any of the 16 tested birds, while Kato et al. (7) showed the presence of this pathogen in 2 (6.06%) of 33 tested pheasants. The results reported by Levrè et al. (13) were similar to our studies; these authors showed that *Y. enterocolitica* was detected in 26 (11.98%) of 217 tested birds.

The isolation of potentially pathogenic *Y. enterocolitica* strains only on PSB medium confirms the need to perform a cold culture in order to assess the epidemiological profile of yersiniosis (1, 9, 11, 19). All isolated *Y. enterocolitica* strains belonged to biotype 1A; this was confirmed by the results of molecular analysis, in which triplex PCR yielded a 180-bp band that indicated the presence of ystB gene amplicons typical of this biotype.

The results of serotyping of isolated *Y. enterocolitica* strains are particularly interesting since three of five isolates belonged to serotype O:8. The strains belonging to serotype O:8 that are typically classified as biotype 1B are regarded as the most pathogenic to humans, whereas the bioserotype 1A/O:8 has rarely been reported (14, 24). Wang et al. (24) describe a similar situation in China, where, for over 20 years, 39 *Y. enterocolitica* strains belonging to biotype 1A serotype O:8 have been collected. The strains were isolated from different animal species (pigs, cattle, goats, and dogs) and from food. Those studies, however, did not include wild animals and birds. Of the strains collected during the studies, none had the typical virulence markers, such as *all*, *ystA*, *yadA*, and *virF* gene amplicons; also, they did not cause lethal effects on experimental mice and they presented a very low capacity to invade Hep-2 cells. These strains, however, had the amplicons of the ystB gene, which is characteristic of strains isolated in our studies. This fact suggests that weakly pathogenic strains can be isolated from mallards and that they constitute a potential source of infection to humans. McNally et al. (14) achieved slightly different results in studies from 1999 to 2000, in which the authors compared *Y. enterocolitica* biotypes isolated from different animal species and from clinical cases of yersiniosis in humans. In these studies, 12 strains of biotype 1A serotype O:8 were isolated from pigs, which corre-

sponded to only 1.84% of all *Y. enterocolitica* strains isolated from this animal species. The results of studies on 164 *Y. enterocolitica* strains isolated from humans with yersiniosis seem far more interesting. In five cases, biotype 1A serotype O:8 strains were the causative agents of the disease; this agrees with previous reports on clinical cases of yersiniosis in humans caused by biotype 1A with the gene encoding the production of YstB enterotoxin (16, 20).

It seems that the standard procedure of bio-serotype determination is an insufficient criterion to assess the pathogenic potential of *Y. enterocolitica* strains (21–23). To sum up, mallards may carry and shed *Y. enterocolitica* and may be a potential source of *Y. enterocolitica* infection. The isolated strains belonged to the rare bioserotype 1A/O:8 and had the amplicon of the ystB gene, which might pose a risk to public health.

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**REFERENCES**


