Research Note

Prevalence of *Yersinia enterocolitica* in Raw Cow’s Milk Collected from Stables of Mexico City

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ABSTRACT

*Yersinia enterocolitica* has been isolated from a batch of pork-derived products, from healthy and diseased animals, and from different types of milk and dairy products, among others. We studied the prevalence and diversity of *Y. enterocolitica* isolated from raw cow’s milk collected from stables in Mexico City. Of the 1,300 samples analyzed, *Y. enterocolitica* was isolated in 454, with an average of 25% positive samples for each stable. Of the total isolated bacteria of the *Yersinia* genus, 44.25% were *Y. enterocolitica*, 18.28% were *Y. kristensenii*, 13.65% were *Y. intermedia*, 14.85% were *Y. frederiksenii*, and 9.14% were *Y. aldovae*. Among the different strains of *Y. enterocolitica*, biotype 1A was present in 70%, biotype 2 in 13.2%, biotype 3 in 8.54%, and biotype 4 in 8.15% of samples. Serotypes O:5, O:3, O:2, and O:9 were found in all biotypes identified. There were no statistically significant differences in the isolation of this bacterium with respect to the stables where they were found, although there was significant difference regarding the time of year. The data obtained in this work show the need to protect public health in Mexico against infections transmitted by raw cow’s milk.

*Yersinia enterocolitica* causes yersiniosis and is an important enteropathogen worldwide (33), responsible for gastroenteritis and other syndromes in humans and animals (7, 36). Human cases have increased in recent years, and more than two-thirds of these infections are manifested by acute diarrhea lasting 1 to 3 weeks, affecting mainly children younger than 5 years. Most of the infections are sporadic, and only a few cases have been verified as outbreaks (37).

In the Americas, strains of *Y. enterocolitica* pathogenic for humans are widely distributed in nature, and they have diverse animal reservoirs such as chinchilla, rabbit, rat, monkey (13, 18), pig (34), dog, cat, chicken, cows (26), and aquatic animals (29). The transmission of infection occurs, however, mainly through contaminated food or water (38). The main foods that have been involved in the isolation of *Y. enterocolitica* are raw pork including the tongue, liver, heart, kidney, loin, tenderloin, chops, ham, beef, and pork sausage (9). The bacterium has also been isolated from untreated water, tofu, raw cow’s milk, pasteurized milk, milk from sick animals, dairy products, asymptomatic handlers (24), clinically normal lactating animals, excrement, and conserved forage in silos (14, 16, 32). Currently, the pathogenic strains identified by serology, according to their somatic antigens, belong to serogroups O:1, 2, 3, O:3, O:8, O:9, O:4,32, O:5,27, O:12,25, O:19, O:20, and O:2. Among the serogroups that cause disease in humans, O:3, O:8, O:9, and O:5,27 are the most prevalent (23, 41).

The statistical livestock service from SIAP/SAGARPA (Food and Fisheries Information Service from the Agriculture, Livestock, Rural Development, Fisheries and Food Secretariat, Mexico City) reported that the production of bovine milk was 10,549,038 kl in 2009, 10,711,620 kl in 2010, 10,742,637 kl in 2011, and as of April, 3,385,509 kl in 2012 (27).

The presence of presumptive enteropathogenic *Y. enterocolitica* in raw milk, pasteurized dairy products, and asymptomatic handlers has been reported in the United States, France, Australia, Brazil, Finland, and China, among other countries (24, 39). Based on the ubiquity of this organism (12, 35) and its ability to grow at refrigeration temperatures (22), *Y. enterocolitica* is a bacterium of interest from a public health point of view.

In Mexico, the presence of *Y. enterocolitica* has been detected in raw pork, chicken, and precooked pork meat (25). There are not precise data on the consumption of raw cow’s milk or on the frequency of this organism. Furthermore, no routine search for this bacterium is performed, making it impossible to know its real epidemiological relevance and the potential health risk for the population consuming raw cow’s milk. For these reasons and because there has been scant research related to food contamination by *Y. enterocolitica*, this work sought to demonstrate the presence of this bacterium in raw milk of...
A total of 1,300 milk samples were analyzed—100 Y. enterocolitica, Y. kristensenii, Y. intermedia, Y. frederiksenii, Y. aldovae, Y. enterocolitica, Y. kristensenii, Y. intermedia, Y. frederiksenii, Y. aldovae, Yersinia—of the positive samples, followed by Y. kristensenii, Y. intermedia, Y. frederiksenii, Y. aldovae, Yersinia, for Tla huac, 23.56 was not isolated in this work. was the species isolated at the highest frequency (11). was determined on days 1, 3, 5, 7, and 10 of incubation at a temperature of 28 ± 2°C. was taken from milk bulk tanks containing milk from 10 to 20 cows and was placed in a plastic bag. The samples were brought to the laboratory in a thermal container at 4 to 6°C, and the time between collection and analysis never exceeded 4 h. was used, with the SPSS v.16.0 software (SPSS Inc., Chicago, IL). every month, 25 milk samples were analyzed from each stable. cows in stables located in Mexico City and to find the most abundant bioserotypes in this milk.

### MATERIALS AND METHODS

#### Sampling.
A total of 1,300 milk samples were analyzed—100 samples each month—from four different stables (Tlahuac, San Lorenzo Tezonco, Iztapalapa, and Milpa Alta, all of them using manual milking and located in the southwest of Mexico City). One daily sample from each stable, approximately 1,000-ml volume, was taken from milk bulk tanks containing milk from 10 to 20 cows and was placed in a plastic bag. The samples were brought to the laboratory in a thermal container at 4 to 6°C, and the time between collection and analysis never exceeded 4 h. The methodology for bacterial isolation and identification was that recommended by the U.S. Food and Drug Administration in the *Bacteriological Analytical Manual* (41).

#### Isolation and selection.
Under aseptic conditions, 25 ml of sample was suspended in 225 ml of phosphate-buffered saline with 10% peptone (11). The mixture was homogenized for 30 s. The presence of presumptive enteropathogenic *Y. enterocolitica* was determined on days 1, 3, 5, 7, and 10 of incubation at a temperature of 10°C. At each time, 1 ml of the medium was mixed with 9 ml of 0.5% KOH (prepared in 0.5% saline solution); 0.1 ml of the mixture was immediately spread in MacConkey agar and cefsulodin-irgasan-novobiocin medium and was incubated at 28 ± 2°C for 24 to 48 h.

### RESULTS

#### Isolation and identification.
Presumptive enteropathogenic *Y. enterocolitica* was isolated from 454 (34.92%) of the 1,300 samples analyzed in this study. The percentage of positive samples per stable was 30.61% for Tlahuac, 24.88% for Milpa Alta, 23.56% for Iztapalapa, and 20.92% for San Lorenzo Tezonco. As shown in Table 1, *Y. enterocolitica* was isolated in all four stables throughout the study time. The greatest number of positive samples were obtained from Tlahuac. The presence of *Y. enterocolitica* had a seasonal effect; the largest numbers of positive samples were obtained in December (68 of 100) and January (62 of 100) (Table 1).

Of the 454 raw cow’s milk samples testing positive, 1,362 isolates with characteristic colony morphology were obtained. After identification, only 725 strains were identified as members of the genus *Yersinia*. *Y. enterocolitica* was the species isolated at the highest frequency (44.25% of the positive samples), followed by *Y. kristensenii* (18.28%), *Y. intermedia* (13.65%), *Y. frederiksenii* (14.85%), and *Y. aldovae* (9.14%) (Table 2). *Yersinia pseudotuberculosis* was not isolated in this work.

### Bioserotyping.
The results of this method are shown in Table 3, with the highest percentage of identification for bioserotypes 1A/O:1, O:2, O:3, O:5, O:27, O:8, O:9 (70%), followed by 2/O:5, O:2, O:3, O:1, O:9, O:8 (13.20%).

### Identification of bacteria.
Three characteristic colonies were analyzed with presumptive tests: oxidase, catalase, Gram stain, and mobility at 28 and 37°C in media with lysolecine, arginine, and bile esculin agar. Their growth was also assessed in triple sugar iron agar medium and in Christensen’s urea medium (in both cases, with incubation for 48 h at 30 ± 2°C), and in methyl red Voges-Proskauer broth, incubated at 35 ± 2°C for 24 h. Subsequent confirmatory tests were performed using the API 20E system (bioMérieux, Hazelwood, MO), according to manufacturer’s instructions.

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3/O:5,27, O:3, O:9, O:8, O:2 (8.54%), and 4/O:5,27, O:3, O:9, O:2 (8.15%) (Table 3). Serotypes O:5, O:3, O:2, and O:9 were found in all biotypes identified. Serotype O:1 was not present in biotypes 3 and 4, and serotype O:8 was not present in biotype 4.

**DISCUSSION**

Milk has exceptional nutritional value, as the most complete and balanced food, exclusively consumed by humans in their very early life and excellent at any age (10). It has been implicated, however, as an important vehicle for pathogens in humans (30). Microorganisms that may be present in raw cow’s milk include Campylobacter jejuni, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Serratia* spp., *Salmonella* spp., *Salmonella enterica* serovar Typhimurium, and *Y. enterocolitica* (17, 20).

*Y. enterocolitica* is an important zoonotic pathogen that can cause yersiniosis in humans and animals (2). Its presence in the milk of diseased animals, in clinically normal lactating animals, as well as in stool and in conserved forage in silos has been documented, and its widespread occurrence increases the likelihood of milk contamination (14). The ubiquity of *Y. enterocolitica* (12) and its ability to grow at temperatures below 4°C probably have an impact on its frequency (21, 22). Yucel and Ulusoy (42) reported that *Y. enterocolitica* can grow in foods stored at a temperature of 0°C, and this quality is a serious problem for the food industry.

In the present work, the presence of presumptive enteropathogenic *Y. enterocolitica* was found in 34.92% of the raw cow’s milk samples analyzed. When the results were analyzed using a nonparametric Friedman test, no statistically significant difference was found in the isolation of this bacterium with respect to its origin in stables.

Some previous studies have reported that the prevalence of *Y. enterocolitica* is variable, which may be due to the time of year, weather, geographical area, size of the stable, number of animals per stable, and hygiene practices and management (21, 23), reflecting a clear regional disparity (40). Some authors claim that it is reasonable to assume that, within a herd, the prevalence of some or all serotypes increases during the cold months (23). Other studies have reported a seasonal variation in the isolation of *Y. enterocolitica* during the summer months (8). The results obtained in this work indicate that *Y. enterocolitica* isolates show a cyclical trend, with an increase in the number of positive samples during the cold months (Table 1). When the χ² test was used to evaluate the effect of season on the presence of *Y. enterocolitica*, statistically significant differences were found. Furthermore, higher levels of contamination could be detected after shorter preenrichment cultures from September to February (data not shown).

The bioserotypes of presumptive enteropathogenic *Y. enterocolitica* identified in the present study are shown in Table 3. It should be noted at this point that the use of cefsulodin-irgasan-novobiocin medium can explain the low frequency of isolation of biotype 4/O:3. It has been reported that bioserotypes 1A/O:3, 1A/O:8, 1A/O:9, 1A/O:5,27; 2/O:5,27, 2/O:3, 2/O:2, 2/O:1, 2/O:9, 2/O:8; 3/O:5,27, 3/O:3, 3/O:8, 3/O:2; and 4/O:5,27, 4/O:3, 4/O:2, 4/O:9 affect humans worldwide. Most strains associated with yersiniosis belong to 1B/O:8, 2/O:5,27, 2/O:9, 3/O:3, and 4/O:3, for which different geographical distributions have been shown (9). The biotype 1A, the most common for *Y. enterocolitica* strains isolated from milk, is considered nonpathogenic to humans, whereas biotypes 1B, 2, 3, 4, and 5 show pathogenicity (21). However, clinical, epidemiological, and experimental evidence suggest that some strains of biotype 1A are increasingly virulent and may cause gastrointestinal disease (31), with symptoms identical to those of strains belonging to pathogenic biotypes (1).

In many European countries, human yersiniosis ranks third in diarrheal disease, after campylobacteriosis and salmonellosis (6). In Mexico, gastrointestinal diseases caused by food consumption numbered 5,003,650 in 2009, with an increase to 5,175,178 cases in 2011 (28). It is noteworthy that a high rate of yersiniosis may be subclinical in the healthy population (5).

The sale of raw cow’s milk implies lower costs than of the treated milk; however, the degree of risk posed by consumption of this product in Mexico is not well documented. Notably, it is customary in our country to make dairy products from unpasteurized milk, although this food can be a vehicle for transmission of pathogens to humans. Although outbreaks by this organism have been reported from consumption of pasteurized milk (15), other studies have shown that this bacterium is not able to survive the pasteurization process, suggesting that contamination of milk in these cases most probably occurred at the postpasteurization stage (19).

In Mexico, there is very little research related to food contamination by presumptive enteropathogenic *Y. enterocolitica*. There are no reports of its presence in raw milk,
pasteurized milk, or milk derivatives; it has only been detected in raw pork, chicken, and precooked pork meat (25). In our country there are no official standards governing the sanitary quality of this food, there are only voluntary standards; NMX-F-700-COFOCALEC-2004 (3) and NMX-F-730-COFOCALEC-2008 (4), which set out requirements for the obtaining, hygienic handling, and pasteurization of milk. These guidelines, being nonbinding, allow the sale of this food through unregulated channels. The data obtained in this work, however, show the need to protect public health in Mexico against infections transmitted by raw cow’s milk.

REFERENCES


