ISOLATION, BIOTYPING, AND SEROTYPING OF YERSINIA ENTEROCOLITICA STRAINS FROM FATTENING PIGS

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Received: April 29, 2010         Accepted: July 28, 2010

Abstract

The study has been taken up to collect more data on Yersinia enterocolitica isolated from pigs as the main reservoir and source of infection with strains pathogenic for humans. Bacteriological examinations, bio- and serotyping were conducted on 616 rectal swabs, taken from 308 fattening pigs. Two samples were taken from each animal to determine the ability of Y. enterocolitica to grow under different temperature conditions (warm and cold culture). It has been proven that low temperature constitutes a suitable culture condition. 138 Yersinia enterocolitica strains were isolated (22.40%), most of which (65.22%) were obtained in cold culture and 99.28% included in biotype 3 (one strain belonged to biotype 2). Serotyping yielded a positive result in 107 strains with the diagnostic serum for antigen O:3, in 18 – with the serum for antigen O:9, and 13 strains were determined to be non-typable. The results indicated that asymptomatic infections with Y. enterocolitica strains of the biotypes and serotypes pathogenic for humans are common in pig population.

Key words: pigs, Yersinia enterocolitica, biotyping, serotyping.

Yersinia enterocolitica has been increasingly important as an aetiological factor in human diarrhoea in Poland (21). Therefore, it seemed prudent to take up studies to collect more data on this microorganism isolated from pigs as the main reservoir and source of infection with strains pathogenic for humans. Correlation between strains isolated from pigs and those, which produce clinical signs in humans has been sufficiently proven, which corroborates the justifiability of selecting this animal species as the study object (1, 7, 9, 10). First reports about the frequency of isolation of Y. enterocolitica from pigs appeared in the 1970’s and concerned butchers’ shops in Belgium where the microbes were isolated from as much as 50% of the examined pig tongues. In the 1980’s, further studies were carried out by Zaremba and Grala-Kałuszna (22) on Y. enterocolitica spread in pig herds and the level of carrier state, showing that it ranged from 4.4% to 18.2%. Numerous studies were conducted in the 1990’s in many countries, including Poland, which resulted in characteristics of the epizootic situation of Y. enterocolitica infections in pig population. The study conducted by Platt-Samoraj (15) in 1992-1993 included examination of 1,200 palatine tonsils, 40 rectal swabs, and 20 samples of pig faeces. Strains of Y. enterocolitica were isolated from 94 (7.83%) palatine tonsils and from five (12.5%) rectal swabs. Studies have been conducted in recent years to determine the epizootic situation of yersiniosis not only in Poland or Europe but also worldwide (5, 8, 12, 18, 20).

The palatine tonsils, intestine contents, and swabs from carcasses of slaughter animals are usually used as the study material for Y. enterocolitica, while rectal swabs, faecal samples, and pharyngeal swabs are taken from live animals. It was decided, on the basis of literature reports (13), to use in the presented study rectal swabs taken in as non-invasive manner as possible from animals, which do not show any clinical signs of the disease. If the bacteria are shown to be present in the gastrointestinal tract, this may be evidence of asymptomatic infection or of the carrier state and excretion of the bacteria into the environment. The bacteriological examinations, as the first stage of the study, belong to the most frequently performed in detecting Y. enterocolitica infection or carrier state. By application of selective culturing media, it can be used for the pre-selection of the studied material and as a starting point for further, more detailed laboratory analyses.

The aim of the study was to isolate Y. enterocolitica strains from fattening pigs and to characterise them by biotyping and serotyping.
Material and Methods

Bacterial strains and culture conditions. The material for the study consisted of 616 rectal swabs taken from 308 fattening pigs, which came from three different pig farms situated in the regions of Warmia and Mazury and Mazowsze (Table 1).

Two samples taken from each animal were cultured simultaneously on ITC medium (broth with irgasan™, ticarcillin, and potassium chloride) – warm culture, and PSB medium (broth with peptone, sorbitol, and bile salts) – cold culture. The ability of Y. enterocolitica to grow under low temperature conditions was determined. Further biochemical identification was carried out according to the PN-EN ISO 10273 standard (16), the same for both kinds of cultures, and this allowed for the preliminary selection of potentially pathogenic strains, which were used for further examinations.

Biotype and serotype determination. The biotype determination of the examined strains was made in accordance with the PN-EN ISO 10273 standard (16) and involved the evaluation of the ability to ferment xylose, trehalose, and esculin as well as to synthesise pyrazinamidase, Tween esterase, and indole.

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 (Grasco) were used as an antigen, and the sera containing antibody for particular somatic antigens O:3, O:5, O:8, and O:9 came from ITEST company (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. The occurrence of agglutination with one of the four used sera, after shaking for 1 min, was considered to be a positive result. In case of lack of agglutination with any serum, the strain was regarded as non-typable.

Results

Altogether 138 strains of Y. enterocolitica were isolated from 616 rectal swabs, taken from 308 fattening pigs, which accounts for 22.4% of all the samples, most of which (65.22%) obtained in cold culture (90 out of 138 strains). This phenomenon was particularly noticeable in farm Z, where over five times more strains were isolated from culture in PSB broth as compared to culture in ITC broth. It is also noteworthy that the results of the isolation of Y. enterocolitica in different farms, from which the swabs were taken, varied with the herd infection index ranging from 41% to 46% in farms X and Z, to the absence of the bacteria in the pig population in farm Y. Results of Y. enterocolitica strains isolation from fattening pigs in the farms are shown in Table 2.

From the 138 Y. enterocolitica strains subjected to the biotyping tests, nearly all (99.28%) belonged to biotype 3, which fermented xylose and trehalose, but did not ferment esculin or produce pyrazinamidase, Tween esterase, and indole. Only one (0.72%) strain produced indole, fermented xylose and trehalose, did not ferment esculin, and did not produce pyrazinamidase and Tween esterase, and therefore, it was included to biotype 2 according to the diagram in PN-EN ISO 10273 (17). However, it should be pointed out that it is extremely difficult to differentiate between biotypes 2 and 3. The differentiation is based on the ability to produce indole, which is absent in biotype 3 strains. Biotype 2 strains can produce indole, but the reaction is often delayed or weak and, consequently, difficult to detect beyond doubt. No strains belonging to biotypes 1A, 1B, 4, or 5 were detected, with the correct results for positive controls 1A, 1B, 2, 3, and 4 descended from our collection of Y. enterocolitica strains.

The serotyping tests of the 138 collected Y. enterocolitica strains yielded 107 (77.54%) positive results with diagnostic serum for the O:3 antigen. A positive result with serum for the O:9 antigen was observed in 18 (13.04%) strains. Absence of the reaction with any of the diagnostic sera resulted in including 13 (9.42%) strains to the non-typable group. Serotyping of the isolated strains was preceded by control tests with strains of the known serotype.

Out of the 48 Y. enterocolitica strains obtained in warm culture, a clear majority – 38 (79.17%) showed a positive reaction with the diagnostic serum for the O:3 antigen. A positive reaction in the plate agglutination test with the O:9 antigen was observed in five (10.42%) cases; a similar number of strains proved to be non-typable.

Of the 90 Y. enterocolitica strains, which were obtained in cold culture, 69 (76.67%) were shown to react positively with the diagnostic serum for the O:3 antigen. A positive reaction in the slide agglutination test with the serum for the O:9 antigen was observed in 13 (14.44%) cases, for eight (8.89%) strains no reaction was observed with any of the diagnostic sera. Serotyping results for Y. enterocolitica strains are shown in Table 3.

Discussion

Increased importance of Y. enterocolitica as an aetiologic factor in human diarrhoea was the basis of studies whose aim was to evaluate the occurrence of this microorganism in the populations of animals, especially pigs. Milnes et al. (12) conducted in 2003 comprehensive examinations of 7,703 samples from the intestines of cattle, sheep, and pigs, obtained from 93 abattoirs in Great Britain, which aimed at isolation of Y. enterocolitica strains. The percentage of positive samples for pigs was 10.2%. Singh et al. (18) did their research in the same year.
They examined 492 pharyngeal swabs from pigs near Delhi and isolated strains of *Y. enterocolitica* from 162, which accounts for 32.9% of all the samples examined. Such a high percentage of positive results, rare in Europe, indicates an increased intensity of the *Y. enterocolitica* carrier state among pigs in India. Research conducted in 2005 in Germany by Gürtler *et al.* (5) shows that *Y. enterocolitica* was isolated from 19.6% out of the 491 faecal samples taken from fattening pigs. This result is similar to that obtained in our study in 2008, in which strains of *Y. enterocolitica* were isolated from 138 (22.4%) out of the 616 rectal swabs from fattening pigs. At the same time, Kot *et al.* (8) conducted a study in Poland on 192 palatine tonsils of pigs, from which they isolated 25 strains of *Y. enterocolitica*, which accounts for 13.02%. This result is similar to that obtained by Wesley *et al.* (20) in the United States in 2008, who observed a positive result in 122 out of the 1,218 tonsils under examination, which accounts for 10.02% of all the samples. Pigs – carriers, from whose tonsils *Y. enterocolitica* was subsequently isolated, were found in 55 out of the 122 examined farms. Frequent isolation of the bacteria from pig herds, as shown by many authors, including the authors of this paper, confirmed the presence of the carrier state, and has become the starting point for many programmes aiming at the prevention of its occurrence. Among them a system of breeding animals free from *Y. enterocolitica* strains pathogenic for humans has been developed (14).

In our study the microbe was shown to be present in two out of three pig farms where swabs from fattening pigs were taken, differing, for example, by the herd size. Interestingly, not the smallest of the farms proved to be free from *Y. enterocolitica*, since no bacteria were isolated in farm Y with 800 pigs, using standard bacteriological methods, either in warm or cold culture. The largest number of *Y. enterocolitica* strains, 40 in warm culture and 44 in cold culture, were isolated from swabs taken in farm X with only 280 animals. These results suggest that there are various factors, which affect the epizootic situation on a farm, among which the age of animals is obviously an important one.
For example, Gürtler et al. (5) showed that no Y. enterocolitica was isolated from any of the 600 examined piglets (from birth to the post-weaning period), whereas strains of the bacteria were recovered from 96 out of 491 (19.5%) examined pigs during the fattening period. Based on these studies, whose findings were confirmed by Wehebrink et al. (19), the age of animals from which the swabs were taken was determined to be 20 (±1) weeks.

Bacteriological examinations, used for isolating Y. enterocolitica strains, are time- and labour-consuming, especially cold culture in PSB broth, where sample incubation period lasts as long as 3 weeks. However, because of higher sensitivity cold culture should not be neglected, which may be confirmed by results obtained in farm Z. In this farm only eight strains of Yersinia enterocolitica were isolated in warm culture, whereas as many as 46 in cold culture. The farm proved to be the most diverse in terms of the number of strains of Y. enterocolitica obtained from two different types of culture. Five times more strains were isolated from culture in PSB broth as compared to culture in ITC broth; this is one of the arguments for conducting both types of culture, especially the cold type as being highly specific for Y. enterocolitica (8, 11, 17).

Biotyping of the collected strains of Y. enterocolitica, conducted in accordance with the Polish standard PN-EN ISO 10273 (16), brought surprising results. It was shown that over 99% of the isolates can be classified to biotype 3, and only one strain showed the biochemical features typical for biotype 2. However, many authors emphasise that it is relatively difficult to distinguish both biotypes, based only on the ability to synthesise indole, which – unlike in biotype 3 strains – can occur in biotype 2 strains, but is usually weak or delayed and therefore difficult to determine (3, 9). No 1A, 1B, 4, and 5 biotype strains were isolated. In previous studies on evaluation of the variety and occurrence of individual biotypes in different animal species, 1A and 4 biotypes dominated in pigs. Kot et al. (8) recently evaluated 48 strains of Y. enterocolitica isolated from pigs and showed biotype 1A as a predominant - 25 strains (52.08%), whereas strains of biotype 4 (22) accounted for 47.92% of the samples.

The isolation of most biotype 3 strains in our study is of particular interest with regard to the serotyping tests, which were conducted simultaneously. A positive result in the slide agglutination test with the diagnostic serum for O:3 antigen was observed in 107 out of the 138 strains of Y. enterocolitica (77.54%). Only 18 strains (13.04%) were classified to serotype O:9, whereas 13 (9.42%) did not react with any diagnostic sera in the agglutination test, therefore they were classified as non-typable. The O:5 serotype strains were not found, which correlates with the absence of 1A biotype among the strains under examinations. Kot et al. (8) performed the serotyping of 48 strains of Y. enterocolitica and showed that 23 strains (47.92%) belonged to serotype O:3, which accounts for a lower percentage than that observed in our study. Furthermore, six strains (12.5%) gave a positive reaction with the serum for the O:5 antigen and seven strains (14.58%) – with the serum for antigen O:6. Twelve (25%) strains were not classified to any of the serotypes. Serum for O:6 antigen was not included in our study, but the number of non-typable strains was much lower than that obtained in the study conducted by Kot et al. (8). Additionally, nearly 30% more strains of the O:3 serotype and 13% more strains of the O:9 serotype were shown to be present.

Obtaining strains of different serotypes from two culture processes, under different conditions is an important aspect of serotyping the strains of Y. enterocolitica collected in our study. The presence of two serotypes in two samples with the same number, which were taken from the same animal, is an indication of mixed infection and makes it necessary to conduct the two types of culture in order to provide the best characterisation of the epizootic situation in a pig herd. Out of the 40 cases when Y. enterocolitica were isolated in both cold and warm culture, different serotyping result was observed in 14 cases.

While introducing the concept of a bioserotype, which is currently used by many scientists who conduct research in this field to describe correlations between the biotype and the serotype of a specific strain of Y. enterocolitica, it has to be stated that 3/O:3 was the dominant bioserotype in our study. This result is different from that obtained by Kot et al. (8), where 1A/O:5, 1A/O:6, and 4/O:3 were shown to dominate. The last bioserotype is considered to be the most frequently occurring in pigs in Poland and in Europe. However, according to Frederiksson-Ahomaa et al. (2), biotype 3 is the most frequently isolated from pigs, ruminants, and humans in the United Kingdom. Since Fukushima et al. (4) isolated strains of Y. enterocolitica biotype 3 (serotype O:3) from healthy pigs and from pork, the strains have been proved to be as equally important in human infections as biotype 4 strains (serotype O:3). According to Kaneko and Maruyama (6), over 70% of Y. enterocolitica strains in Japan are classified to biotype 3 (serotype O:3), with the same pathogenic properties as biotype 4 (serotype O:3). The authors even suggest that it is necessary to conduct examinations of the global range of this bioserotype strains, especially in the context of the study conducted by Kuehni-Boghenbor et al. (9), who genotyped strains of Y. enterocolitica isolated from pigs and humans. This study has shown that 83%-87% of Y. enterocolitica strains classified to bioserotypes 2/O:9, 3/O:3, and 4/O:3 were phylogenetically similar. It was confirmed that human infection may be caused also by strains from bioserotype 4/O:3 with known pathogenic properties, because there is a strong correlation between strains of biotypes 3 and 4, isolated from humans and animals (9).

The results of our study indicate widespread asymptomatic infections in pig population with Y. enterocolitica strains of the bio- and serotypes pathogenic for humans, and show that the study should be continued in its both epidemiological and molecular aspects.
Acknowledgments: The study was supported by the Committee of Scientific Research (KBN, grant No. N N308 320235) and EU within the European Social Fund.

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