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SEROEPIDEMIOLOGIC STUDY ON THE OCCURRENCE OF ANTIBODIES AGAINST *YERSINIA ENTEROCOLITICA* AND *YERSINIA PSEUDOTUBERCULOSIS* IN URBAN AND RURAL POPULATION OF THE LUBLIN REGION (EASTERN POLAND)

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Abstract: The aim of this study was to assess the seroprevalence of antibodies against *Yersinia* in the rural and urban population and to determine the frequency of particular serotypes of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. 472 sera were examined, 257 of rural inhabitants and 215 of urban inhabitants. The survey was carried out by passive hemagglutination test with the antigens of *Yersinia* serotypes considered pathogenic for humans: *Y. enterocolitica* 03, 05, 06, 08, 09 and *Y. pseudotuberculosis* I and III. In the examined rural population positive reactions to *Yersinia* antigens were significantly more frequent than in the examined urban population (42% versus 20%, $p<0.0001$). The most frequent reactions were against *Y. enterocolitica* serotypes 05 and 08.

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INTRODUCTION

Yersinia spp. in general and *Y. enterocolitica* and *Y. pseudotuberculosis* in particular are widespread Gram-negative bacteria. They were isolated from animals (healthy, as well as ill) belonging to various taxonomic groups, from mammals to insects. Pigs are considered as the basic hosts for these bacteria, in particular for the serotype *Y. enterocolitica* O3 [2, 3, 4, 14, 16, 23, 29].

These microorganisms were isolated from soil, waste and water of different purity: well water, spring water bacteriologically stated as clean, surface waters classified into the 1st and the 2nd class of purity, and heavily contaminated water [15, 25]. They were also found in food (meat, milk, eggs, vegetables) contaminated with feces of infected animals or secondarily during the technologic process [1, 2, 4, 8, 9, 12, 16].

Y. enterocolitica multiplies and produces enterotoxin at 4ºC. This enables long survival of these bacteria in cold water or refrigerated food.

Y. enterocolitica comprises over 60 serotypes but only a few are pathogenic for humans. The most virulent serotype is *Y. enterocolitica* O3, which is also the most frequent. *Y. enterocolitica* O9 has also been mentioned as pathogenic.

There were 490 cases of diagnosed yersiniosis in Poland in the years 1972-1979. *Y. enterocolitica* O3 was diagnosed as a cause in 72% of these cases, *Y. enterocolitica* O9 in 21%, mixed infection with these two serotypes in 6%, and infections with other serotypes in 1% of all cases [28].

Some later studies have shown that in our climatic zone antibodies against serotype *Y. enterocolitica* O5 are also found [11, 24]. In Germany, yersiniosis is caused by *Y.*

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enterocolitica O3, O9 and O5 [22]. Serotype O8 was dominant in the United States until 1980, but now serotype O3 is more often isolated. Serotype O8 is frequently found in Japan and Europe. It has been stated that serotype dominance in different regions is fluent and the reason is so far not explained [20, 29].

The majority of *Yersinia* infections is food-borne. Pork and pork products are considered as the main source of infection. The spread of the disease is mainly dependent on the degree of hygiene standard at every step of the technological process of food processing, storing and distribution [20, 27].

Assuming that food is the main source of infection, yersiniosis is expected to spread mainly in urban areas. Urban population using canteens, pre-processed food and food often long stored in cold stores is thought to be more exposed to these bacteria. Nevertheless, there is also a high risk of occupational exposure to *Yersinia* during handling of animals, meat, meat products and other products of animal origin, what is common among rural inhabitants. Serological survey among farmers and people working at animal breeding and slaughtering showed higher seroprevalence when compared to other occupational groups [17, 21].

In Poland, almost every farmer breeds animals although often their number is low. Usually, there are several pigs, cows, birds, cats and dogs on almost every farm. Rodents (mice and rats) also often occur, as well as insects, mainly flies. Slaughtering animals on farms and manual processing of meat in improper hygienic conditions creates a risk of raw meat contamination with microorganisms, including pathogenic ones [1, 14, 26]. Meat and meat products are often stored for long periods in unacceptable conditions. Ubiquitous rodents may contaminate environment and food not only of animal origin. In the rural environment, food is produced mainly for own use but is sometimes retailed in places with inadequate hygienic facilities.

Yersiniosis in man is a sporadic or endemic disease with various clinical symptoms and often is misdiagnosed. The most frequent picture is mild enterotoxemia spontaneously cured but it may also have a severe course. Recurrences and complications may also occur. Reactive arthritis is the most frequent complication [6, 7, 10, 26, 29]. Ostroff suggests that *Yersinia* infections pose a growing problem world-wide [20].

In Poland, there is no precise epidemiologic evidence concerning yersiniosis because of the lack of duty of outbreaks registration except for documented group enterotoxemia. Anusz regards yersiniosis in Poland as a sporadic disease [2]. Zaremba *et al.* assessed that in Poland enteritis is caused by *Yersinia enterocolitica* O3 in 2-5% of cases [29]. On the other hand, Kałużewski *et al.* has found antibodies anti-*Yersinia* in the sera of 96% of children aged 7-14 years [11]. In the people with disease of rheumatoidal character, where rheumatoidal factor was excluded, antibodies anti-*Yersinia* were found in 43.2% of examined cases [24].

The aim of this study was the assessment of seroprevalence of antibodies against *Yersinia* in the rural and urban populations and the frequency of particular serotypes of *Yersinia*.

MATERIALS AND METHODS

Examined population

A total of 472 human sera, comprising 257 sera of rural inhabitants and 215 sera of urban inhabitants were studied. Examined population may be divided into three groups: persons suspected of other zoonoses, healthy persons working in forestry, and healthy persons working in other environments.

Persons suspected of other zoonoses. Sera from 177 persons were collected in this group. 110 samples came from rural inhabitants and 67 from inhabitants of towns, mainly Lublin. Average age in these groups was relatively low: 32.5 and 27.8 years, respectively. The examined people represented various occupations. Rural inhabitants were mainly farmers maintaining traditional small farms. Urban inhabitants were mainly clerks and workers without occupational contact with animals and products of animal origin. Examined persons had symptoms which could suggest zoonoses, mainly toxoplasmosis. They were patients of gynecologists, obstetricians, ophtalmologists, neurologists, rheumatologists, haematologists, gastrologists, laryngologists. Nobody in this group was suspected of yersiniosis.

Healthy persons working in forestry. This group consisted of 140 persons, mainly from the Forest District Włodawa. 105 of them lived in a rural area and 35 in an urban area (Włodawa, Lublin). These persons were employed in different work conditions. They worked as foresters, woodcutters and also as managers and clerks. A part of them were hunting or picking mushrooms in their leisure time. The majority of persons living in the rural area also maintained small farms. Average age of people coming from the rural area was 44.9 and from the urban area 47.6.

Healthy persons working in other environments. This group consisted of 155 persons, 42 of them from villages of Firlej community and 113 persons living in the city of Lublin. Average age of Firlej inhabitants was 42.3 and their occupation was traditional farming. Average age of Lublin inhabitants was 38.6. In Lublin, there were examined 63 employees of a scientific institute and 50 blood donors having no occupational contact with animals, meat and meat products.

All persons from the rural area in this study stated that they had contact with bred animals particularly pigs, cattle and birds, and also with domestic animals as dogs and cats. People employed in forestry also had contact with wild animals. The majority of village people used

Table 1. Prevalence of positive reactions to *Yersinia* antigens.

Population	Group	N	Total positive (number, percent)	Reactivity to particular antigens in the groups of positive reactants (number, percent)						
				Y.e. O3	*Y.e.* O5	*Y.e.* O6	*Y.e.* O8	*Y.e.* O9	*Y.p.* I	*Y.p.* III
Rural	Suspected of other zoonoses	110	45 40.9 **	7 15.6	25 55.6	0	7 5.5	0	3 6.7	3 6.7
	Healthy forestry workers	105	44 41.9 **	7 15.9	16 36.4	10 22.7	10 22.7	0	1 2.3	0
	Healthy persons of other professions	42	19 45.2 **	3 15.8	3 15.8	0	10 52.6	0	2 10.5	1 5.3
	Total	257	108 42.0 ***	17 15.8*	44 40.7***	10 9.2	27 25.0*	0	6 5.5	4 3.7
Urban	Suspected of other zoonoses	67	13 19.4	2 15.4	1 7.8	6 46.2	0	0	0	4 30.8
	Healthy forestry workers	35	4 11.4	0	2 50.0	0	2 50.0	0	0	0
	Healthy persons of other professions	113	26 23.0	2 7.7	5 19.2	0 0	9 34.6	0	6 23.1	4 15.4
	Total	215	43 20.0	4 9.3	8 18.6	6 13.9	11 25.6	0	6 13.9	8 18.6
Total (rural+urban)		472	151 32.0	21 13.9	52 34.4	16 10.6	38 25.2	0	12 7.9	12 7.9

*–**–*** Percent of positive reactions significantly higher compared to urban envirenment: *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$. *Y.e.* - Y*ersinia enterocolitica, Y.p.* - Y*ersinia pseudotuberculosis.*

own well-water, the rest used water from local waterworks. The majority of people from the rural area was drinking unboiled water and milk. They consumed mainly self produced food (meat and meat products, milk and eggs, vegetables and fruits).

Persons from the urban area stated that they had contact with domestic animals (dogs and cats), laboratory animals (guinea pigs, mice, hamsters) and birds (parrots and canaries). These persons consumed food bought in markets (vegetables, fruit, milk, eggs and meat). The majority used processed food. Urban population used tap water, rarely unboiled.

Serological examination

Blood serum samples were examined by passive haemagglutination test using commercial sets produced by the National Institute of Hygiene, Warsaw, Poland. As antigens were used lipopolysaccharides (antigens O) obtained with modified Boivin method from *Yersinia enterocolitica* strains O3, O5, O6, O8, O9 and *Y. pseudotuberculosis* strains I and III, coated on sheep erythrocytes and fixed with glutaraldehyde. Before the test, serum samples were inactivated for 30 min at 56ºC and then adsorbed on fresh sheep red blood cells for 30 min at 37ºC. Such prepared sera were diluted in 0.25% gelatine gel and placed in microtiter plates with V-shaped wells and mixed with all antigens. Results were read after 4 hours of incubation at the room temperature and complete sedimentation of red blood cells. Titers 20 or higher were considered as positive.

RESULTS AND DISCUSSION

In the rural population, positive results were found in 42.0% of the total examined persons. Out of 7 antigens used, 6 reacted positively, at least once. The total results are shown in Table 1. In the rural population, the majority of positive cases were obtained with *Y. enterocolitica* O5 (40.7%), and *Y. enterocolitica* O8 (25.0%). As much as 15.8% of positive results were found with the antigen *Y. enterocolitica* O3 and 9.2% with *Y. enterocolitica* O6. No positive results were found with the antigen *Y. enterocolitica* O9. Positive reactions with *Y. pseudotuberculosis* were rare amounting for 5.5% to serotype I and 3.7% to serotype III.

In the urban population, 20.0% of cases were found to be positive with the same 6 out of 7 available antigens but in different proportions. In this group, positive reactions occurred frequently with *Y. enterocolitica* O8 antigen (25.6%) and *Y. enterocolitica* O5 antigen (18.6%). There were also found 9.3% of positive reactions with *Y. enterocolitica* O3 antigen and 13.9% with *Y. enterocolitica* O6 antigen. Significantly more positive reactions compared to the rural population were found with *Y. pseudotuberculosis* - 18.6% with *Y. pseudotuberculosis* III ($p < 0.01$) and 13.9% with *Y. pseudotuberculosis* I ($p < 0.05$). This is probably the result of part of the whom

Table 2. Combinations of positive reactions to particular *Yersinia* antigens.

Antigens	Numbers of positive reactions					
	Rural environment			Urban environment		
	Suspected of other zoonoses	Healthy forestry workers	Healthy persons of other professions	Suspected of other zoonoses	Healthy forestry workers	Healthy persons of other professions
Y.e. O3 alone	2	3	1	2	0	1
Y.e. O5 alone	18	6	1	1	2	2
Y.e. O6 alone	0	2	0	4	0	0
Y.e. O8 alone	5	8	6	0	2	5
Y.p. I alone	1	1	2	0	0	6
Y.p. III alone	1	0	1	2	0	4
Y.e. O3 + *Y.e.* O5	3	2	0	0	0	0
Y.e. O3 + *Y.e.* O8	0	2	2	0	0	1
Y.e. O5 + *Y.e.* O6	0	8	0	0	0	0
Y.e. O5 + *Y.e.* O8	0	0	2	0	0	3
Y.e. O6 + *Y.p.*III	0	0	0	2	0	0
Y.e. O3 + *Y.e.* O5 + *Y.p.* III	2	0	0	0	0	0
Y.e. O5 + *Y.e.* O8 + *Y.p.* I	2	0	0	0	0	0

Y.e. – Yersinia enterocolitica, Y.p. – Yersinia pseudotuberculosis

inhabitants maintaining laboratory animals - a known reservoir of these bacteria [22, 26, 28].

Among persons reacting positively and coming from the rural population, 28.9% reacted simultaneously with more than one antigen (Tab. 2). This may suggest different sources of infection in the same person. In the urban population, persons reacting with more than one antigen amounted to 16.2%.

The titers of seropositive reactions were relatively low in both populations, although a little higher in the rural population. They reached subminimal, minimal or a little above minimal levels compared to manufacturer's recommendations [19] (Tab. 3). These levels of antibodies indicate the asymptomatic infection with *Yersinia* or chronic process where antibody level is often low.

This picture is coherent to clinical status of population where *Yersinia* infection was not diagnosed but many persons had suffered in the past from enterotoxemia caused by an unknown etiologic factor which could have been *Yersinia*. There might also be an association with past infection with *Yersinia* in examined persons having rheumatoidal symptoms. It has been proved that anti-*Yersinia* antibodies may be found in serum for long periods of time. Their presence was detected after 9 years in patients suffered from reactive arthritis caused by *Yersinia* [6].

Table 3. Titers of seropositive results.

Antigens	Population	
	Rural	Urban
Y.e. O3	40–320	20–160
Y.e. O5	160–1280	80–640
Y.e. O6	40–80	20–80
Y.e. O8	80–640	80–320
Y.p. I	20–80	20–160
Y.p. III	20–80	20–160

Y.e. – Yersinia enterocolitica, Y.p. – Yersinia pseudotuberculosis

Analysing the distribution of positive results in particular groups of examined persons with regard to place of residence, differences were found which may be due to a different way of life (including personal hygiene standard), occupational exposure (including contact with animals and meat), food quality, feeding habits and food processing standard.

In all analysed groups of the rural population, seropositive results with all antigens were at least twice as high than in the urban population. In the group of patients suspected of zoonoses, seropositive results were found in 40.9% of the rural population and in 19.4% of the urban population.

In forestry workers living in villages positive reactions were found in 41.9% while in forestry workers living in towns only in 11.4% of examined individuals. In healthy persons of other professions living in villages anti-*Yersinia* antibodies were found in 45.2% while in corresponding group living in towns only in 23.0%.

Totally, among 472 examined persons coming from Lublin macroregion positive results with *Yersinia* antigens were found in 151 persons, i.e. 32.0%. This result is in accordance with the assessment made by the authors of ELISA method *Microgen* who estimated that 30% of

population has IgG antibodies against *Yersinia* [18]. Our study taking into consideration the environment of life, indicates the significant difference between rural and urban environment amounting for 42.0% versus 20.0% ($p < 0.0001$).

Analysis of the summarised results both from the rural and urban environments concerning the presence of antibodies against various serotypes of *Yersinia* indicates the dominance of *Y. enterocolitica* O5 (34.4%) and *Y. enterocolitica* O8 (25.2%) in examined area. The antibodies were also found to the serotypes *Y. enterocolitica* O3 (13.9%) and *Y. enterocolitica* O6 (10.6%). No reactions were found to *Y. enterocolitica* O9. The frequency of reactions to *Y. pseudotuberculosis* in both environments was 7.9%.

CONCLUSIONS

1. In the examined rural population positive reactions to *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* antigens were significantly more frequent than in the examined urban population.

2. In the examined population the most frequent were antibodies to the *Y. enterocolitica* serotypes O5 and O8.

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REFERENCES

1. Andersen JK, Sorensen R, Glensbjerg M: Aspects of the epidemiology of *Yersinia enterocolitica*: a review. *Int J Food Microbiol* 1991, **13**, 231-237.

2. Anusz Z: *Zapobieganie i Zwalczanie Zawodowych Chorób Odzwierzęcych*. ART, Olsztyn 1995.

3. Colins FM: *Pasteurella, Yersinia,* and *Francisella*. **In**: Baron S (Ed): *Medical Microbiology*. University of Texas, Houston 1996.

4. D'Amario C, Fischetti M, Melino C: Yersiniosis. *Clin Ter* 1992, **141**, 51-58.

5. Donelly CW: Concerns of microbial pathogens in association with dairy foods. *J Dairy Sci* 1990, **73**, 656-661.

6. Garwolińska H, Noworyta J, Brasse-Rumin M: Odległe obserwacje chorych na reaktywne zapalenie stawów po zakażeniu pałeczkami *Yersinia enterocolitica*. *Reumatologia* 1998, **3**, 255-259.

7. Garwolińska H, Noworyta J, Brasse-Rumin M: Reaktywne zapalenie stawów po infekcji *Yersinia enterocolitica*. *Reumatologia* 1993, **4**, 386-398.

8. Greenwood MH, Hooper WL: Excretion of *Yersinia* spp. associated with consumption of pasteurized milk. *Epidemiol Infect* 1990, **104**, 345-350.

9. Hartung M, Gerigk K: *Yersinia* in effluents from the food-processing industry. *Rev Sci Tech* 1991, **10**, 799-811.

10. Hoogkamp-Korstanje JA: *Yersinia* infections. *Ned Tijdschr Geneeskol* 1996, **140**, 128-130.

11. Kałużewski S, Połowniak-Pracka H, Szych J, Zaleska M: Występowanie i poziom przeciwciał dla O antygenów *Y. enterocolitica* wykrywanych u wybranych grup ludzi odczynem hemaglutynacji biernej. *Med Dośw Mikrobiol* 1985, **37**, 147-164.

12. Kapperud G: *Yersinia enterocolitica* in food hygiene. *Int J Food Microbiol* 1991, **12**, 53-65.

13. Kapperud G: *Yersinia enterocolitica* infection. Epidemiology, risk factors and preventive measures. *Tidsskr Nor Laegeforen* 1994, **114**, 1606-1608.

14. Kapperud G, Ostroff SM, Nesbakken T, Hutwagner LC, Bean NH, Lassen J, Tauxe RV: Risk factors for sporadic *Y. enterocolitica* infection in Norway: a case control study. **In:** Ravagnana G, Chiese C (Eds): *Yersiniosis*: Present and Future, 25-28. Karger, Roma 1994.

15. Krogulska B, Maleszewska J: Występowanie bakterii z rodzaju *Yersinia* w wodach powierzchniowych. *Rocz Państw Zakł Hig* 1992, **43**, 295-300.

16. Logue C, Sheridan JJ, McDowell DA, Blair IS: A survey of the incidence of *Yersinia* on Irish meat and meat products. **In:** Ravagnana G, Chiese C (Eds): *Yersiniosis*: Present and Future, 77-79. Karger, Roma 1994.

17. Merilahti-Palo R, Lahesmaa R, Granfors K, Gripenberg-Lerche C, Toivanen P: Risk of *Yersinia* infection among butchers. *Scand J Infect Dis* 1991, **23**, 55-61.

18. Mikrogen molekular-biologische Entwicklungs-GmbH: Enzyme immunoassay with recombinant antigens for the detection of IgG, IgA or IgM antibodies against *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in human serum or plasma. Information for use. München 1998.

19. National Institute of Hygiene: Metodyka Hemaglutynacji Biernej z Zastosowaniem do Diagnostyki Zakażeń Wywołanych przez Pałeczki *Yersinia*. Warszawa 1994.

20. Ostroff S: *Yersinia* as an emerging infection. **In:** Ravagnana G, Chiese C (Eds): *Yersiniosis*: Present and Future, 1-4. Karger, Roma 1994.

21. Seuri M, Granfors K: Antibodies against *Yersinia* among farmers and shaughterhouse workers. *Scand J Work Environ Health* 1992, **18**, 128-132.

22. Stojanka A, Beckmühl J, Hanns-Herbet W: Epidemiology of *Y. pseudotuberculosis* in Germany 1983-1993. **In:** Ravagnana G, Chiese C (Eds): *Yersiniosis*: Present and Future, 55-58. Karger, Roma 1994.

23. Stojek N: *Jersinioza* u ludzi i zwierząt. *Med Ogólna* 1996, **2**, 30-33.

24. Stojek N, Sroka J: Obecność przeciwciał anty-*Yersinia* w surowicach osób ze zmianami reumatycznymi. *Med Ogólna* 1998, **4**, 431-436.

25. Stojek N, Stroczyńska-Sikorska M, Kłapeć T: Badania wody studziennej w kierunku bakterii z rodzaju *Yersinia*. **In**: Sozański M (Ed): *Zaopatrzenie w Wodę Miast i Wsi*, 217-223. PZITS, Poznań 1994.

26. Stypułkowska-Misiurewicz H: Jersinioza. **In:** Magdzik W (Ed): *Choroby Zakaźne i Pasożytnicze*, 415-419. Vesalius, Kraków 1993.

27. Windyga B: Jakość mikrobiologiczna żywności. *Rocz Państw Zakł Hig* 1995, **46**, 247-250.

28. Zaremba M: Kompleksowe badania nad *Yersinia enterocolitica* i występowanie jersiniozy w Polsce w latach 1972-1979. *Pol Tyg Lek* 1979, **47**, 1849-1851.

29. Zaremba M, Borowski J: *Podstawy Mikrobiologii Lekarskiej*. PZWL, Warszawa 1994.