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Characterization of Rabbit Antibodies for Immunochemical Detection of *Yersinia enterocolitica*

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ABSTRACT. Rabbit IgG raised against whole cells of *Yersinia enterocolitica* O:3, O:9 and against a group of pathogenic *Y. enterocolitica* strains (serotypes O:3, O:5,27, O:8. and O:9) were prepared. The antibody limiting titers were within the range of $1:9.5 \times 10^4$–$1:7.5 \times 10^5$. The immunoblotting analysis of *Yersinia* lipopolysacchides separated by SDS-PAGE showed that IgG against the single serotype O:3 interacted with high-molar-mass LPS of O:3 whereas other antibodies were bound to low-molar-mass LPS of serotypes O:3, O:5,27, O:9 and strain *Y. enterocolitica* (CNCTC Y 2/68). IgG against the group of pathogenic serotypes also weakly interacted with low-molar-mass LPS of serotypes O:5, O:6,30, and O:10. The cross-reactivity of the antibodies with *Y. pseudotuberculosis* Ia and/or *Y. rohdei* b, d, e, f, i, which was observed by means of dot-blotting procedure using the whole bacterial cells as an antigen, was shown not to be caused by LPS of these bacteria. The prepared antibodies were used in the development of indirect competitive ELISA. At the optimum concentration of the immunoreactants the detection limits were within the range of $3–7 \times 10^6$ colony-forming units per mL.

Abbreviations

BSA	bovine serum albumin	PAGE	polyacrylamide gel electrophoresis
CELISA	competitive ELISA	PBS 7.4	10 mmol/L phosphate-buffered saline, pH 7.4
ELISA	enzyme-linked immunosorbent assay	PBST	PBS 7.4 containing 0.05 % Tween 20
IgG	immunoglobulin G	Px	horseradish peroxidase
IPBS	isotonic phosphate buffered saline, pH 7.0	SDS	sodium dodecyl sulfate
LPS	lipopolysaccharide		

Yersinia enterocolitica is a heterogeneous species comprising pathogenic and nonpathogenic strains that are widely distributed in both terrestrial and aquatic ecosystems. The organism has been isolated mainly in regions of Europe and North America with mild or cooler climate (Cover and Aber 1989). Strains pathogenic for man belong to serotypes O:3, O:5,27, O:8 and O:9 (Bissett *et al*. 1990; Kapperud 1991; Bottome 1997). Pigs were recognized as the main reservoir of the strains associated with human yersiniosis (Hurvell 1981; Schieman 1989; Kapperud 1991). The organisms are present in the oral cavity, especially on the tongue and in the tonsils and in the intestine and feces.

The infection caused by *Y. enterocolitica* is manifested as a self-limiting gastroenterocolitis with symptoms: abdominal pain, bloody or watery diarrhea, mild fever and infrequent vomiting. The incubation period is ≈3–7 d. Post-infectious sequelae, such as polyarthritis, multisystem Reiter's syndrome, erythema nodosum and septicemia with presumably subsequent glomerulonephritis and myocarditis have been described (Laitenen *et al*. 1972; Foberg *et al*. 1986; Bottome 1997; Smego *et al*. 1999).

The major route of transmission of *Y. enterocolitica* to man is a consumption of contaminated food. The pathogenic and nonpathogenic strains have been isolated from beef, pork, poultry, milk, cheese, mussels, and lettuce (Vidon and Delmas 1981; Schieman and Toma 1981; Walker and Gilmour 1986; Fukushima *et al*. 1997; Nortjé *et al*. 1999; Filetici *et al*. 2000; Capita *et al*. 2002); the transmission is facilitated by the ability of this microorganism to grow at low temperature. Contamined water is another important reservoir of *Y. enterocolitica* (Kapperud 1977; Schiemann 1978; Shayegani *et al*. 1981; Ostroff *et al*. 1994; Waage *et al*. 1999). This microorganism can survive for several weeks in natural aquatic environment (Tashiro *et al*. 1991; Terzieva and McFeters 1991).

We focused on the preparation and characterization of polyclonal antibodies for enzyme immunoassay of pathogenic *Y. enterocolitica* strains.

MATERIAL AND METHODS

Bacteria were purchased from the *Czech Collection of Microorganisms* (CCM) (Brno, Czechia), the *Czech National Collection of Type Cultures* (CNCTC) in Prague or they were obtained from the collection of microorganisms at the *Department of Biochemistry and Microbiology, Institute of Chemical Technology* (DBM).

Swine anti-rabbit IgG–Px conjugate (7.62 mg/mL, molar ratio Px/IgG = 1.63) was purchased from *Sevapharma* (Czechia). Unless otherwise indicated the analytical substances were purchased from *Sigma-Aldrich*.

Immunogen and standard preparation. *Yersinia enterocolitica* O:3 (CNCTC Y 7/71), *Y. enterocolitica* O:5,27 (CNCTC Y 10/71), *Y. enterocolitica* O:8 (CNCTC Y 13/71), and *Y. enterocolitica* O:9 (CNCTC Y 14/71) were chosen as immunogens for preparation of rabbit antibodies. The microorganisms were cultivated in Nutrient broth (*Oxoid*, USA) for 1–2 d at 28 °C. The cell enumeration was done by a plate-count procedure on Plate count agar (*Oxoid*). After cultivation, the cells were separated from cultivation broth by centrifugation (5000 ***g***, 15 min, 4 °C) and then thrice washed with 10 mmol/L phosphate-buffered saline (pH 7.4) (PBS 7.4). Formaldehyde-killed bacterial cells were prepared according to Rice *et al.* (1997) as the immunogen. In the case of a standard preparation the cells were resuspended in 10 mL PBS 7.4 and stored at 0 °C until used.

Immunization and antibody preparation. New Zealand White rabbits were used as the source of hyperimmune antisera. The animals were challenged with 10^7 cells per dose diluted initially in complete Freund's adjuvant, and lately Al-Span-Oil (*Sevapharma*) or incomplete Freund's adjuvant, respectively. Each animal obtained six doses at 3-week intervals. Specific antibody titer dynamics was evaluated by indirect CELISA with ethanol-fixed bacteria. Titers were controlled within the whole long-time immunization schedule.

Preparation of IgG fractions from collected sera was performed by affinity chromatography using glass-bead immobilized protein A (Prosep A high capacity; *Bioprocessing*, UK). After binding the sera diluted in PBS 7.4 and washing of the column, the globulin fraction was eluted using 0.1 mol/L citrate buffer (pH 3.0). The end-use form of the fractions was prepared by freeze-drying from ammonium hydrogencarbonate neutral buffer.

Dot blotting. Cell suspensions of different bacterial strains at concentration $\approx 10^8$ CFU/mL were used for screening. Bacterial samples (1 μL per spot) were applied to nitrocellulose membrane (pore size 0.45 μm), dried with hot air and saturated for 1 h with 5 % solution of non-fat dried milk in PBS 7.4 at room temperature. The membrane was then washed thrice for 5 min with PBS 7.4 containing 0.05 % (*V*/*V*) Tween 20 (*Fluka*, Germany) (PBST) and incubated for 1 h in 5 mL of rabbit IgG (10 μg/mL) raised to particular *Y. enterocolitica* serovar at room temperature. After washing, 5 mL of horseradish peroxidase-labeled swine anti-rabbit IgG antibody (concentration 1.5 μg/mL) was added and kept for 1 h to react at room temperature. The membrane was washed thrice in PBST for 5 min and once for 5 min in deionized water, and then immersed into a substrate solution containing 0.06 % (*W*/*V*) 3-amino-9-ethylcarbazole, 20 % (*V*/*V*) formamide, and 0.042 % (*V*/*V*) hydrogen peroxide in 0.05 mol/L citrate/phosphate buffer (pH 5.0). After washing with deionized water, the membrane was dried.

Preparation of LPS by proteinase K digestion of whole-cell lysates. A procedure slightly modified from Mandatori and Penner (1989) was used to isolate LPSs. Bacteria were harvested from a single Nutrient agar plate (*Oxoid*) in 2 mL of cold IPBS (pH 7.0; 0.35 % (*W*/*V*) Na_2HPO_4 in 0.85 % NaCl) and diluted in IPBS to the turbidity of 8 McFarland's units. Cell suspension (1.5 mL) was transferred to Eppendorf tube and centrifuged in a microcentrifuge for 5 min. The supernatant was discarded and the remaining cell pellet was solubilized in 0.2 mL lysis buffer (20 % (*V*/*V*) glycerol; 5 % (*V*/*V*) 2-sulfanylethanol; 2 % (*W*/*V*) SDS; 0.6 mmol Tris-HCl, pH 6.8; 50 ppm (*W*/*V*) bromophenol blue). The mixture of lysed cells was heated for 10 min at 100 °C, cooled to room temperature and 50 μL proteinase K (3 mg/mL) in lysis buffer was added. After a 1-d incubation at 37 °C, enzyme-treated lysates were incubated for another 2 h at 65 °C to carry out the autodigestion of the enzyme. Then the samples were boiled for 5 min and subsequently centrifuged in a microcentrifuge for 10 min before electrophoresis.

SDS-PAGE and silver staining of LPS. LPS preparations (5 μL per lane) were separated by PAGE in discontinuous Laemmli (1970) system. SDS-PAGE was done in 0.7 mm thick slab gels with the 4 % stacking gel and 12 % separating one. The electromigration carried out under the constant current of 35 mA. After SDS-PAGE, the gels were fixed and stained according to Tsai and Frasch (1982). Briefly, the gels were immersed in fixative solution (40 % (*V*/*V*) ethanol, 5 % (*V*/*V*) acetic acid) for 18 h and subsequently incubated in an oxidizing solution (0.7 % (*W*/*V*) periodic acid, 40 % (*V*/*V*) ethanol, 5 % (*V*/*V*) acetic acid) for 5 min. The gels were washed thrice in deionized water for 15 min each and then immersed in a staining solution

containing 2 mL of concentrated ammonium hydroxide, 28 mL of 0.1 mol/L sodium hydroxide, 5 mL of 20 % (*W*/*V*) silver nitrate and 115 mL deionized water, and incubated for 10 min at room temperature. The gels were immersed thrice in deionized water for 15 min each and incubated in developing solution consisting of 0.018 % formaldehyde in 0.2 mmol/L citric acid. After washing, the gels were stored in deionized water.

Immunoblotting analysis of LPS. The electrophoretic transfer of LPS fractionated by SDS-PAGE from gel to nitrocellulose sheet (pore size 0.45 μm) was performed by means of the Trans Blot system (*Bio-Rad*, USA) using the Towbin's transfer buffer (Towbin *et al.* 1979). After the SDS-PAGE separation, the gel was immersed in the transfer buffer (25 mmol/L Tris, 192 mmol/L glycine, 20 % (*V*/*V*) methanol) for 30 min and applied to the nitrocellulose sheet. Blotting was performed under constant voltage of 100 V for 3 h. After transfer, the nitrocellulose sheet was immersed in 5 % (*W*/*V*) skimmed milk in PBS 7.4 for 1 h. The sheet was washed thrice in PBST for 10 min each. Then 10 mL of rabbit IgG raised to the particular *Y. enterocolitica* serovar at the concentration 5 μg/mL was applied and incubated at room temperature for 1 h. The nitrocellulose sheet was washed thrice in PBST and then 10 mL of swine anti-rabbit IgG–Px conjugate diluted with 5 % (*W*/*V*) skimmed milk in PBST to the concentration 3.8 μg/mL was applied. The incubation was done for 1 h at room temperature. The sheet was washed, stained by 3-amino-9-ethylcarbazole and stopped as previously.

Indirect CELISA. The suspension of antigen (whole cells of *Y. enterocolitica*) was diluted by PBS 7.4 to optimum concentration (Table I). Diluted antigen (100 μL) was pipetted into the wells of a polystyrene microplate (type U; *Costar Corning*, USA). The content of the wells was incubated for 1 h at 37 °C followed by an additional overnight incubation at 4 °C. Then 50 μL of 0.5 % (*V*/*V*) glutaraldehyde was added, and after a 15-min incubation at room temperature, the reaction mixture was removed. The microplate wells were washed thrice with 0.2 mL of PBST. As a blocking agent, BSA (*Imuna*, Slovakia; 0.1 mL of 1 % (*W*/*V*) BSA in PBS) was added for 1 h at room temperature. Microplates were washed thrice with 0.2 mL PBST. In the next step, 50 μL diluted standard was added into the wells. Then 50 μL of rabbit IgG raised to the particular *Y. enterocolitica* serovar diluted with PBS to optimum concentration was added and kept to react for 1 h at room temperature. The reaction mixture was removed and the wells were washed thrice with 0.2 mL PBST. Then 0.1 mL of swine anti-rabbit IgG–Px conjugate diluted with 0.1 % (*W*/*V*) BSA in PBST to the optimum concentration was pipetted into microplate wells. The conjugate was kept for 1 h at room temperature. The wells were then washed four times with 0.2 mL of PBST, once with 0.2 mL of deionized water and, finally, 0.1 mL of 2.7 mmol/L benzene-1,2-diamine dihydrochloride and 0.03 % (*V*/*V*) H_2O_2 in 50 mmol/L citrate–phosphate buffer (pH 5.0) was added. The enzyme reaction was terminated after 20 min by the addition of 50 μL 2 mol/L H_2SO_4. The absorbance A_{492} was measured directly in the wells using the microplate reader SLT RainBow (*Tecan*, Switzerland).

Table I. Optimum concentration of immunoreactants used in indirect CELISA of *Y. enterocolitica*

Sample	Immobilized antigen CFU/nL	Antibody μg/mL	Conjugate μg/mL
Strain O:3	79	5.0	0.4
Strain O:9	23	2.5	0.4
Group of serotypes	79[a]	5.0	0.4

[a]The cells of serotype O:9 were used as an immobilized antigen.

Evaluation of experimental values. The calibration curve has been calculated according to the four-parameter equation (Karpinsky 1990):

$$A = C + (D - C)/\{1 + \exp[-2(\alpha + \beta x)]\}$$

where C and D are the lower and upper asymptotes, respectively, α and β are the indicators of the location and steepness of the curve; x represents the logarithm of analyte concentration, A is absorbance.

The detection limit was calculated as the average value of absorbance at zero standard concentration minus three standard deviations.

RESULTS AND DISCUSSION

Rabbit sera raised against whole cells of *Yersinia enterocolitica* O:3, O:9 and against a group of pathogenic *Y. enterocolitica* strains (serotypes O:3, O:5,27, O:8. and O:9) were prepared *via* immunization of New Zealand White rabbits using whole bacterial cells as immunogens. The limiting titers of sera against serotypes O:3 and O:9 and against a group of pathogenic *Y. enterocolitica* strains were $1:2.0 \times 10^5$–7.5×10^5,

1 : 7.1 × 10^5–7.2 × 10^5, and 1 : 9.5 × 10^4–1.0 × 10^5, respectivelly. (The titer of the antibody against the group of *Yersinia* serotypes is valid for *Y. enterocolitica* O:9.)

The specificity of prepared antibodies was evaluated using dot-blotting and whole cells of 19 *Yersinia* strains and 6 non-*Yersinia* species; the cross-reactivity of antibodies is given in Table II. Immunoglobulins raised against a single *Y. enterocolitica* serotype were specific. The antibody raised against four pathogenic *Y. enterocolitica* serotypes (O:3, O:5,27, O:8 and O:9) showed (under the conditions of the dot-blotting) affinity only to serotype O:9. On the other hand, this antibody weakly cross-reacted with *Y. enterocolitica* O:6,30, *Y. enterocolitica* O:10, *Y. bercoverii* O:8, and *Y. pseudotuberculosis* Ia. All antibodies also interacted with strain *Y. rohdei* b, d, e, f, i. No cross-reactivity was observed with tested non-*Yersinia* species. All prepared immunoglobulins were potentially useful for the development of immunoassays for detection of pathogenic *Y. enterocolitica* serotypes in foods and environmental samples. The weak undesirable cross-reactivity was removable *via* saturation of the antibodies with heterologous antigens.

The SDS-PAGE and silver staining of LPS prepared by means of proteinase K-digestion of whole cell lysates were used for investigation of components interacting with prepared immunoglobulins. On comparing electrophoretic profiles of different *Y. enterocolitica* serotypes and *Y. bercoverii* O:8, *Y. pseudotuberculosis* Ia, and *Y. rohdei* b, d, e, f, i (Fig. 1), the main differences among tested *Y. enterocolitica* serotypes were observed in the O-polysaccharide profiles. The serotype O:8 expressed long-chain LPS migrating into

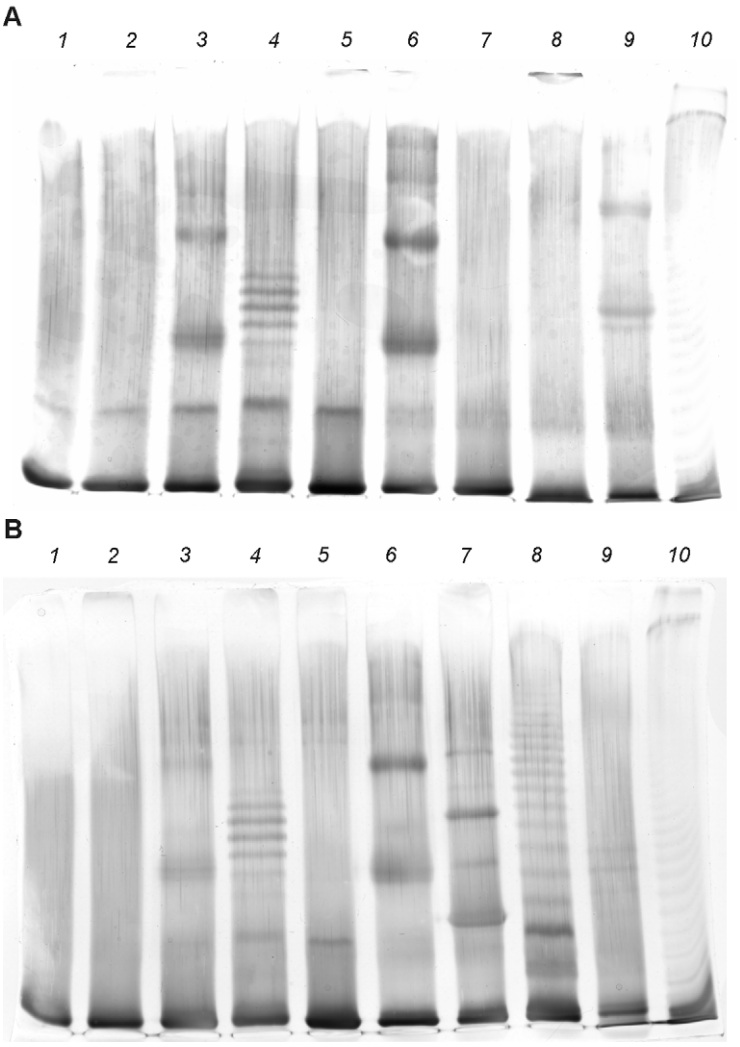

Fig. 1. Silver-stained LPS profiles of *Yersinia* strains. **A**: *1*, *2* – *Y. enterocolitica* O:3, *3* – O:5,27, *4* – O:8, *5* – O:9, *6* – *Y. enterocolitica* CNCTC Y 2/68, *7* – O:10, *8* – *Y. pseudotuberculosis* Ia, *9* – *Y. rohdei* b, d, e, f, i, *10* – *Salmonella enterica* serovar enteritidis; **B**: *1*, *2* – *Y. enterocolitica* O:3, *3* – O:5,27, *4* – O:8; *5* – O:9, *6* – O:5, *7* – O:6,30, *8* – *Y. aldovae* O:17, *9* – *Y. bercoverii* O:8, *10* – *Salmonella enterica* serovar enteritidis.

a ladder-like banding pattern, whereas serotypes O:3 and O:9 expressed long-chain LPS migrating into unresolvable diffuse bands. As reported by Gorshkova *et al*. (1985), the repeating units of O-specific polysaccharides of *Y. enterocolitica* O:3 involved single saccharide 6-deoxy-L-altrose residues linked by β-L-1,2 glycosidic bonds. Similarly, the O-chain of *Y. enterocolitica* O:9 is a linear homopolymer composed of 1,2 linked 4,6-dideoxy-4-formamido-α-D-mannopyranosyl units (Caroff *et al*. 1984). Since repeating units of such mo-

lecule are monosaccharides, molecules differing by in a single repeat unit could not be distinguished by the SDS-PAGE procedure. In comparison with other tested *Y. enterocolitica* serotypes, the strain O:5,27 produced high-molar-mass LPS with a limited range of polysaccharide chain lengths. The complex O-chain was observed in LPS from serotype O:8. In this case, the pentasaccharide unit contains *N*-acetyl-α-D-galactosamine, L-fucose, D-galactose, D-mannose, and 6-deoxy-D-gulose. The repeating units are linked together by 1,4 glycosidic bonds between *N*-acetyl-α-D-galactosamine and D-mannose residues (Skurnik and Bengoechea 2003).

Table II. Cross-reactivity of rabbit IgG raised against pathogenic serotypes of *Yersinia enterocolitica* detected by dot blotting

Strain	Number[b]	Cross-reactivity[a] against *Y. enterocolitica*		
		O:3	O:9	group of serotypes
Yersinia enterocolitica O:3	CNCTC Y 7/71	+	–	–
O:5,27	CNCTC Y 10/71	–	–	–
O:8	CNCTC Y 13/71	–	–	–
O:9	CNCTC Y 14/71	±	+	+
O:5	CNCTC Y 9/71	–	–	–
O:6,30	CNCTC Y 41/71	–	–	±
O:7,8	CNCTC Y 12/71	–	–	–
O:10	CNCTC Y 15/71	–	–	±
O:13,7	CNCTC Y 21/71	–	–	–
Yersinia enterocolitica	CNCTC Y 2/68	+	–	–
Yersinia aldovae O:17	CNCTC Y 49/84	–	–	–
bercoverii O:8	CCM 4206	–	–	±
bercoverii O:58,16	CCM 4205	–	–	–
frederiksenii	CCM 3555	–	–	–
intermedia serovar 17	CCM 3558	–	–	–
kristensenii O:1,2a	CNCTC Y 47/83	–	–	–
molaretii O:59	CCM 4208	–	–	–
pseudotuberculosis 1a	CNCTC Papt 10/72	–	±	±
rohdei b, d, e, f, i	CNCTC Y 57/88	+	±	+
Citrobacter freundii	DBM 3127	–	–	–
Escherichia coli O:157:K88:a,c:H19	CNCTC Eck 267/75	–	–	–
Morganella morganii	DBM 3074	–	–	–
Salmonella anatum 3,10:e,h:1,6	CNCTC Sk 78	–	–	–
milwaukee 43:f,g:–	CNCTC Sk 733/56	–	–	–
Bacillus cereus	DBM 3035	–	–	–

[a](+) – strong, (±) – weak, (–) – none.

[b]CCM – *Czech Collection of Microorganisms, Faculty of Science, Masaryk University*, Brno; CNCTC – *Czech National Collection of Type Cultures*, *National Institute of Public Health*, Prague; DBM – collection of microorganisms at the *Department of Biochemistry and Microbiology, Institute of Chemical Technology*, Prague.

The strains *Y. aldovae* O:17 and *Y. bercoverii* O:8 expressed long-chain LPS, producing ladder-like banding patterns that were observed in other members of the *Enterobactericeae*. *Y. enterocolitica* serotypes O:5, O:6,30, *Y. enterocolitica* CNCTC Y 2/68, and *Y. rohdei* b, d, e, f, i produced long-chain LPS but with a limited range of polysaccharide-chain lengths (Figs 1 and 2). *Y. pseudotuberculosis* Ia produced O-polysaccharides migrating into a poorly visible ladder-like banding pattern. It is not evident if the bacterium produces a considerably smaller amount of O-polysaccharides in comparison with core LPS (Chart and Cheasty 2006) or, if these high-molar-mass LPS are unstainable with silver as it had been described for *Campylobacter jejuni* strains (Preston and Penner 1987).

The antibody against *Y. enterocolitica* O:3 interacted (as follows from the results of immunoblotting analysis) with homologous high-molar-mass LPS. Negligible interactions of this antibody were observed with core LPS and lipid A from serotypes O:3, O:5,27, and O:9 (Fig. 2). On the other hand, antibodies raised against serotype O:9 and those raised against the spectrum of the main pathogenic serotypes showed interactions with the fast migrating LPS of serotypes O:3, O:5,27 and O:9 and the strain *Y. enterocolitica* CNCTC Y 2/68. Both antibodies recognized the set of tiny bands between the fastest migrating main ones (Figs 2 and 3). All these interactions are weak, probably due to a steric hindrance; they could not therefore be detected under conditions of dot-blotting in which the whole cells were used as an antigen. In addition,

the antibody raised against the spectrum of the pathogenic serotypes weakly interacted with core LPS and lipid A of *Y. enterocolitica* O:5, O:6,30, and O:10. As there is only a limited information on the composition of the *Y. enterocolitica* core region a simple and clear elucidation is unattainable. In serotype O:3, the O-LPS are attached to the inner core region of the LPS. In addition to the O-polysaccharides, the outer core hexasaccharide (that consists of glucose, *N*-acetyl-galactosamine, galactose, and *N*-acetyl-fucosamine) is attached to the inner core, forming a short branch in the LPS molecule (Skurnik and Bengoechea 2003).

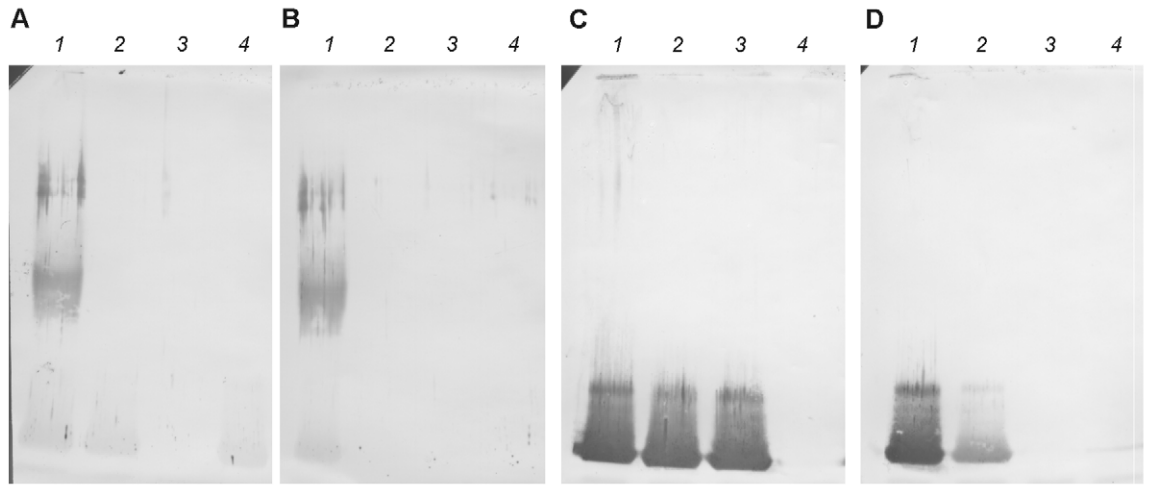

Fig. 2. Interaction of rabbit IgG against *Y. enterocolitica* O:3 (**A**, **B**) and O:9 (**C**, **D**) with LPS of different *Yersinia* strains detected by immunoblot. **A**: lane *1* – *Y. enterocolitica* O:3, *2* – O;5,27, *3* – O:8, *4* – O:9; **B**: *1* – *Y. enterocolitica* O:3, *2* – *Y. enterocolitica* CNCTC Y 2/68, *3* – *Y. pseudotuberculosis* Ia, *4* – *Y. rohdei* b, d, e, f, i; **C**: *1* – *Y. enterocolitica* O:9, *2* – O:3, *3* – O:5,27, *4* – O:8; **D**: *1* – *Y. enterocolitica* O:9, *2* – *Y. enterocolitica* CNCTC Y 2/68, *3* – *Y. pseudotuberculosis* Ia, *4* – *Y. rohdei* b, d, e, f, i.

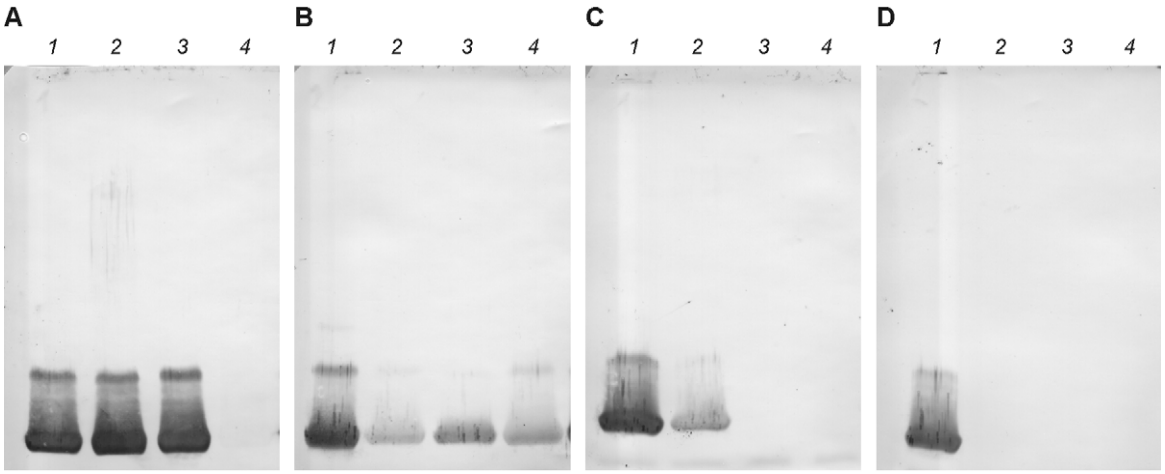

Fig. 3. Interaction of rabbit IgG against a group of pathogenic *Y. enterocolitica* serotypes (O:3, O:5,27, O:8, and O:9) with LPS of different *Yersinia* strains detected by immunoblot. **A**: *1* – *Y. enterocolitica* O:9, *2* – O:3, *3* – O:5,27, *4* – O:8; **B**: *1* – *Y. enterocolitica* O:9, *2* – O:5, *3* – O:6,30, *4* – O:10; **C**: *1* – *Y. enterocolitica* O:9, *2* – *Y. enterocolitica* CNCTC Y 2/6), *3* – *Y. pseudotuberculosis* Ia, *4* – *Y. rohdei* b, d, e, f, i; **D**: *1* – *Y. enterocolitica* O:9, *2* – *Y. aldovae* O:17, *3* – *Y. bercoverii* O:8, *4* – *Salmonella enterica* serovar enteritidis.

The *immunoblotting analysis* also indicated that LPS of *Y. bercoverii* O:8, *Y. pseudotuberculosis* Ia, and *Y. rohdei* b, d, f, i do not contribute to cross-reactivity of tested antibodies with these bacteria (Table II, Fig. 4). The interactions among the *Y. bercoverii* O:8, *Y. pseudotuberculosis* Ia, *Y. rohdei* b, d, e, f, and i cells and tested antibodies are probably mediated by protein components of the bacterial cell wall. The influence of cell-wall proteins on the cross-reactivity is a subject of further investigation.

Development of indirect CELISA (Fig. 4). Optimum concentrations of all reactants were established (Table I). The detection limits were 7/nL CFU (*i.e.* 7×10^6 CFU per mL) and 3/nL CFU of serotypes O:3 and O:9, respectively. If the antibody raised against the whole spectrum of pathogenic serotypes was used,

the detection limit reached 7/nL CFU. In this case, cells of O:9 were used both as an immobilized antigen and as a standard. The assays have to be further validated using naturally contaminated and spiked foods in comparison to conventional detection methods.

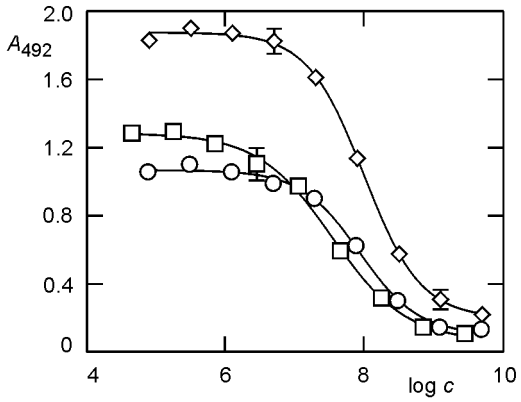

Fig. 4. Sensitivity (log *c*, *c* – detection limit in CFU per mL) of indirect CELISA using rabbit IgG against *Y. enterocolitica* O:3 (*circles*), *Y. enterocolitica* O:9 (*squares*), *Y. enterocolitica* O:3, O:5,27, O:8 and O:9 (*diamonds*).

The standard microbiological methods for detecting *Y. enterocolitica* are very sensitive but also very laborious, time consuming and/or expensive. The detection and identification of pathogenic *Yersinia* strains performed according to *ČSN ISO 10273* (1996) protocols takes 6–12 d. On the other hand, ELISA (or CELISA) was developed as one of the most rapid, inexpensive, sensitive and specific techniques for detection of many different bacteria. The sensitivity of these immunochemical methods varies among 10^4–10^7 CFU per mL depending on the quality of antibody and the assay format (Kaneko and Maruyama 1989; Padhye and Doyle 1991; Hübner *et al.* 1992; Kerr *et al*. 2001; Hochel *et al.* 2004) and microorganisms can be usually detected in less than 20 h. This procedure can be therefore recommended for routine screening of food samples.

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