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*The usefulness of serological tests for detection of Salmonella enteritidis antibodies in poultry muscle juice**

Przydatność odczynów serologicznych do wykrywania przeciwciał dla *Salmonella enteritidis* w soku z tkanki mięśniowej ptaków

Over 2,000 food poisoning serotypes of salmonella exist and the prevalence of individual serotypes constantly changes. Moreover, salmonellosis continues to become a major problem both in terms of infections and food poisonings. In Poland 92.5% of food born intoxications in humans in 1992-1994 was associated with salmonellae (16). They were associated largely with *Salmonella enteritidis* (6, 12, 17, 20, 21). The main source of *S. enteritidis* for human beings are birds, mainly poultry undercooked meat, poultry meat products, bacteria contaminated eggs and egg products. Hence, the successful control of zoonotic salmonellosis requires intervention at many points of food chain. Most important is to prevent or greatly diminish salmonella infections in poultry by regular monitoring of poultry flocks.

Several methods of determining the salmonella status of flocks have been devised. Infections in mature birds can be identified by serological tests. The enzyme-linked immunosorbent assay technique (ELISA) was used to detect salmonella carriers in poultry flocks (1, 2, 8, 10, 12, 13, 15, 21, 22, 25). ELISA appeared to be a very sensitive test, indispensable to detect both quantitatively and qualitatively antigens and antibodies in various biological materials. The indirect-ELISA

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with the labelled immunoglobulins is used to detect specific anti-salmonella antibodies in poultry. By this procedure antibodies both against flagellar salmonellae (*S. enteritidis, S. typhimurium*) and non-flagellar (*S. gallinarum, S. pullorum*) can be detected (1, 6, 10, 15, 21, 22).

In the ELISA for antibody various types of antigens are postulated, for example flagellar or fimbrial antigens (SEF 14), structures of the outer membrane of bacterial cell such as proteins or lipopolysaccharides (1, 3, 6, 7, 15, 17, 24, 25, 26, 27). The later is successfully used because of very high sensitivity of the test. Unfortunately, when this antigen is used, cross reactions between serovar of the group B and D are found (1, 3, 14, 20, 22) because of the presence the common antigen 0-12 in both groups. Immunoblotting test has showed that this antigen possesses a character of dominating epitope in *S. enteritidis* and other salmonella serovars (6).

The ELISA test is recommended for detecting the antibodies in poultry sera and in egg yolk. Antibodies present in the egg yolk may be useful for serological diagnostics of infections in poultry. First, the yolk antibodies can be easily extracted, second, for screening it is easier to obtain eggs than sera, and large numbers of eggs may be used for testing, moreover, eggs contrary to sera are obtained without any stress, tests may be repeated many times (9, 11, 12, 13, 18, 21, 24). Cleaning procedure of yolk is one factor limiting the used egg as material for testing.

The objective of the paper was to compare the efficacy of the own ELISA test with other commonly used serological tests for detection of anti-*Salmonella enteritidis* antibodies in poultry muscle juice.

MATERIALS

Blood serum, egg yolk and muscle juice of hens at the age of about 20 months, Isa Brown breed, from a positive flock in a plate agglutination test with Pullognost were examined. Thirty samples of each material were used. Bacteriological examination for *Salmonella* of feces and yolk eggs of the flock was negative.

Moreover, there were tested blood sera, egg yolk and muscle juice of hens of the Isa Brown at the age of about 12 months free from salmonella infection. Ten samples of each material was tested. Plate agglutination test with Pullognost and bacteriological examinations of the flock were negative.

Also 23 samples of blood serum and 23 samples of meat juice each from 20 broiler flocks; *Lohman* breed and *Arbor Acres* breed; at the age of 6-8.5 weeks were included for examinations. Samples of blood and muscles were randomly chosen in the poultry producing units.

METHODS

Lipopolysaccharide extract (LPS) was prepared from a field strain of *S. enteritidis*, phage type 1 (determined at the Institute of Marine and Tropical Medicine in Gdynia) by the method of H a s s a n et al. (15). The bacterium was suspended in 10 ml of the Oxoid CM 67 broth and incubated for 24 h

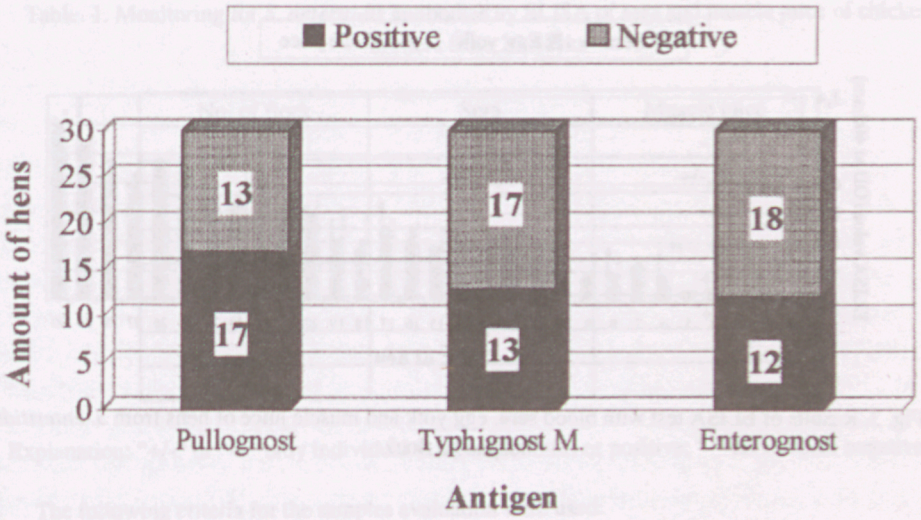

Fig. 1. Plate agglutination test with a fresh blood drop using three different antigens

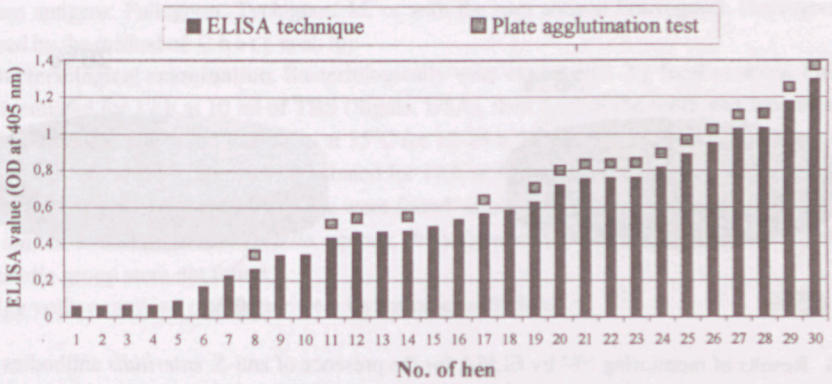

Fig. 2. Comparison of the results of ELISA and plate agglutination test with Pullognost for poultry sera

at 37°C agitated 100 times per minute. 10 ml of the obtained bacterial suspension (about 10^9 cfu/ml) was transferred into 1 L of TBS medium (Sigma) and incubated at 37°C for 12 hr.

Supernatant from egg yolks was obtained by the method of B o l l e n et al. (4). In the ELISA test the diluted supernatant (1:10) was used.

Muscle juice was obtained from frozen femoral and pectoral muscles then sawed at room temperature. Diluted (1:10) juice was used in the test. Blood sera for the ELISA was diluted 1:100.

ELISA technique. Wells in microplates PolySorp Nunc (Denmark) were first coated with 0.96 µg LPS/ ml of carbonate buffer (pH 9.6) at room temperature for 18 h. The unbound antigen was removed by washing with PBST (PBS plus 0.5% Tween 20). Then the wells were filled with the tested material (100 µl/well). Two wells for each individual sample were used. Control wells were filled with a

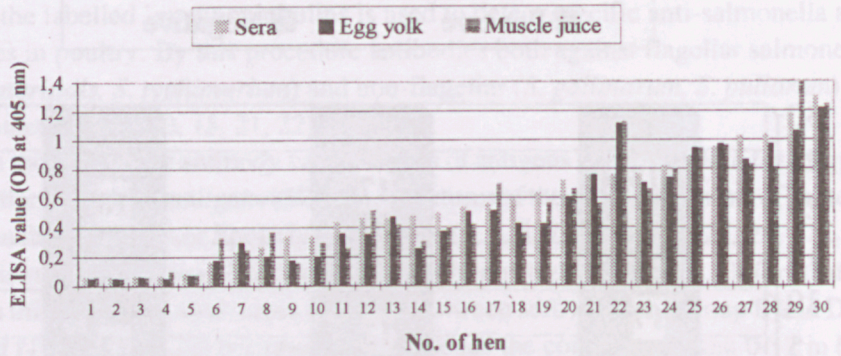

Fig. 3. Results of ELISA test with blood sera, egg yolk and muscle juice of hens from *S. enteritidis* suspected flock

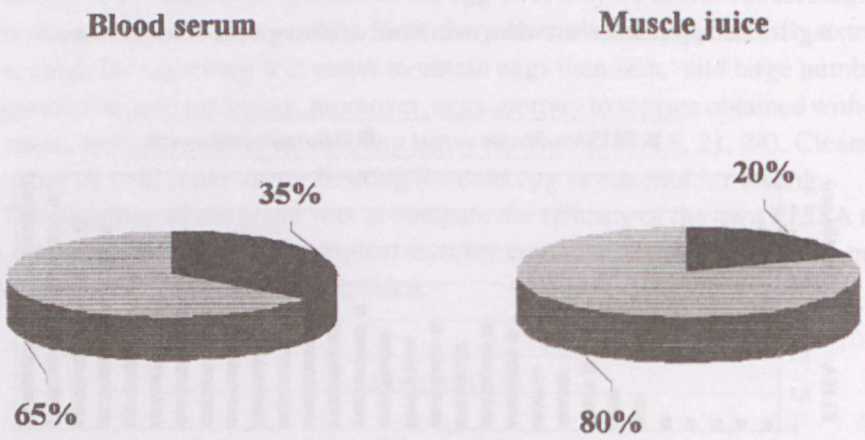

Fig. 4. Results of monitoring (%) by ELISA for the presence of anti-*S. enteritidis* antibodies in sera and muscle juice of chicken broilers from 20 flocks (grey – all samples negative; black- positive and doubtful samples V)

positive serum (S+) derived from a hen infected with salmonella of OD equal to OD of a control positive serum of commercial kit for examination of poultry sera for detection of anti-*S. enteritidis* antibodies (Bommeli company), and a negative (S-) antiserum. After 30 min incubation at room temperature the plate was three times automatically washed (Labsystem, Multiwash, Finland) with PBST. After washing the wells were filled with 100 µl of a conjugate of antibodies against hen IgG (Jackson Immuno Res. USA) at a concentration 1:5000. The plates were incubated for 30 min at room temperature. After washing with PBST 100 µl of the enzymatic substrate ABTS (Sigma, USA) with hydroxide peroxide was added to each well. The color intensity was estimated by a microplate spectrophotometer (Multiscan, Multisoft, Labsystem) at 405 nm at the moment when OD for the positive control (S+) trespassed 0.400.

Table. 1. Monitoring for *S. anteritidis* antibodies by ELISA of sera and muscle juice of chicken broilers from 20 flocks

No. of flock	Sera	Muscle juice
1-13.	-	-
14.	+/-, +, +	+/-, +
15.	+	-
16.	+/-, +	+/-, +, +
17.	+/-, +	+
18.	+/-, +, +	+
19.	+	-
20.	+/-	-

Explanation: "+/-" or "+" only individual samples doubtful or positive; "-" all samples negative

The following criteria for the samples evaluation were used:

$S/P = OD_{\text{examined sample}} - OD_{S-} / OD_{S+} - OD_{S-} \times 100\%$

Samples of sera and muscle juice: negative S/P< 25%, doubtful S/P 25-35%, positive S/P>35%.

Plate agglutination test. The test was done with a fresh blood drop and/or serum using three different antigens: Pullognost, Typhignost M. or with the own antigen Enterognost. Enterognost was prepared by the method of C h a r t et al. (6).

Bacteriological examination. Bacteriologically were examined 1-2 g fecal samples. First they were incubated for 12 h at 10 ml of TBS (Sigma, USA), then 1 ml of the broth was inoculated into 10-15 ml of SF medium and incubated at 35°C for 12-24 h. Then McConkey's agar was inoculated with 2 drops of the SF culture and incubated for 12 h at 37°C. Bacteria isolated were identified by the API 20E test. The following bacteria were found: *Escherichia coli, Enterobacter agglomerans, Ent. cloacae, Ent. sakazakii, Klebsiella oxytoca, Proteus mirabilis, Citrobacter freundii*. Members of *Salmonella* group were not found.

Egg yolks examined on SS agar were bacteriologically free.

RESULTS AND DISCUSSION

When infected domestic animals, particularly poultry, frequently become asymptomatic carriers of salmonella for variable periods. In this situation quick and effective diagnosis of infection based on detection of specific antibodies or salmonella antigens is necessary for epidemiological and economical reasons. For testing of poultry flocks and for sampling different materials various serological techniques, including ELISA are postulated.

In the presented examinations, first, samples of fresh blood from 30 hens from a salmonella suspected flock have been examined by the plate agglutination test using three different antigens: Pullognost, Typhignost M and Enterognost. Using Pullognost 17, Typhignost M 13 and Enterognost 12 birds in

the flock reacted positively (Fig. 1). Comparable results with the plate agglutination test noted C h a r t et al. (6). They used two antigens: Pullognost and Enterognost of their own production. Most positive reactors with Pullognost also reacted positively with Enterognost. The presence of a common somatic antigen O=12 in serotypes of *Salmonella* from group B and D may explain this situation (1, 3, 14, 20, 23). O=12 antigen is present in such serovars as *S. enteritidis* (O:1, 9, 12; H: g.m.), *S. typhimurium* (O:1, 4, 5, 12; H:i,1, 2) and *S. pullorum* (O: 9,12).

When sera of hens from this flock were tested by ELISA 24 (80%) individuals possessed significantly increased mean value of OD. For birds no from 7 to 30 a mean value of OD exceeded 0.200. Among birds positively reacting in the ELISA test 17 (57%) also reacted positively with Pullognost at the plate agglutination test (Fig. 2). Similar results were obtained by other authors. N i c h o l a s et al. (22) in the flock bacteriologically positive for *S. enteritidis* noted 75% of positive results in the LPS-ELISA, but at the plate agglutination test with Pullognost they noted positive results with 37% of sera examined. F u r r e r et al. (10) screening poultry for *S. enteritidis* obtained positive results with the LPS-ELISA for 62% of individuals and only for 33% of birds by the plate agglutination test with Pullognost.

In the next phase of studies mean values of OD for sera, yolk egg and meat juice obtained for hens have been compared (Fig. 3). It was found that birds of a low mean serum values of OD also have low mean OD values for egg yolk and muscle juice. Contrary, birds of sera of a higher mean OD values showed high mean value of OD for egg yolk and meat juice. These observations are in agreement with data of many authors (1, 10, 21, 23). In hens experimentally infected with salmonella or in flocks naturally infected by salmonella titer of specific anti-*Salmonella* IgG antibodies in sera and yolk egg of the same individual were almost identical. D a d r a s t et al. (8) found that in the *S. enteritidis* flock of egg laying hens 60% of egg yolks showed significantly higher titer of IgG antibodies in the LPS-ELISA. S u n w o o et al. (24) had examined a flock experimentally infected with *S. typhimurium*. They found similar titers of antibodies in sera and egg yolks, and that they decreased in very similar manner. Moreover, they determined concentration of IgG in sera and in egg yolks. Serum IgG concentration was about 6.0 mg/ml, egg yolk concentration 10 mg/ml. N i c h o l a s et al. (21) examining *S. enteritidis* infected flocks showed in the LPS-ELISA 75% positive sera and 55% positive egg yolk.

The LPS-ELISA was used for the first time to check the level of antibodies in poultry muscle juice. There have been no data on the use of ELISA for this purpose so far. Muscle juice was used for the first time for *S. enterica* surveillance and control in Danish slaughter swine herds (5, 19).

The examination of control flock of hens confirmed the results of the ELISA for different tissues including muscle juice. In this *Salmonella* uninfected birds the value of ELISA test was low.

The most interesting was monitoring of 20 broiler flocks in which blood sera and meat juice samples were applied. It was found (Table 1, Fig. 4) that 35% of blood sera and only 20% of meat juice samples reacted positively or doubtfully. It is worthy to note that samples blood sera and muscle juice examined have been obtained from different individuals. Therefore, for monitoring for salmonella infection of poultry, especially in slaughter houses, meat juice samples may be used for serological tests. Using meat juice it is possible to examine any number of samples even after slaughter. It can be concluded that muscle juice like blood serum is very useful to determine immunologic patterns of poultry flocks in a district. Any deviations from the pattern could be estimated as reflecting changes in immune status of birds.

There is a possibility to use yolk egg and meat juice for serological sampling towards *Salmonella* infection of poultry instead of blood serum. The level of specific antibodies in these three types of samples obtained from birds at the identical time is almost identical.

CONCLUSIONS

The own ELISA may be used for testing both muscle juice and blood sera or egg yolk.

The own ELISA test is a very sensitive and rapid method for serological control of poultry flocks. Moreover, the results are repeatable and the test enables to examine at a short time a great number of samples.

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STRESZCZENIE

Coraz powszechniej jest wykorzystywany odczyn pośredni ELISA do wykrywania u drobiu zakażeń wywołanych przez *Salmonella enteritidis*. Jako materiał badany oprócz surowicy krwi jest coraz częściej wykorzystywany ekstrakt mięśni (sok mięśniowy) oraz wyciąg z żółtka jaja. W badaniach porównano przydatność dwóch testów serologicznych: ELISA i aglutynacji płytowej z użyciem Pullognostu, Typhignostu M. i Enterognostu własnej produkcji jako antygenów do wykrywania przeciwciała specyficznych dla *S. enteritidis* w surowicy krwi, żółtku jaja kurzego i soku mięśniowym pochodzących od kur wolnych od zakażenia i zakażonych tym drobnoustrojem. W teście ELISA zastosowano LPS *S. enteritidis* typ fagowy 1, koniugat przeciwciał przeciwko immunoglobulinom kurzym klasy IgG, substrat enzymatyczny ABTS. Część badanego materiału reagowała pozytywnie, zaś część negatywnie w teście ELISA. Występowała przy tym ścisła korelacja pomiędzy wynikami testu ELISA, dla wszystkich trzech rodzajów próbek użytych do badań serologicznych. Test ELISA, w którym zastosowano własnej produkcji LPS jest bardzo przydatny do wykrywania obecności przeciwciał dla *S. enteritidis* w surowicy, żółtku jaja oraz soku mięśniowym.