Screening of *Salmonella* Enteritidis Specific O and H Antiserum Potency against Isolated Field Strains of *Salmonella* from Aegean Region

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**ABSTRACT**

**Background:** The increasing prevalence of foodborne human salmonellosis caused by *Salmonella* Enteritidis is a major foodborne illness throughout the world. Prevention from *Salmonella* infection is more important in an effective ongoing screening program. The slide agglutination test could be more widely used in developing countries to obtain local and regional data due to its rapid, valid, and relatively cheap and ease application. The main purpose of this study is to characterize *Salmonella* Enteritidis O and H specific polyclonal antibodies for the detection of *Salmonella* Enteritidis in field samples by comparing with different species of *Salmonella* field samples isolated from Aegean Region.

**Materials, Methods & Results:** The polyclonal antibodies used in this study were produced in rabbits as a diagnostic tool in our previous study. A total of 70 *Salmonella* field samples isolates collected between the years 2009 and 2012 from the layers and broilers poultry farms in Aegean Region were examined against *Salmonella* Enteritidis O and H specific polyclonal antibodies by slide agglutination test. Isolated *Salmonella* suspect colonies were subjected to biochemical identification by VITEK 2 compact microbial identification system and further serotyping to identify the serovar was done in the *Salmonella* National Reference Laboratory of Turkey. Seventy isolates of *Salmonella*, representing 8 different serotypes were obtained from field samples. In serotyping, the strains together with *Salmonella* Schwarzengrund, *Salmonella* Enteritidis, *Salmonella* Gallinarum, *Salmonella* Mbandaka, *Salmonella* Infantis, *Salmonella* Kentucky, *Salmonella* Virchow and *Salmonella* Corvallis were determined in accordance with the Kauffman White Le Minor scheme. The results of *Salmonella* Enteritidis O and H hyperimmune rabbit sera reactivity with a variety of field strains of *Salmonella* by slide agglutination test demonstrated that the polyclonal antibodies showed stronger binding capacity to *Salmonella* Enteritidis without showing cross reactivity between O and H antigen in different field strains. The avidity test result verified a range between 15 and 31 s for O antisera, whereas H antisera showed a range from 15 to 26 s.

**Discussion:** The prevalence of *Salmonella* may possibly vary from country to country attributable to the geographical properties, sampling strategies and isolation methods performed in the countries. In this study, *Salmonella* strains were isolated from the Aegean region and polyclonal antibodies were examined for the detection of possible antigenic differences among isolated field strains of *Salmonella* by using slide agglutination test. Comparing our result with previous studies in literature, the isolated field strains of *Salmonella* from layers and broilers poultry farms were parallel to those obtained by other researchers. Additionally, according to the study results for the distribution of *Salmonella* serotype, *Salmonella* Enteritidis was found the predominant serovar in the Aegean region of Turkey which is also the most frequently isolated serotype worldwide. The results confirm that previously produced polyclonal antibodies were able to identify all *Salmonella* isolated serotypes without generate any false negative positive results interpreted in accordance with National Reference Laboratory findings. Consequently, the findings show that the prepared polyclonal antibodies can be used in detecting *Salmonella* Enteritidis from field and clinical samples. From this point of view, importance of the study was clearly put forward that the development of novel diagnostic reagent against *Salmonella* Enteritidis is very critical in our country since the diagnostic reagents have been exported from various countries with higher cost.

**Keywords:** Polyclonal antibodies, poultry, *Salmonella* Enteritidis, diagnosis
INTRODUCTION

Salmonella is one of the most harmful bacterial pathogens in foods representing a serious risk to public health [19,27,29,34]. Salmonella Enteritidis, only rose to its predominant position in human and poultry in the mid 1980s in many parts of the world resulting in enormous cost each year [10,15]. Contaminated poultry meat and meat products are known to be a significant reservoir for Salmonella and the most important source of Salmonella Enteritidis infection in humans [26]. Prevention of Salmonella infection has great significance both for human health, poultry health and for the food industry, which can only be achieved by good monitoring and effective screening programs [23]. Serological assays in the screening of Salmonella Enteritidis have played a significant role in the development of rapid, specific and sensitive diagnostic tests. Serological monitoring systems include agglutination, latex test and enzyme-linked immunosorbent assays (ELISA) most commonly by using polyclonal antibodies (Pab) gained broad range of commercial application for high throughput screening of Salmonella [3,9,24,36,37].

In this study, as part of our continuing studies on the development of cost effective diagnostic reagent for the detection of Salmonella Enteritidis, we conducted a study on that Pabs have been produced in rabbits as a diagnostic tool in our previous study [25] and pre-characterized for their specificity, cross-reactivity, avidity and titers for the detection of Salmonella Enteritidis [25]. For this purpose, previously produced Pabs were further characterized with isolated different field strains of Salmonella from Aegean Region of Turkey.

MATERIALS AND METHODS

Samples

A total of 70 Salmonella isolates were collected during the period of 2009 and 2012 under Bornova Veterinary Control Institute (Izmir, Turkey) National Salmonella monitoring surveillance programs for poultry from the layers and broilers poultry farms in Aegean Region of Turkey. S. Gallinarum field isolates were identified previously before 2004

Isolation and identification of Salmonella

The International Organization for Standardization 6579 (ISO 6579) procedure was applied to isolate and identify Salmonella spp. with a determined detection limit of 10 cfu/mL [7]. Briefly, environmental samples (dust, swab sample from surface) and faecal samples (cloacal swab, drag swab) taken from the hatcheries and breeding flocks were tested. Pre-enrichment of the samples was performed in 10 mL buffered peptone water (BPW) and blended for 2 min. This homogenate was incubated for 18 h in 37°C. After this pre-enrichment step, 0.1 mL from this mixture was transferred into 10 mL of Rappaport Vassiliadis Soy Broth (RVS) and incubated in 41.5°C for 24 h. In the mean time, 1 mL from this pre-enrichment mixture was transferred into 10 mL of Muller Kauffmann Novobiocin Broth (MKTTnB) and incubated at 37°C for 24 h. Following primary enrichment, 20µl from the RVS and MKTTnB culture were streaked onto Xylose Lysine Tergitol 4 Agar (XLT4) and Brilliant Green Novobiocin Agar (BGN) and incubated aerobically at 37°C for 24 h and 48 h. Salmonella suspect colonies were subjected to biochemical identification by VITEK 2 Compact microbial identification system.

Serotyping

Salmonella isolates were serotyped in accordance with the White Kauffmann Le Minor scheme [13]. These isolates were serotyped for O and H antigens by using commercially available antisera based on slide agglutination tests. Salmonella isolates were sent for further serotyping to identify the serovar using specific antisera to Salmonella National Reference Laboratory (Central Veterinary Control and Research Institute, Etlik, Ankara,Turkey). Salmonella Enteritidis O and H specific hyperimmune sera have previously been prepared in rabbits and pre-characterized by Nalbantsoy et al. [25] to be used in Salmonella Enteritidis-specific isolate determination. Briefly, Salmonella Enteritidis O and H specific antisera were obtained from rabbits immunised subcutaneously using formalin treated whole bacterial cells as antigens. Salmonella Adeyo was used in order to eliminate cross reactivity as an antigen for the production of H specific polyclonal antibody. The immunisation of rabbits with prepared Salmonella Enteritidis and Salmonella Adeyo O and H antigens resulted in good agglutination and ELISA titres ranged from 273,300 ± 0.041 to 312,200 ± 0.028 at 492 nm for both O and H antigens, respectively. Stained O and H antigen preparations were achieved with a stronger binding degree to the hyperimmune rabbit sera and without being shown cross reactivity between O and H antigens.
Slide agglutination test

The slide agglutination test was performed with saline using standard methods at room temperature. The rabbit antisera against each of the different Salmonella suspensions and O and H stained antigen against specific antisera were tested for agglutinin formation. The agglutination efficiency was determined using the four-grade system (from + to ++++) as described previously [14,24].

Avidity test

This test was used to determine the time required for visible for agglutination to occur. The slide agglutination method was used as described earlier [17].

RESULTS

Pab against Salmonella are found to be enhanced to monoclonal antibodies in capturing and concentrating target molecules and are used in immunomagnetic or immunobead based captures. Sheep, goats and rabbits are the most common animals used for polyclonal antibody production, although chickens have also occasionally been used [2,3,16,17,25]. In this study, Salmonella Enteritidis O and H antigen specific antisera, which have previously been produced [25], were screened against Salmonella isolates, which were formerly collected from the layers and broileres poultry farms during the screening period of 2009 and 2012. Seventy isolates of Salmonella, representing 8 different serotypes were obtained from field samples. In serotyping, the strains including Salmonella Schwarzengrund, Salmonella Enteritidis, Salmonella Gallinarum, Salmonella Mbandaka, Salmonella Infantis, Salmonella Kentucky, Salmonella Virchow and Salmonella Corvallis were determined according to the Kauffman White Le Minor scheme (Figure 1). The produced sera reactivity with a variety of field strains of Salmonella was checked by slide agglutination and the results were summarized in Table 1. The avidity test result demonstrated a range between 15 and 31 s for O antisera, while H antisera showed a range from 15 and 26 s.

<table>
<thead>
<tr>
<th>Field strains</th>
<th>Number of screened strain (s)</th>
<th>O antiserum O:9 Negative</th>
<th>Positive</th>
<th>H antiserum 16:g,m Negative</th>
<th>Positive</th>
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<td>Salmonella Schwarzengrund</td>
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<tr>
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<td>-</td>
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<tr>
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<td></td>
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<tr>
<td>Salmonella Gallinarum</td>
<td>1</td>
<td>-</td>
<td>1/1****</td>
<td>1/1</td>
<td>-</td>
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<tr>
<td>(1,9,12:-:)</td>
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<td></td>
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<td></td>
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<tr>
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<td>6</td>
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<td>-</td>
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<tr>
<td>(6,7,14: z10: e,n,z15. [z37],[z45].</td>
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<tr>
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<td>Salmonella Corvallis</td>
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Table 1. Summary of results for reactivity of Salmonella O and H antigen specific hyperimmune rabbit sera with a number of Salmonella strains by slide agglutination assay.
DISCUSSION

Conventional methodology for identifying Salmonella is labor intensive and time consuming process and requires several enrichment and selective cultivation procedures with subsequent biochemical and serological assays [1,21,34,35]. Still, the most frequently used conventional method for identification of the presence of Salmonella is the slide agglutination test since the diagnostic reagents have been exported from different countries had higher cost. The test originally developed by Runnels, Coon, Farley, and Thorp [31] which is applied to serum samples of poultry. This test, which was then adapted by Schaffer [33] found wide use in developing countries [5,11] due to its low cost and easy conductance [2,12,20]. In the present study, we focused on further characterization of Pab that had been produced in our pilot work for larger applications in poultry, and food industry in collaboration with Bornova Veterinary Control Institute (Izmir, Turkey) scientists. From this point of view, preisolated Salmonella from different layers and broilers in the Aegean region of Turkey were screened against Salmonella Enteritidis O and H antisera by slide agglutination tests.

The prevalence of Salmonella in poultry may differ from country to country due to the geographical properties, sampling strategies and isolation methods performed in the various countries [4,6,7,12,30]. Comparing our result with previous studies in literature, our findings showed us that the isolated field strains of Salmonella from layers and broilers poultry farm were similar to those obtained by other researchers [4,6-8,12,22,30,32,]. Salmonella Enteritidis was found as the predominant serovar in the Aegean region of Turkey, as similar to the worldwide frequent isolation of this serotype reported by CDC [8]. This study examined the possible antigenic differences among field strains of Salmonella could be detected by using these Pabs. The results indicated that the slide agglutination test using these Pabs is accurate compared to Salmonella National Reference Laboratory serotyping results, using commercial antisera for isolated field strains. The results confirm that Pabs were able to identify all Salmonella serotypes without generate any false negative positive results interpreted in accordance with National Reference Laboratory findings. Negative results should be confirmed with other test system, such as ELISA with Vi antigen against Salmonella Enteritidis. The diagnostic Pab are developed for bacterial detection, and immunologists and microbiologists were commonly used for their ability to react with a wide variety of epitopes to characterize Salmonella antigen [3,21]. Most of the commercial assays use Pab which is the assay format can be in the form of agglutination, precipitation or ELISA [2]. However, one of the most commonly used conventional methods is the rapid slide agglutination test [3,5]. From this point of view, importance of our study was clearly put forward that the development of novel diagnostic reagent against Salmonella Enteritidis is very critical in our country.
since the diagnostic reagents have been exported from various countries with higher cost. Additionally, the avidity test results obtained in this study are comparable with our earlier study results [25] indicating the antisera showed good correlation among the field strains, as well.

In conclusion, in this report we evaluated and determined that the produced Pabs have a good potential to be used for the detection of *Salmonella* Enteritidis from samples against this serotype in poultry, food and human samples. In addition, the present work showed that the produced Pabs were useful for *Salmonella* Enteritidis screening from the field samples.

**REFERENCES**


