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Cross Reaction of Serum in *Salmonella enteritidis*-Vaccinated Chicken to Some *Salmonella enterica* Serotypes

(REAKSI SILANG SERUM AYAM YANG DIVAKSIN DENGAN SALMONELLA ENTERITIDIS TERHADAP BEBERAPA SEROTIPE SALMONELLA ENTERICA)

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ABSTRACT

Salmonella spp. has been recognized as the major cause of food-borne illness in humans worldwide causing remain relevant to public health. Poultry vaccination is one promising strategy to mitigate *Salmonella* infection in poultry and, in turn, in humans as well. The objective of this study was to assess the potential of cross-reaction of serum in *Salmonella enteritidis*-vaccinated chicken to some serotype of *Salmonella enterica.* Four female, Isa Brown layer chickens (20 weeks old), were vaccinated with *S. enteritidis* strain Sm24/Rif12/Ssq (intra vena) to induced the production of specific antibodies in serum. Cross-reaction of serum in *S. enteritidis*-vaccinated chicken were assess with agar gel immunodiffusion test (AGID) with *S. enteritidis*, *S. pullorum*, *S. typhimurium*, *S. typhi*, and *Escherichia coli* antigens. Serum could react with *S. enteritidis* and all types of *S enterica* used in this study (*S. pullorum*, *S. typhimurium*, *S. typhi*), but could not react with *E. coli.* The potential of cross-reaction of serum in *S. enteritidis*-vaccinated chicken to some serotypes of *S. enterica* may play a role in reducing the infection caused by that serotype.

Key words: vaccine; *Salmonella enteritidis*; *Salmonella enterica;* cross-reaction.

ABSTRAK

Salmonella spp. dikenal sebagai penyebab utama penyakit yang bersumber dari makanan pada manusia yang mengakibatkan dampak serius terhadap kesehatan masyarakat di seluruh dunia. Vaksinasi unggas merupakan salah satu strategi yang menjanjikan untuk mengurangi infeksi *Salmonella* pada unggas dan pada akhirnya pada manusia. Tujuan penelitian ini adalah untuk mengetahui potensi reaksi silang dari serum yang dihasilkan oleh ayam yang divaksinasi dengan *S. enteritidis* terhadap beberapa serotipe *S. enterica*. Sebanyak empat ekor ayam petelur galur Isa Brown (umur 20 minggu) divaksinasi dengan vaksin *S. enteritidis* strain Sm24/Rif12/Ssq (intra vena) untuk menginduksi pembentukan antibodi spesifik di dalam serum. Reaksi silang serum ayam yang divaksin dengan *S. enteritidis* diuji dengan menggunakan teknik *agar gel immunodiffusion test* (AGID) menggunakan antigen *S. enteritidis*, *S. pullorum*, *S. typhimurium*, *S. typhi* dan *Escherichia coli*. Serum dapat bereaksi dengan *S. enteritidis* dan semua serotipe *S. enterica* yang digunakan pada penelitian ini (*S. pullorum*, *S. typhimurium*, dan *S. typhi*), namun tidak dapat bereaksi dengan *E. coli.* Potensi reaksi silang dari serum ayam yang divaksin dengan *S. enteritidis* dengan beberapa serotipe *S. enterica* berperan dalam mengurangi infeksi oleh serotipe tersebut.

Kata-kata kunci: vaksin; *Salmonella enteritidis*; *Salmonella enterica;* reaksi silang

INTRODUCTION

Salmonella spp. has been recognized as the major cause of food-borne illness in humans worldwide causing remain relevant to public health (Yang *et al.,* 2015). Although many different foods have been implicated in salmonellosis outbreaks, most cases are usually caused by the consumption of poultry and eggs (Okamura *et al.,* 2012; Goh *et al.,* 2015). Intense international trade of animals and products of animal origin facilitates the spread of these bacteria (Liebana *et al.,* 2013), making salmonellosis an international public health concern that is responsible for serious economic losses to the poultry industry and governments worldwide (Plym and Wierup, 2006). The *Salmonella enteritidis* and *S. typhimurium* serotypes are the most frequent causes of food contamination leading to salmonellosis in humans (Gustavsson *et al.,* 2015). On the other hand, *S. pullorum* caused a significant problem in commerciel egg and poultry production (Foley *et al.,* 2011). *Salmonella* control within farms that breed and rear poultry is a significant public health issue (Dhanani *et al.,* 2015).

Poultry vaccination is one promising strategy to mitigate *Salmonella* infection in poultry and, in turn, humans as well (Okamura *et al.,* 2012). A variety of *Salmonella* strains are known to cause extraintestinal infections in poultry (Johnson *et al.,* 2010). *Salmonella* can infect humans and are the main causes of food contamination (Gallati *et al.,* 2013). Although these infections do not cause severe symptoms in poultry, the eggs and meat of infected animals can become a reservoir for infection of human consumers (Elsheimer-Matulova *et al.,* 2015). These a symptomatic bird carriers play a major role in *Salmonella* propagation and in food contamination. *Salmonella* infect chickens via the fecal-oral route, colonize the alimentary tract, invade internal organs such as the liver and spleen, and finally spread to the reproductive tract. The *S. enteritidis* bacteria can be transmitted to the eggs, which can be transmitted to humans through consumption of these contaminated eggs, which is a major public health issue (Lee, 2015).

The vaccines are targeted for the most often reported serovars of human infections namely *S. enteritidis* and *S. typhimurium*. Vaccination has a limited effect on improving animal health and welfare and is used primarily for public health reasons. Vaccines can decrease public health risk caused by *Salmonella* in poultry products by reducing the colonisation of reproductive tissues as well as reducing faecal shedding (Jawale and Lee, 2014).

Cross-protection can enhance the clearance of pathogens through the acquired immune response. Several live vaccine strains have conferred high levels of protection against infection with heterologous serovars. A vaccine constructed for a single serovar of *Salmonella* may induce immunity against other heterologous serovars of *Salmonella* (Nandre *et al.,* 2015). It has been demonstrated that *Salmonella* live vaccines can elicit cross immunity against members of the same Kauffmann–White scheme serogroup (Chacana and Terzolo, 2006). The objective of this study was to assess the potential of cross-reaction of serum in *S. enteritidis*-vaccinated chicken to some serotypes of *S. enterica.*

RESEARCH METHODS

Isolate Vaccine and Vaccination

The vaccine strain used in this study is commercial vaccine of *S. enteritidis* strain Sm24/Rif12/Ssq (AviPro®, PT. Lohmann Animal, Health Indonesia). Four female, Isa Brown layer chickens (20 weeks old), were used in this study. The chickens were reared in individual cage (40 x 40 x 80 cm) at 22°C with free access to water and feed.

The antibody production technique in chickens refer to Wibawan *et al.* (2010). Chickens were vaccinated in the first week with 1 mL live attenuated *S. enteritidis* vaccine (10^9 cfu/mL, intra vena). The vaccination was repeated in the second week and the third week with 1 mL antigen suspension (three times, intra vena) respectively. One week after the last vaccination, the serum was collected.

Preparation of Dissolved Antigen for Cross-Reaction Test

The bacteria used for the cross-reaction test are the antigen vaccine *S. enteritidis* strain Sm24/Rif12/Ssq, *S. enteritidis* ATCC 13076, *S. enteritidis* BCC B2691 (collection of The Indonesian *Research* Centre for *Veterinary* Science), *S. enteritidis*, *S. typhimurium*, *S. pullorum* (collection of laboratory of Microbiology, Faculty of Veterinary Medicine, Bogor Agricultural University), *S. typhi* ATCC 35250, and *E. coli* ATCC 25250.

The soluble antigen was produced by an extraction technique using HCl. Extraction using HCl used for extract surface antigens. *Enteribactericeae* have complex surface antigens. The main surface antigens are somatic antigens (O antigen), flagellar antigens (H antigen), and envelope or capsule antigens (Vi antigen). The antigenic structure is used to differentiate organism within a genus, species, or serotype. Technique HCl extraction based on that of Abrar *et al*. (2012) with minor modifications. All isolates were inoculated in 50 mL brain heart infussion broth and incubated at 37°C for 18-24 hours. Bacteria was harvested with centrifuge 10.000×g for 15 minutes. The supernatant was disposed, the pellet was added with 0.35 mL of 0.2 N HCl, one drop of phenol red, and one drop of 1 N NaOH. This suspension was heated on waterbath at 56°C for two hours. Suspension will centrifuge and the supernatant will use as a dissolved antigen.

Agar Gel Immunodiffusion Test

Cross-reaction tests done by using the agar gel immunodiffusion test (AGID). Soluble antigens of all selected bacterial isolates were used in this test. A total of 0.5 g of agarose and 0.05 g of sodium azide were dissolved into 25 mL of phosphate-buffered saline (PBS) and 25 aqua bidest. The mixture was heated and poured on a glass object up to 4 mL, then cooled, and hexagonal holes were made with a diameter of 4 mm, depth of 3-4 mm, and the distance between holes of 2 mm. A total of 25 µL of serum resulted from vaccination was put into in the hole located in the center, and the other holes were filled with soluble antigen of each bacteria in the same volume. Gel was incubated at 25°C in a moist state for 18-24 hours. Cross-reactions were observed for the visible precipitation lines between the serum hole and the isolate hole.

RESULTS AND DISCUSSION

Cross-reaction tests were performed on the serum produced by the chickens vaccinated with the commercial vaccine *S. enteritidis*. A total of nine bacterial antigens were used in the cross-reaction tests. These tests were carried out using the agar gel immunodiffusion (AGID) technique. Positive results were characterized by the formation of precipitation lines between serum well with antigen wells (Figure 1). The precipitation line is a visualized antigen-antibody complex, thus it can be seen by the eye. The presence of precipitation lines between serum well with antigen wells indicates that the serum contains immunoglobulins which are specific to the antigen tested. Table 1 presents the results of cross-reaction test of serum in chickens vaccinated with the commercial vaccine *S. enteritidis* strain Sm24/Rif12/SSQ to some bacteria.

Table 1. Results of cross-reaction test of serum in chickens vaccinated with the commercial vaccine *S. enteritidis* strain Sm24/Rif12/SSQ to some bacteria using agar gel immunodiffusion test

No	Antigens	Precipitation line
1	*S. enteritidis* vaccine strain Sm24/Rif12/Ssq	+
2	*S. enteritidis* ATCC 13076	+
3	*S. enteritidis* BCC B2691	+
4	*S. enteritidis*	+
5	*S. typhimurium*	+
6	*S. pullorum*	+
7	*S. typhi* ATCC 35250	+
8	*E. coli* ATCC 25250	-

Vaccination is likely to have a central role in the reduction of *Salmonella* in poultry, because research studies indicate that it may reduce both the horizontal and vertical transmission of *Salmonella.* Vaccination works by reducing the prevalence of *Salmonella* or by increasing the passive immunity and blocking the horizontal transmission of *Salmonella* in poultry (Dórea *et al.,* 2010). The isolates used as a master seed vaccine must have the ability to induce the formation of antibodies against the disease agent contained on field. This study uses some antigens *S. enteritidis* to test the ability of antibodies produced by vaccinated chickens to recognize such types of *S. enteritidis* isolates. *S. enteritidis* BCC and *S. enteritidis* collection of laboratory of Microbiology, Faculty of Veterinary Medicine, Bogor Agricultural University are the on-field isolates of *S. enteritidis* in Indonesia. The AGID test results showed positive results on all isolates of *S. enteritidis* used in this study. This indicates that the serum produced by chickens vaccinated with *S. enteritidis* strain Sm24/Rif12/Ssq contains antibodies capable of recognizing all *S. enteritidis* isolates used in the study. The serum contains the antibodies specific to the on-

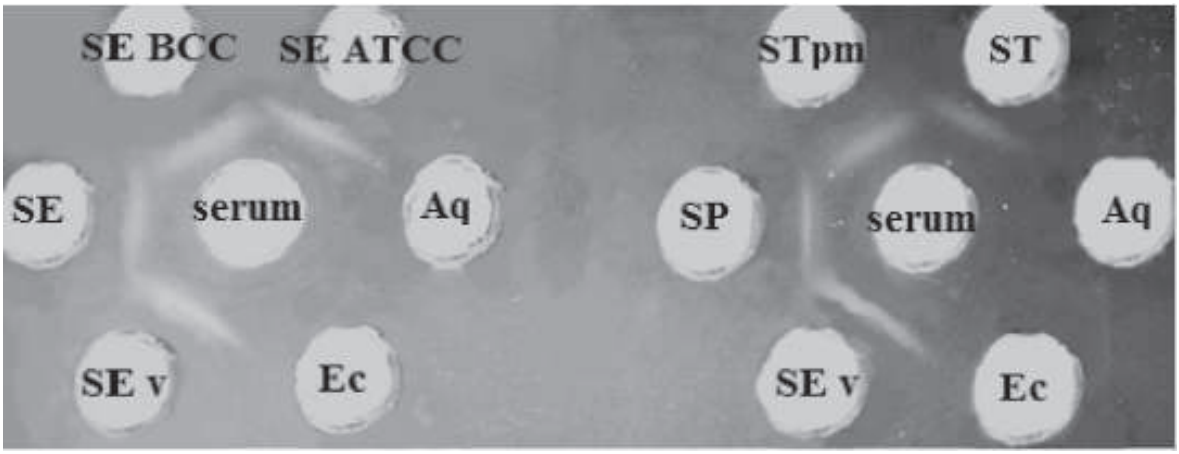

Figure 1. Agar gel immunodiffusion (AGID) test of serum in chickens vaccinated with the commercial vaccine *S. enteritidis* strain Sm24/Rif12/SSQ to some bacteria (SE v: *S. enteritidis* vaccine strain Sm24/Rif12/Ssq; SE: *S. Enteritidis*; SE BCC: *S. enteritidis* BCC B2691; SE ATCC: *S. enteritidis* ATCC 13076; SP: *S. Pullorum*; STpm: *S. Typhimurium*; ST: *S. typhi* ATCC 35250; Ec: *E. coli* ATCC 25250; Aq: Aquadest) (left side is cross-reaction test of serum with *S. enteritidis* vaccine strain Sm24/Rif12/Ssq, *S. enteritidis*, *S. enteritidis* BCC B2691, *S. enteritidis* ATCC 13076, and Aquadest; right side is cross-reaction test of serum with *S. pullorum*, *S. typhimurium*, *S. typhi* ATCC 35250, *E. coli* ATCC 25250, and Aquadest).

field *S. enteritidis* isolates of Indonesia. The presence of specific antibodies against the on-field isolates can hamper commercial poultry infections on field. This implies that the vaccine is expected for an effective use in poultry farms in Indonesia.

Live *Salmonella* vaccines have been reported to induce cross-immunity against related serovars (Matulova *et al.,* 2013; Nandre *et al.,* 2015; Lee, 2015). Cross-reactions test of the serum in *S. enteritidis* strain Sm24/Rif12/Ssq-vaccinated chickens showed positive results when they were reacted with the antigens of *S. typhimurium*. This is consistent with the study by Nandre *et al.* (2015). Nandre *et al.* (2015) reported that vaccination with an *S. enteritidis* strain induced significantly higher levels of systemic IgG and mucosal sIgA antibodies specific for *S. typhimurium*.

Based on Kauffmann-White scheme, *S. enteritidis* and *S. typhimurium* have similar somatic antigens of O1 and O12 (Grimont and Weill, 2007). The formation of precipitation lines between the serum in *S. enteritidis* strain Sm24/Rif12/Ssq-vaccinated chickens with the *S. typhimurium* antigen showed compatibility between immunoglobulin contained in serum and its specific antigen. This is because the serum recognizes the specific somatic antigens of O1 and O12 on *S. typhimurium* antigen that has similarity to the somatic antigens of *S. enteritidis*.

It has been demonstrated that *Salmonella* live vaccines can elicit cross immunity against members of the same Kauffmann-White scheme serogroup (Chacana and Terzolo, 2006). The *S. enteritidis*, *S. pullorum*, and *S. typhi* belong to the serogroup of D1 in Kauffmann scheme (Grimont and Weill, 2007). The D1 serogroup is composed of those serotypes having the somatic antigen O9. This study found the presence of precipitation lines between the serum in *S. enteritidis* strain Sm24/Rif12/Ssq-vaccinated chickens with the antigens of *S. pullorum* and *S. typhi*. This is because the serum contains specific immunoglobulins that recognize the antigen section similar to that of the vaccine isolates. This antigen is likely to be the somatic antigen O9. The *S. pullorum* has similarities in all somatic antigen O with *S. enteritidis*, namely, somatic antigens O1, O9, and O12. This indicates that the formation of precipitation line between the serum and the *S. pullorum* antigen is influenced by not only the similarity of somatic antigen O9 but also the similarity of somatic antigen O1 and O12. The same thing happened between the serum with the *S. typhi* antigen. The *S. typhi* and *S. enteritidis* had similarity not only in the somatic antigen O9 but also in the somatic antigen O12. The

presence of the somatic antigens of O9 and O12 on *S. typhi* has caused serum of *S. enteritidis*-vaccinated chicken react positively to *S. typhi*.

The test results showed that chickens vaccination using a live vaccine *S. enteritidis* could induce humoral immunity that recognize not only *S. enteritidis*, but also some other serotypes of *S. enterica*, namely *S. typhimurium*, *S. pullorum* and *S. typhi*. Systemic antibodies are essential to kill *Salmonella* bacteria, which escape from infected cells to reach distant tissue sites and form new foci of infection (Dougan *et al.,* 2011). The ability of systemic antibodies to recognize some strains of *Salmonella* is expected to inhibit the infection by these strains. This can increase the efficient use of the vaccine. The potential cross-reaction of the serum from chickens vaccinated with *S. enteritidis* to some serotypes of *S. enterica* is expected to play a role in reducing the infection by the serotypes.

The serum of *S. enteritidis*-vaccinated chickens could not react with the *E. coli* antigen. Cross-reaction testing for *E. coli* was done to see the presence of immunoglobulin specific to the bacteria included in the *Enterobactericeae* family. The negative result of the cross reaction test for *E. coli* indicates that the serum does not contain the immunoglobulin specific to this bacteria. *Enterobactericeae* have complex surface antigens. The main surface antigens are somatic antigens (O antigen), flagellar antigens (H antigen), and envelope or capsule antigens (Vi antigen). The antigenic structure is used to differentiate organism within a genus, species, or serotype. The difference of surface antigens of *Salmonella* and *E. coli* resulted in specific antibodies against *Salmonella* do not recognize surface antigens of *E. coli*. This implies that the use of *S. enteritidis* vaccines could not induce the formation of antibodies specific to other *Enterobactericeae*, particularly *E. coli*, so it could not inhibit the infection by this bacteria.

CONCLUSION

The serum in *S. enteritidis* strain Sm24/Rif12/Ssq-vaccinated chickens can react with some on-field *S. enteritidis* isolates of Indonesia. It can recognize some serotypes of *S. enterica* such as *S. pullorum*, *S. typhimurium*, and *S. typhi*, but cannot react with other *Enterobactericeae* bacteria, especially *E. coli*. *S. enteritidis* vaccine is potential to be used against several serotypes of *S. enterica.*

SUGGESTION

The serum in *S. enteritidis* strain Sm24/Rif12/Ssq-vaccinated chickens can react with some serotypes of *S. enterica* such as *S. enteritidis, S. pullorum*, *S. typhimurium*, and *S. typhi*. This vaccine can be used to against that bacteria in poultry farm.

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