ADVERSE EFFECTS OF SALMONELLA ENTERICA SEROVAR ENTERITIDIS VACCINE IN CHICKENS

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Abstract

This study was designed to find out whether lesions developing after the vaccination against salmonellosis were the result of an adjuvant action. Four-week-old 41 broiler breeders were assigned randomly to receive a Salmonella Enteritidis vaccine, containing lipid (n=29) or aluminium hydroxide (n=12) adjuvant, injected subcutaneously in the neck region. The birds were sacrificed by cervical dislocation 8 weeks after vaccination for morphological and histopathological evaluation. Diffuse nodular structures of varying sizes with whitish and yellowish pus were observed in the cervical and temporal region of 19 and 7 chickens injected with lipid and aluminium hydroxide adjuvant, respectively. Microscopic evaluation of these nodules revealed that they were granulomatosus focuses with coagulation necrosis in the centre surrounded by lime accumulation, necrotic cell residues, and pycnotic heterophilic leukocytes. Moreover, epithelioid histiocytes, lymphocytes, macrophages, and foreign body giant cells were also present peripherally. These cells were encapsulated by fibroblast, fibrocyte, and collagen bundles. The results of this experimental study ascertained the legitimacy of occurrence of post-vaccinal lesions. However, histopathology of the lesions did not differ by the type of the adjuvant.

Key words: chicken, Salmonella, vaccine, adjuvant, pathology.

Salmonella organisms are Gram-negative bacilli, members of the Enterobacteriaceae family (9, 16). The Enterobacteriaceae are distributed worldwide and found in the soil, water, fruits, vegetables, grains, flowering plants, and trees, and in the animals from insects to man (16). Some Salmonella organisms cause specific diseases in mammals and birds. Salmonella infections in avian are divided into three groups. The first group includes S. gallinarum and S. pullorum. S. gallinarum causes an acute or chronic septicaemic disease, especially in mature birds known as fowl typhoid. S. pullorum affects chicks and young fowl and causes pullorum disease. Both Salmonella species have special host selectivity to birds. The second group covers a number of Salmonella serovars causing non-systemic paratyphoid infection in poultry (7, 9, 14). Predominant serovars include S. Enteritidis and S. Typhimurium and in a lesser extent S. Heidelberg, S. Hadar, S. Montevideo, S. Kentucky, S. Agona, S. Newport, S. Saintpaul, S. Senftenberg, and S. Barely. The third group includes only S. enterica subsp. arizonae that causes avian arizonosis, associated with substantial losses among turkeys (7, 27). For its diagnosis, biochemical evaluation of the agent is necessary, because the clinical signs cannot be discerned from those caused by other group of bacteria (7, 9, 14). Among these three groups, S. Enteritidis is the most widespread organism causing infections in birds (24).

Avian species are usually asymptomatic carriers of paratyphoid Salmonella bacteria. The organisms colonise the intestinal tract and sometimes the reproductive tract. Vertical transmission of the bacteria to carcasses and eggs in infected birds increases the risk of food poisoning in humans (7, 9, 14). Thus, salmonellosis is a big threat to poultry production and a significant zoonosis. Despite serious surveillance and control measures, it has not been successfully eradicated even in developed countries (1).

Vaccination against Salmonella is an efficient prophylactic method allowing avoiding the infections (9). In technical standpoint, one of the problems with salmonella vaccines is that lesions may develop at the injection sites. The adverse side effects may be related to action of the adjuvant (3, 8, 13, 15, 17, 22). The objective of this experimental study was to evaluate the pathomorphological lesions at the site of the injection of the vaccines against S. Enteritidis, containing two different adjuvants.
Material and Methods

Forty-one broiler breeders, at the age of 4 weeks, were obtained from the commercial breeder company. The chickens were randomly assigned to vaccination against *S. Enteritidis* differing in adjuvant. The vaccine (0.5 cc) containing lipid or aluminium hydroxide adjuvant was applied subcutaneously to the dorsal neck region of 29 and 12 chickens, respectively. The vaccination was repeated 4 weeks later as prescribed by the provider companies.

Eight weeks after the first vaccination, chickens were euthanised by cervical dislocation for necropsy. The tissue samples from the site of vaccination were fixed in a-10% formalin, processed routinely, and embedded in paraffin. Then, 5-6 micron sections were prepared and stained with haematoxylin-eosin (HE), Ziehl-Neelsen method, and Brown and Brenn Gram stains for light microscopy (18).

Results

Macroscopical findings. In chickens vaccinated with lipid adjuvant, diffuse nodular structures located subcutaneously from 2/3 of the cervical region to temporal region of the skull were present in 19 of 29 chickens. Of these, the smallest and largest ones were in the dimensions of 15 x 5 x 5 and 40 x 19 x 12 mm, respectively. Moreover, there were yellowish coloured abscesses in 2 of 29 chickens in the sizes of 31 x 20 x 19 mm to 30 x 27 x 15 mm, with an apparent leakage of pus (Fig. 1). There were also nodular structures in dimensions ranging from 5 x 5 x 1 to 35 x 19 x 10 mm, located on the similar areas in 7 of 12 chickens administered the vaccine with aluminium hydroxide adjuvant. Sectional surface of these nodular structures were whitish and yellowish coloured. Leakage of milky fluid from the sections was also present, especially in response to compression.

Microscopical findings. Histopathological findings of lesions from both groups of the chickens were similar. These lesions were predominantly granulomatous focuses (Figs 2a-c). In the centres of these focuses, there was coagulation necrosis surrounded by, in order of lime accumulation, necrotic cell remnants, and pyecnotic heterophil leukocytes. Epithelioid histiocytes, lymphocytes, macrophages, and foreign body giant cells were also present peripherally (Figs 3a-b) in 11 and 5 chickens administered the vaccine with lipid and aluminium hydroxide adjuvant, respectively. These cells were encapsulated with fibroblast, fibrocyte, and collagen bundles varying in the thickness. Moreover, it was also observed that inside and around the granulomas, there were many macrophages containing very large lipid vacuoles.

Discussion

Avian salmonellosis is still a serious problem at a farm level and it threatens humans (2, 9). Failure of its treatment and resistance against antibiotics (1, 9), emphasises the importance of preventive measures (9). Vaccination would be an important tool among the preventive measures. However, sporadic complaints resulting from physical and chemical effects of adjuvant in vaccines are valid. In order to enhance the immune response, various adjuvants have been used in different vaccines. An ideal adjuvant should be harmless for the treated animals, have a low level of toxicity, prolong the immunisation with minimal injection, be easily injectable, be applicable to wide array of antigens, be effective in various species, and stimulate a high and continuous antibody production (17). An adjuvant providing all these benefits is unfortunately non-existent. Although at least 100 kinds of adjuvants have been tried in vaccine production, no practical achievement was obtained mainly due to limitation and difficulties of their usage and economic infeasibility. The most frequently used adjuvants are saponins, mineral salts, lipid emulsion, microbiological products, and synthetic products. The properties of these adjuvants limit their usage in practice. For example, saponins usually have a haemolytic effect, which limits their intraperitoneal and intravenous administrations. Moreover, their subcutaneous or intramuscular administration may result in the development of local reactions (17).

Adjuvants that contain lipid emulsions are Freund’s complete or incomplete adjuvant. Lipopeptide adjuvant was presented as an alternative to Freund’s adjuvant, with advantageous of being non-toxic, non-pyrogenic, and having no cellular damaging effects (5). Recent studies have shown that Freund’s adjuvant causes abscesses, fibrosis, milary granulomas, and inflammations at the injection site (8, 17). Histological examination of tissues around injection sites revealed the existence of granulomatous inflammations with focal necrosis, consisting of pseudo-eosinophilic granulocytes, lymphocytes, epithelioid cells, foreign body giant cells, plasma cells, and lipid droplets (8).

In a study comparing new 4 types of adjuvants (H1, H2, H3, H4) with Freund’s adjuvant, it was shown that there was no lesion in pectoral muscles in chickens injected with H1 adjuvant, but varying size of yellowish-white focal areas in those injected with H2 and H3 adjuvants (22). In calves, injected with a complete Freund adjuvant, diffuse granulomas and cutaneous erythemas were observed and microscopically significant tissue necrosis was found (26). Additionally, after the application of *S. Typhimurium* vaccine with an incomplete adjuvant, slightly granulomatous reactions developed (11). In disagreement with the present study, the absence of foreign body cells in chicken vaccinated with lipopolypeptide adjuvant was reported (8).

Adjuvants of mineral salts, aluminium hydroxide, or aluminium phosphohydrate, are in gel form. Aluminium adjuvants are injected via subcutaneous or intramuscular routes in order to stimulate a primary immunological response (12, 29).
**Fig. 1.** Abscesses with pus at the temporal region (arrows).

**Fig. 2a-c.** Granulomatous changes (arrows) and lipid vacuoles (LV), HE, x100.

**Fig. 3a-b.** Foreign body giant cells (arrows), HE, 400x.
Aluminium hydroxide may lead to macrophagic myofascitis through hyperactivation of macrophages via triggering T cells (4) and haemolysis via increasing permeability and releasing lactate dehydrogenase. Granulomatous inflammatory reactions accompanied by macrophages with foamy cytoplasm, lymphocytes, and giant cells corroborate damaging effect of aluminium hydroxide (10). That could be related to its insolubility (6). Nevertheless, it appears that main benefit from aluminium hydroxide is to amplify immunological response through its regulatory effects on adsorbing basic proteins, presenting antigen, and releasing mediators (12, 19, 20, 21, 23, 25). Higher antibody production in response to aluminium hydroxide could also be linked to its low cytotoxicity (24). Acute inflammatory changes, macrophage accumulation, and formation of cysts and fibrosis were less when oily adjuvant (ISA-70) was compared with aluminium hydroxide and Freund’s incomplete and complete adjuvants (29). The development of granulomas was noted 48 h after the vaccination with zinc chloride adjuvant (3). Regardless of the nature of vaccine, our macroscopic and microscopic findings were mostly in agreement with current literature.

In conclusion, this experimental study revealed that complaints upon vaccination against Salmonella (and may be others) are legitimate. However, the necessity of lesions at the injection site may suggest that tissue responsiveness to different chemical natures of adjuvant was lacking. The further studies should be designed to distinguish the physical effect of adjuvant injection and responsiveness to the adjuvant depending on its nature at cellular level.

References


7. Classification of Salmonella bacteria into serotypes according to the Kauffman-White classification scheme: Online: http://www.safe-poultry.com/AboutSalmonella.asp.


