Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

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To cite this article: Evelyn Campagnari, Giulia Rossi, Carlo Franciosi, Luigi Sperati Ruffoni, Davide Giovanardi, Antonia Ricci, Ezio Bianchi & Francesco Prandini (2007) *In vitro* evaluation of live attenuated vaccines against *Salmonella enteritidis*: humoral immune response, Italian Journal of Animal Science, 6:3, 297-300, DOI: 10.4081/ijas.2007.297

To link to this article: https://doi.org/10.4081/ijas.2007.297

In vitro evaluation of live attenuated vaccines against *Salmonella enteritidis*: humoral immune response

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ABSTRACT

Salmonella enteritidis (SE) vaccination is one of the major tool to reduce the infection in commercial poultry. In this work, techniques, evaluating the presence of Ig-G and Ig-A in serum, tears and intestinal secretions, were studied to improve the knowledge of the humoral immune response following SE live attenuated vaccination. The Ig-G and Ig-A ELISA are important and easy tests to plan and optimize SE vaccination programs.

Key words: Salmonella enteritidis, Live vaccine, Humoral response.

RIASSUNTO

VALUTAZIONE *IN VITRO* DI UN VACCINO VIVO ATTENUATO CONTRO SALMONELLA ENTERITIDIS: RISPOSTA IMMUNITARIA UMORALE

In questo studio sono state messe a punto alcune tecniche di valutazione della risposta immunitaria anticorpale per poter ottimizzare l'utilizzo dei vaccini vivi per Salmonella enteritidis (SE)*. In particolare, è stata effettuata la ricerca di Ig-G nel siero e Ig-A nel siero, lacrime e secrezioni intestinali. La metodica ELISA per la ricerca di Ig-G e Ig-A è risultata essere una tecnica facilmente applicabile e in grado di ottimizzare l'efficacia dei programmi di vaccinazione.*

Parole chiave: *Salmonella enteritidis, Vaccino vivo, Risposta umorale.*

Introduction

Salmonellosis is one of major food-borne disease worldwide. Much effort has been made to develop effective control strategies against SE infection in laying hens, including immunization with vaccines. Several studies have suggested that live attenuated vaccines are more effective than killed vaccines in preventing SE infection (Okamura *et al.*, 2004). Attenuated vaccines con-

tain modified live bacteria that are no longer capable of causing clinical disease but still capable to multiply and immunize the animal (Dee Grifin *et al.*, 2002). Immunization of animals with live attenuated *Salmonella* vaccine is usually associated with stimulation of mucosal, systemic and cell-mediated immune response. Vaccination by killed vaccines or bacterins does not lead to the induction of either mucosal or cellular immune response, and humoral immunity may be relatively short lived (Curtiss *et al.*, 1993).

The aim of this study was to evaluate the efficacy of *in vitro* test to optimize the use of a live commercial SE vaccine checking the humoral immune response.

Material and methods

Animals and immunization

Twenty 1 day-old commercial laying hens were housed and eyedrop vaccinated at 14 and 28 days following the label instructions (Gallivac SE, Merial, France). Ten birds were reared separately as negative controls.

Every week tears, sera and intestinal secretions were collected.

ELISA Test

Salmonella enteritidis antigen was purified as described by Beal *et al.* (2004).

ELISA SE Ig-G test was performed as described by Bertholot-Herault *et al.* (2003), with peroxidase-labeled affinity purified antibody to chicken Ig-G (KPL). Sera were diluted 1/500.

ELISA SE Ig-A test was performed as described by Bertholot-Herault *et al.* (2003), with horseradish peroxidase-labeled goat anti-chicken IgA (Bethyl laboratories). Sera were diluited 1/100, tears 1/50, and intestinal secretions were diluted with collection.

ELISA *Salmonella enteritidis* Test Kit (IDEXX) was compared with the above "home made" ELISA SE Ig-G; respectively the OD (Optical Density) and the titres had the same trend (results not shown).

Results and discussion

Humoral immunity contributes to eliminate *S. enteritidis* from the intestine (Berthelot-Herault *et al.*, 2003) and to lesser extend from liver and spleen (Desmidt *et al.*, 1998). Antibodies may not protect against the intracellular stage of infection, but may provide an efficient macrophage-mediated opsonization and a final destruction of bacteria (Lillehoj *et al.*, 2003). One week post vaccination (PV), we observed that the Ig-G titres were negative and reached the peak two weeks after the second vaccination (Figure 1).

Secreted Ig-A antibodies play a significant role by inhibiting the adherence of microorganism to mucosal cell to preventing the translocation from the gastrointestinal tract (Desmit *et al.*, 1998) to the GALT (Gut-Associated Lymphoid Tissue). Berthelot-Heraultl *et al.* (2003) suggest that IgA antibody response also contribute to the later elimination of the pathogen from the intestine.

In this study Ig-A antibodies were detected in tears, intestinal secretions and sera, showing a Ig-A peak during one week post booster vaccination. Tears had significantly higher ELISA OD values than intestinal secretions and sera. Intestinal secretions, obtained through mucosal washing, showed the lowest OD titres because they were more diluted than other samples (Figure 2).

Conclusions

Salmonella live vaccine programs are influenced by immunity status, somministration route and infection pressure; for these reasons, useful information about humoral immunity is needed to optimize the use of these vaccines in layer hens. Ig-G and Ig-A ELISA tests are easy techniques for this purpose. These antibodies are reliable to assay the animal resistance in the long period of time, because humoral immunity acts later during the infection while cell mediat-

Figure 1. Serum Ig-G titres in vaccinated birds (black columns) and not vaccinated (white columns) one week post vaccination (PV), two weeks PV and one week and two weeks post booster (PB) vaccination. Cut-Off 100 OD.

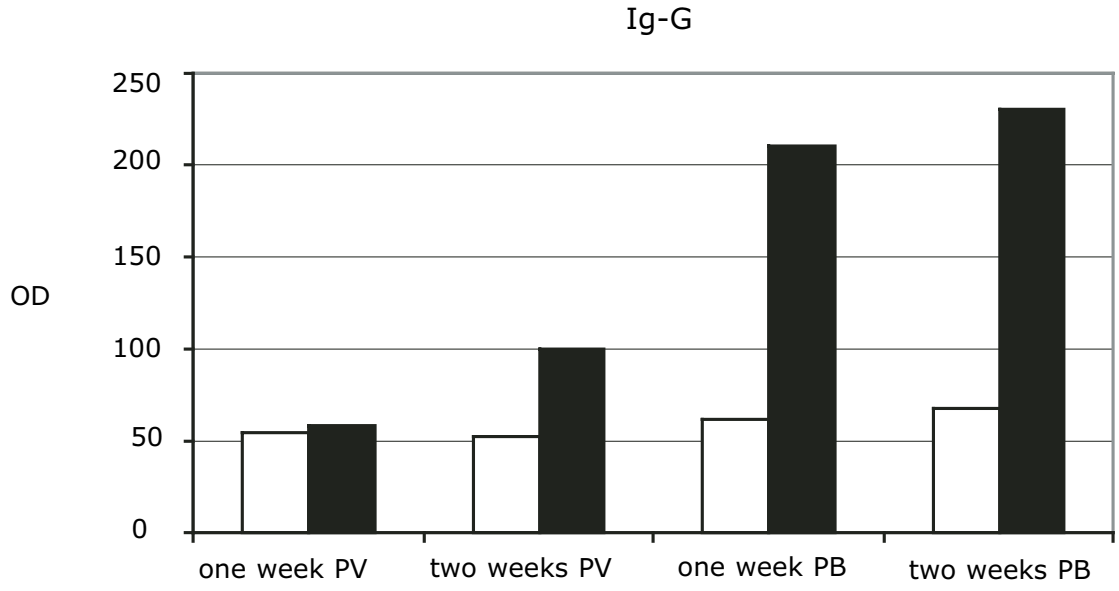
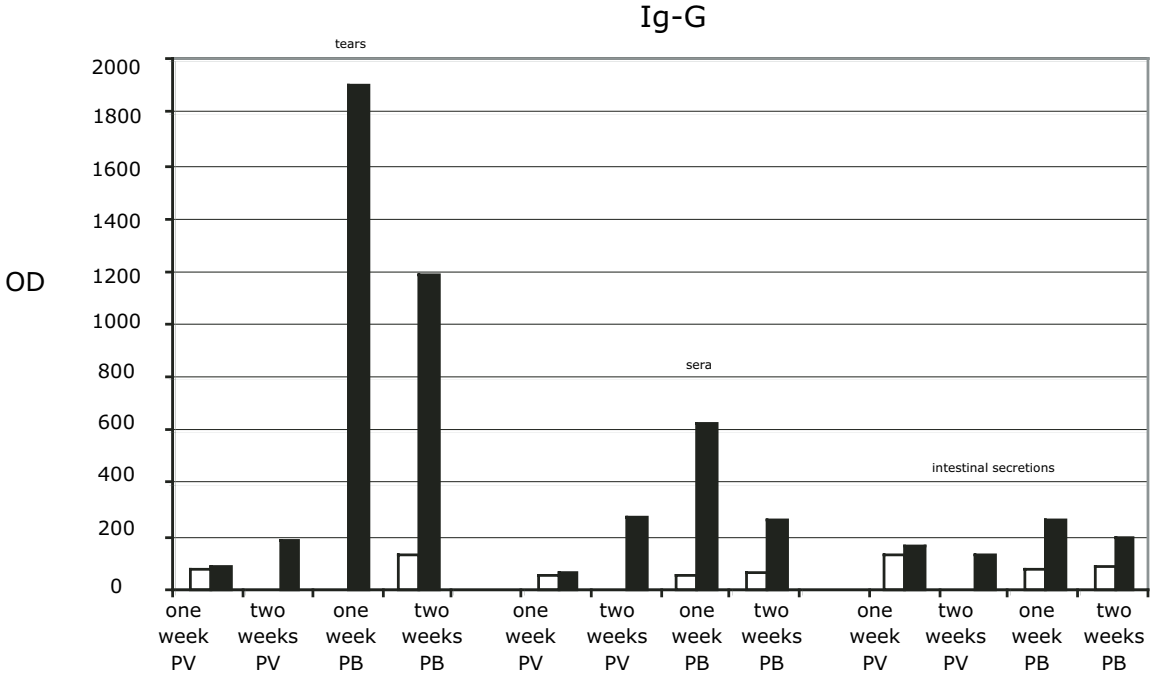


Figure 2. From left to right: tears, sera, intestinal secretions Ig-A titres in birds vaccinated (black columns) and not vaccinated (white columns) one week post vaccination (PV), two weeks PV and one week and two weeks post booster (PB) vaccination. Cut-Off 200 OD.



ed immunity has a important role the earlier phases of the infection. Moreover both interact each other, for example INF-γ increases the later humoral response in chickens (Poli *et al.*, 1999).

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