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Serological responses of chickens experimentally infected with *Salmonella enteritidis* PT4 by different routes

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SUMMARY

Commercially reared chickens were challenged with *Salmonella enteritidis* phage-type (PT) 4 by aerosol, or via the conjunctiva. Inhalation of $2{\cdot}9 \times 10^2$ or $4{\cdot}2 \times 10^3$ *S. enteritidis* resulted in the production of IgG antibodies to the lipopolysaccharide (LPS) of *S. enteritidis* PT4. When the aerosol inoculum was increased to $2{\cdot}4 \times 10^5$ bacteria per bird the antibody produced were predominantly of the IgM-class. Chickens challenged with 10^3 *S. enteritidis* PT4 via the conjunctiva mounted only a poor immune response. Increasing the challenge dose to 10^8 *S. enteritidis* resulted in the production of high-titre serum antibodies of both the IgG and IgM classes. Results from this study suggest that aerosols containing small numbers of *S. enteritidis* PT4 might be responsible for intraflock infection of poultry.

INTRODUCTION

During 1990, the Public Health Laboratory Service Division of Enteric Pathogens identified 18840 strains of *Salmonella enteritidis* phage-type (PT) 4 isolated from human infections in England and Wales. Chickens are a major reservoir of this organism and eggs and poultry products represent important vehicles of infection [1, 2]. The association between poultry and human disease has focused attention on the relationship between *S. enteritidis* PT4 and the chicken host. Bacteriological examination of chickens naturally infected with *S. enteritidis* PT4 has shown that infection involves the colonization of many of the body tissues. Infection of the oviducts can result in the production of eggs containing *S. enteritidis* PT4, and eggs may also become coated with excreta containing *S. enteritidis* PT4 [3–5]. Chickens infected with *S. enteritidis* have also been shown to produce high levels of antibodies of the IgG class to the O = 12 antigen on the lipopolysaccharide (LPS) of this bacterium, and high-titred sera gave a strong agglutination with a commercial *S. pullorum* antigen [3]. The development of serological tests has provided a useful means of studying the immune response of chickens experimentally and naturally infected with *S. enteritidis* PT4 [3, 4].

Challenging hens with *S. enteritidis* PT4 using various regimes has shown that factors such as age of bird, and inoculum size have a significant influence on the pathogenesis of disease and on the type of immune response of infected birds [6. 7]. The routes of infection and the infective doses of chickens naturally infected with *S. enteritidis* PT4 have not been fully elucidated. The studies of Gast and Beard [8] demonstrated the ease with which *S. enteritidis* can spread from infected to uninfected hens even without bird to bird contact. These observations might suggest that the birds may have been infected by airborne bacteria with possible routes of infection being either the respiratory tract or the conjunctiva, and that the infective dose was probably very small.

To investigate the role of airborne bacteria in infections of chickens with *S. enteritidis* PT4, commercial laying hens were exposed to the organism in the form of an aerosol [9]. The ability of this organism to infect birds *via* the conjunctiva was also examined.

MATERIALS AND METHODS

Bacteria

A strain of *S. enteritidis* PT4, isolated from the contents of an egg laid by an infected hen, was used [8]. For experimental challenge, bacteria were grown on blood agar prior to suspension in 0·1% proteose peptone water. Bacteria were isolated from infected tissues and faeces by selenite enrichment medium.

Birds

A total of 49 commercial hybrid laying hens 22–24 weeks old each weighing 1·2–1·5 kg were used. Birds were housed singly in wire-floored cages, fed an irradiated pelleted diet and allowed water *ad libitum*.

Aerosol and conjunctival infection

Birds were infected by exposing them individually to a small particle size aerosol of *S. enteritidis* PT4 generated by a collision spray in a mobile Henderson apparatus [9]. Birds received $2{\cdot}9 \times 10^2$, $4{\cdot}2 \times 10^3$ or $2{\cdot}4 \times 10^5$ viable bacteria. Conjunctival infection was carried out by instilling 0·1 ml of suspension of *S. enteritidis* in peptone water into the conjunctival sac of the right eye of 13 birds. Four birds received 10^3 organisms and 8 were given 10^8 bacteria by this method [10].

Lipopolysaccharide and ELISA

Lipopolysaccharide (LPS) was prepared from *S. enteritidis* PT4 using hot-phenol as described previously [3]. ELISA plates were coated with 0·1 μg LPS preparation in coating buffer and reacted with 100 μl chicken serum diluted ($\times 500$) in phosphate-buffered saline (PBS). Antibodies of the IgG class were detected using an alkaline phosphatase-conjugated goat anti-chicken IgG antibody (Southern Biotechnology). Antibodies of the IgM class were detected by reacting plates with a goat anti-chicken IgM antiserum (Nordic Immunology Ltd), followed by an alkaline phosphatase conjugated rabbit anti-goat Ig antibody (Sigma Chemical Co).

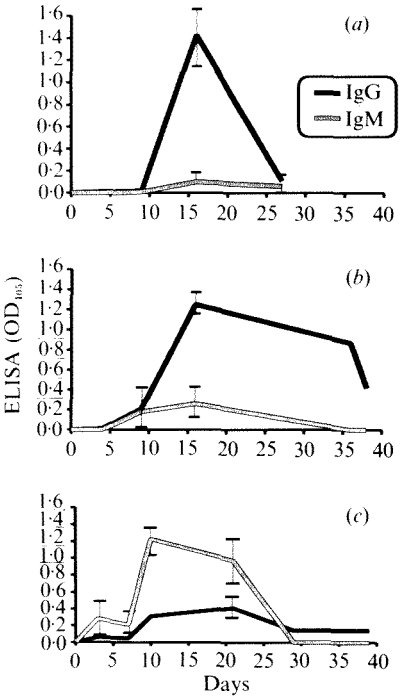

Fig. 1. The kinetics of serum antibody production to *S. enteritidis* LPS by chickens infected by aerosol. Birds challenged with 10^2 bacteria (*a*) produced predominantly an IgG antibody response. Birds given 10^3 *S. enteritidis* PT4 produced a similar antibody response but of longer duration (*b*). Chickens given 10^5 bacteria produced mainly IgM antibodies with a weaker IgG antibody response (*c*).

Salmonella whole-cell agglutinations

A *Salmonella pullorum* antigen obtained from Ministry of Agriculture Fisheries and Food, Weybridge, England, was used for serum agglutination reactions [3].

RESULTS

Aerosol challenge

Nine chickens were challenged with $2{\cdot}9 \times 10^2$ viable *S. enteritidis* PT4 by aerosol inhalation. Pre-infection sera were tested along-side sera from infected birds and values obtained with pre-infection sera were subtracted from those obtained with post-infection sera. Pre-infection sera were found to contain very low levels of IgG and IgM antibodies. Birds challenged with $2{\cdot}9 \times 10^2$ bacteria produced high levels of IgG antibodies, which peaked at 16 days post-infection (p.i.) with a mean ELISA value of 1·4 (OD_{405}), and dropped to levels of $< 0{\cdot}2$ by day 26 (Fig. 1*a*). Only very low levels ($< 0{\cdot}2$) of IgM antibodies were detected. Sera from one bird gave a strong agglutination reaction. Chickens given the higher inoculum of $4{\cdot}2 \times 10^3$ *S. enteritidis* also produced maximum levels of IgG antibodies by day 16

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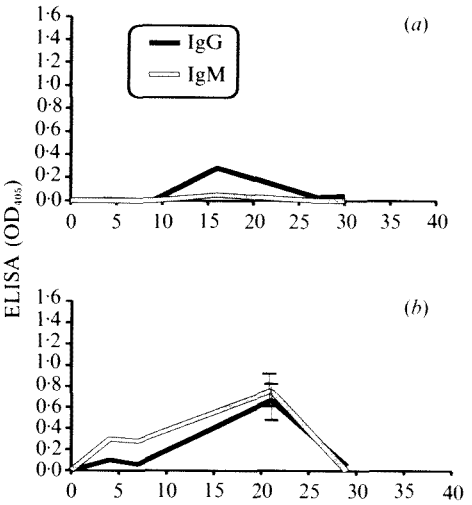

Fig. 2. The kinetics of serum antibody production to *S. enteritidis* LPS by chickens infected via the conjunctiva. Birds challenged with 10^3 bacteria (*a*) produced a very weak IgG antibody response, whereas chickens given 10^8 *S. enteritidis* PT4 (*b*) produced antibodies of both IgG and IgM classes.

with an OD_{405} of approximately 1·3; however, the IgG antibody titres remained high until almost 40 days p.i. Although IgM antibodies were detected, titres did not exceed an OD of $> 0{\cdot}3$ (Fig. 1*b*). Two birds had sera giving a strong agglutination reaction. In complete contrast, birds receiving $2{\cdot}4 \times 10^5$ *S. enteritidis* PT4 by aerosol produced high levels of IgM antibodies ($OD_{405} > 1{\cdot}2$) by day 10 which decreased to levels $< 0{\cdot}1$ by approximately 15 days later (Fig. 1*c*). Levels of IgG antibodies followed a similar pattern but titres did not exceed 0·4. Five of these birds produced very strong slide agglutination reactions.

Conjunctival challenge

Chickens were also infected via the conjunctiva with 10^3 or 10^8 viable *S. enteritidis*. All four birds receiving 10^3 *S. enteritidis* PT4 became infected. *S. enteritidis* was detected in the faeces (1), caecum (1), ileum (1), oviduct (1), ovary (2) and kidney (2). Increasing the inoculum to 10^8 viable *S. enteritidis* PT4 resulted in all birds becoming infected with bacteria in the faeces (3), caecum (1), ileum (2), jejunum (1), ovary (2), oviduct (2), liver (1), spleen (1), kidney (1) and lung (1).

Chickens were also examined for antibodies to the LPS of *S. enteritidis* PT4 during experimentation. As before, pre-infection sera were tested alongside sera from infected birds and values obtained with pre-infection sera were subtracted from values obtained with post-infection sera. Birds challenged with 10^3 bacteria produced detectable levels of IgG antibodies ($< 0{\cdot}4$) (Fig. 2*a*) but levels of IgM antibodies were very low ($< 0{\cdot}1$), and agglutinating antibodies were not detected. However, chickens given the higher inoculum of 10^8 *S. enteritidis* PT4 produced levels of both IgG and IgM antibodies with titres in excess of 0·5, and two of these birds gave strong agglutination reactions.

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DISCUSSION

In the present study laying hens were challenged with *S. enteritidis* PT4 via the respiratory tract by means of an inhaled aerosol, or via the conjunctiva. The inhalation of $2{\cdot}9 \times 10^2$ to $2{\cdot}4 \times 10^5$ bacteria resulted in systemic infection involving the colonization of body organs and tissues [9, 10] and a proportion of these birds excreted *S. enteritidis* PT4 in the faeces. The fact that a low dose of bacteria caused disease supported our theory that only a small inoculum of *S. enteritidis* PT4 in the form of an aerosol was needed to cause an infection, and might explain why in a previous study [6] control chickens sharing a room with infected birds became infected. Also, since it is likely that only a proportion of the 100 bacteria used for challenging chickens, in the present study, actually entered the respiratory tract of birds, the infective aerosol challenge dose would probably be less than 10^2 bacteria. Birds receiving 10^2–10^3 bacteria by inhalation were found to produce high levels of IgG antibodies to LPS, this resembled the response encountered with naturally infected flocks [3]. Whether dose size influences the immune response of naturally infected birds remains unknown. However, previous studies involving experimental challenge of chickens with various doses of *S. enteritidis* PT4 [7] showed that the immune response was dose-dependent, and that inocula of approximately 10^3 bacteria administered into the crop resulted in both an IgG and an IgM response to *S. enteritidis* LPS.

Chickens were also challenged via the conjunctiva. Birds receiving 10^3 organisms of *S. enteritidis* PT4 became infected, as indicated by bacteria in extra intestinal tissues, but the immune response was generally poor. Increasing the inoculum to 10^8 bacteria resulted in birds producing predominantly IgM antibodies to LPS but titres were considerably lower than those observed in birds challenged by aerosol. This suggested that penetration of the conjunctive by *S. enteritidis* PT4, is probably a less important route by which chickens become infected.

The results of this study suggest that the infective dose of *S. enteritidis* PT4 for chickens can be as low as $2{\cdot}9 \times 10^2$ bacteria when in the form of an aerosol. Since the immune response of these and naturally infected birds was similar in involving the production of antibodies of the IgG class, we suggest that the aerosol model of infection described here might indicate an important route of infection in naturally infected poultry flocks.

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