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# Lachrymal antibody response of specific pathogen free chickens and chickens with maternal immunity after infectious bronchitis virus vaccination

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**Abstract**

Lachrymal fluid specific IgG and IgA were detected by ELISA in chickens with specific maternal antibodies and in chickens free of antibodies (SPF), after vaccination at 1 day of age with the H-120 vaccine strain of infectious bronchitis virus. Samples were obtained at 3 day intervals and until Day 37 of age Optical densities obtained were in all instances low but significant differences could be detected within and between the experimental groups. Both class-specific immunoglobulins showed a similar kinetic pattern. Nevertheless, the SPF group increased its IgA level on Day 13 while chickens with maternal immunity increased their level on Day 16. The antibody levels of both IgG and IgA were also different, being higher in the SPF group In both chicken groups, higher levels of IgA than IgG were detected.

## Introduction

Avian infectious bronchitis (IB) continues to cause economic losses worldwide (King and Cavanagh, 1991). The mechanisms of immunity in chickens infected with this virus remain poorly understood. Lack of association between circulating antibody levels and resistance to challenge with IB virus (IBV) was originally reported by Raggi and Lee (1965).

The presence of local specific antibodies in nasolachrymal secretions has been associated with protection against IBV infection in chickens (Gillette, 1981; Davelaar et al., 1982). Specific antibodies have been detected in lachrymal fluid of chickens that have been exposed to different antigens such as IBV, Newcastle disease virus (NDV) and Brucella abortus (Davelaar et al., 1982; Hawkes et al., 1983; Montgomery and Maslin, 1989; Toro et al., 1991). Lütticken et al. (1988) detected a short and low specific IgA response after

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two inoculations with the IB vaccine strain H-120 in 5-week-old specific pathogen free (SPF) chickens. Interference of maternally acquired antibodies with the vaccinal response of chickens to different viral antigens (e.g. NDV and IBV) has been reported (Raggi and Lee, 1958; Davelaar and Kouwenhouven, 1977). Until now, no information has been available on the possible influence of passive serum antibodies on the lachrymal antibody response of chickens after vaccination.

This paper considers the assessment of IBV-specific IgG and IgA levels in lachrymal fluid in chickens which had maternal immunity and in SPF chickens, after vaccination of both groups at 1 day of age with the Massachussetts-type vaccine strain H-120.

## Material and methods

### *Chickens*

Twenty-four 1-day-old SPF chickens (HY-VAC Lab., USA) and 40 1-day-old Leghorn chickens obtained from a commercial farm were included in this study. The commercial chickens, being the progeny of IB vaccinated breeders, showed a mean serum IgG level of 0.12 (expressed as optical density (OD)) with values varying between 0.08 and 0.16, as measured by ELISA (mean value of OD obtained in ten chickens). Each group (SPF and commercial type) was separated randomly (using a table of random digits) into two subgroups and maintained under isolated conditions in four Horsfall-type chambers throughout the experimental period of 37 days.

### *IBV strain*

The H-120 IBV vaccine strain (Intervet, Boxmeer, NL) was used for vaccinating experimental birds and for sensitising ELISA microplates. After one passage in 9-day-old SPF chicken embryos, this strain showed a titre of $10^{5\,23}$ egg infectious doses 50% ($EID_{50}$) $ml^{-1}$ determined according to Villegas and Purchase (1989). The total protein content of this viral material was 0.887 $\mu g\,\mu l^{-1}$ as determined according to the method described by Bradford (1976).

### *Vaccination*

One subgroup of 14 SPF chickens (A1) and one subgroup of 20 Leghorn chickens (A2) were vaccinated at 1 day of age via the ocular route. Each bird received 0.25 ml of the vaccine suspension, approximately $2.5 \times 10^{4\,23}$ $EID_{50}$. The remaining groups, SPF (B1) and commercial (B2), were unvaccinated controls.

*Lachrymal fluid samples*

Tears (50–200 μl from each bird) were obtained as described previously (Toro et al., 1993) employing crystalline sodium chloride (NaCl) as a mild local irritant. These samples were obtained at 3 day intervals, starting on the day prior to vaccination and continuing until Day 37 post vaccination. Samples were stored frozen (−20°C) until tested for specific antibodies by ELISA.

*Enzyme-linked immunosorbent assay (ELISA)*

ELISAs were conducted as described (Voller and Bidwell, 1986) with modifications published elsewhere (Collingwood-Selby and Toro, 1992). Briefly, microplates were coated with the semipurified IBV antigen diluted in PBS pH 7.4 (40% v/v). Plates were blocked with bovine serum albumin/PBS 10A (BSA/PBS). Tear samples were diluted 1/100 (v/v) in BSA/PBS and 100 μl were added to each well. Washing was performed with Tween 20/PBS

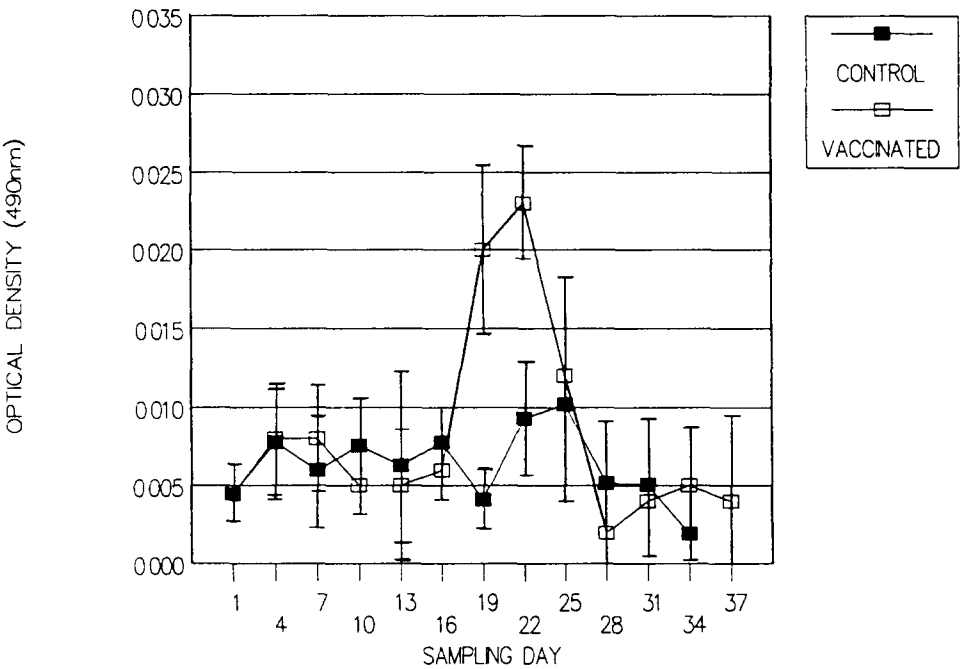


Fig. 1. Infectious bronchitis virus IgA levels detected by ELISA in lachrymal fluid of chickens with maternal immunity after vaccination at 1 day of age with the H-120 IBV vaccine strain. Mean of optical densities obtained in 20 vaccinated and in 20 control birds (bars indicates standard deviations). Progeny of vaccinated breeders from a commercial farm showing a mean serum specific IgG level of OD 0.12; values varying between 0.08 and 0.16.

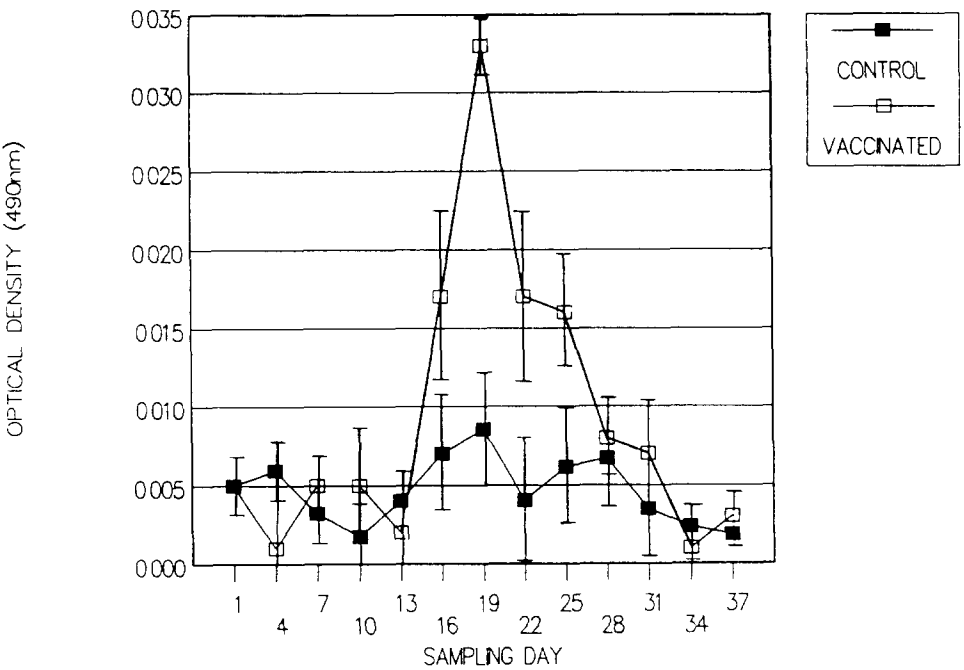


Fig. 2. Infectious bronchitis IgA levels detected by ELISA in specific pathogen and antibody free chickens (Hy-Vac Laboratory Egg Company, Iowa, USA) after vaccination at 1 day of age with the H-120 IBV vaccine strain Mean of optical densities obtained in 14 vaccinated and in 10 control birds (bars indicates standard deviations)

0.05%. Chicken IgG and IgA were detected using commercially available rabbit isotype-specific immunoglobulins (Nordic Immunological Labs., Tilburg, NL). A goat anti-rabbit IgG conjugated with horseradish peroxidase from the same source was added and incubated. The reaction was detected using orthofenildiamine (OPD) (Sigma, St. Louis, USA) and stopped with hydrofluoric acid (0.12% v/v). Optical densities were measured using an interference filter at a wavelength of 490 nm. OD values obtained in each chicken group were compared between the different sampling days. Furthermore, OD values of all four chicken groups were compared between them, at each sampling day. All comparisons were made by repeated measures of analysis of variance considering the model 'antibody level (OD) = source + vaccination status + time + chicken'. Scheffe's test was used for multiple comparisons.

## Results

### *IgA levels*

The commercial-type vaccinated birds (A2) showed baseline IgA levels similar to their control counterparts (B2) until Day 16 post vaccination (p.v.)

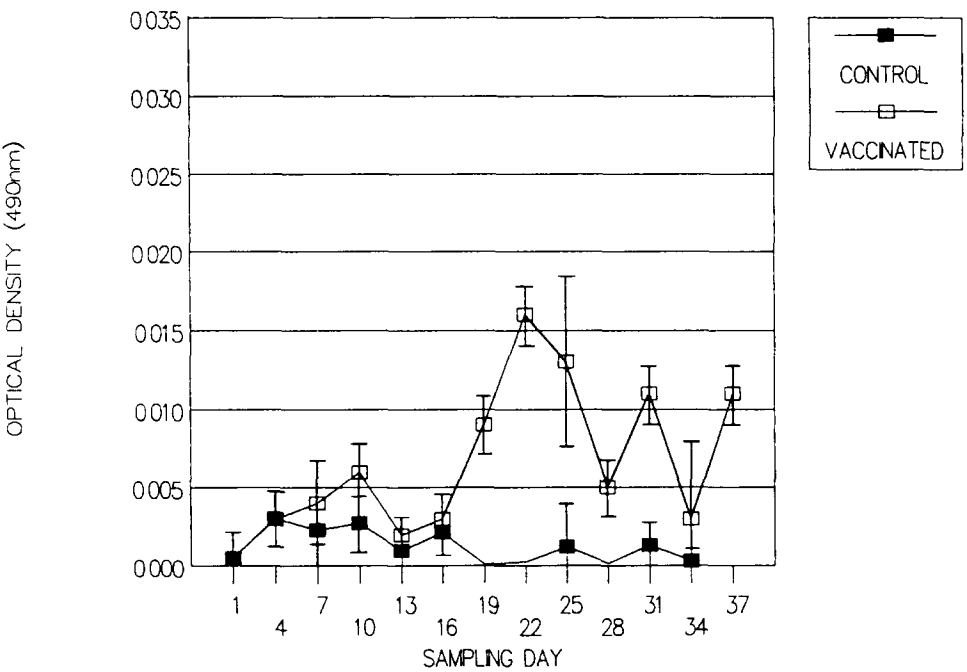


Fig. 3. Infectious bronchitis virus IgG levels detected by ELISA in lachrymal fluid of chickens with maternal immunity after vaccination at 1 day of age with the H-120 IBV vaccine strain Mean of optical densities obtained in 20 vaccinated and in 20 control birds (bars indicates standard deviations). Progeny of vaccinated breeders from a commercial farm showing a mean serum specific IgG level of OD 0.12; values varying between 0.08 and 0.16.

(Fig. 1). After Day 16, group A2 showed a significant increase of its IgA level ($P<0.05\%$) achieving a maximum mean value on Day 22 p.v. (OD 0.024). As shown in Fig. 1, this value declined reaching baseline levels on Day 28 p.v. The control group (B2) maintained baseline levels, not varying significantly from 0.002 to 0.01 OD throughout the experimental period.

Group A1 (vaccinated SPF) maintained IgA baseline levels until Day 13 p.v. (Fig. 2). This group then showed a significant increase in OD values ($P<0.05\%$) achieving a maximum (OD 0.033) on Day 19 p.v. These levels subsequently declined, reaching baseline values on Day 28. Group B1 showed OD values varying between 0.00 and 0.008 with no significant variations.

*IgG levels*

The commercial-type vaccinated birds (A2) showed baseline IgG levels similar to their control counterparts (B2) until Day 16 p.v. (Fig. 3). These chickens then showed a significant increase in lachrymal IgG levels

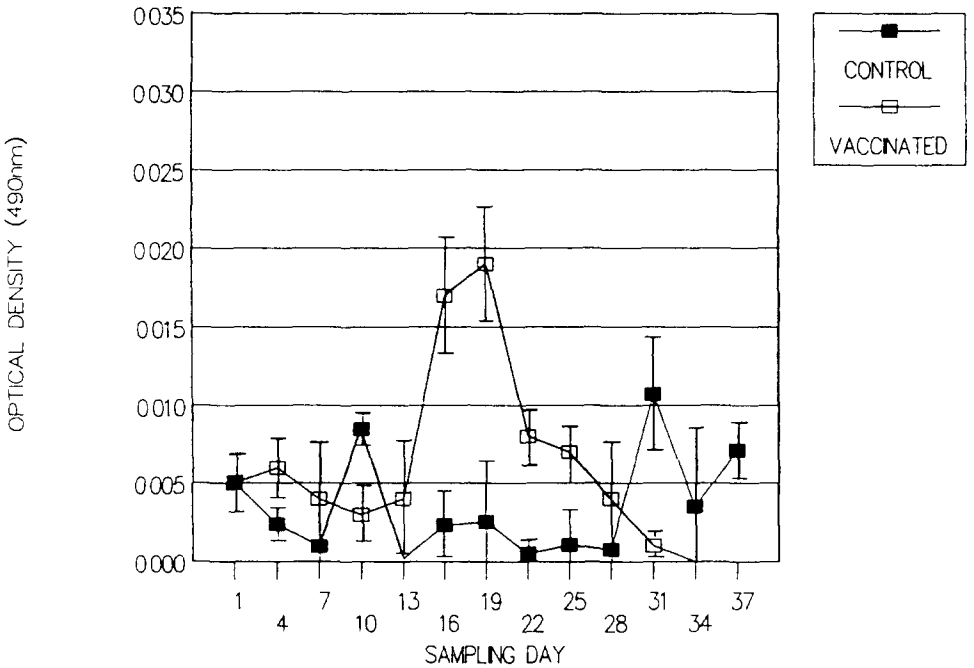


Fig. 4. Infectious bronchitis virus IgG levels detected by ELISA in lachrymal fluid of specific pathogen and antibody free chickens (Hy-Vac Laboratory Egg Company, Iowa, USA) after vaccination at 1 day of age with the H-120 IBV vaccine strain. Mean of optical densities obtained in 14 vaccinated and in 10 control birds (bars indicates standard deviations)

($P<0.05\%$) achieving a maximum (OD 0.016) on Day 22 p.v. As shown in Fig. 3, these values subsequently declined, reaching baseline levels on Day 28 p.v. The control group (B2) maintained baseline levels varying from 0.00 to 0.003 throughout the experimental period.

Group A1 maintained IgG baseline levels (OD 0.004) until Day 13 p.v. (Fig. 4) followed by an increase in OD values, achieving a maximum on Day 19 p.v. (OD 0.019). These levels declined subsequently, reaching baseline values on Day 28. Group B1 showed OD values varying between 0.00 and 0.011 with no significant variations.

When comparing lachrymal-specific IgA and IgG levels at their peaks in both SPF and commercial chickens, significantly higher IgA levels were detected as compared with IgG levels. Commercial birds showed mean antibody levels of 0.023 and 0.016, respectively, whereas SPF chicks reached mean values of 0.033 and 0.019, respectively.

## Discussion

ODs obtained were low in all instances. This can be explained by the use of semipurified antigen for coating the plates. However, a low protein concen-

tration is expected in lachrymal fluid according to values obtained in tears by other authors (Lütticken et al., 1988). In a previous report (Toro et al., 1991), slightly higher optical densities were obtained in this fluid, but a lower dilution of the samples (1/10) was used. In spite of these low antibody levels, significant differences were detected within and between the experimental chicken groups considered in this study.

Both chicken groups responded to the vaccinal stimulus as shown by the rise in antibody levels (expressed as OD). The controls maintained baseline levels throughout the experimental period.

Statistically significant differences in the time of appearance and magnitude of the lachrymal IgA and IgG levels were detected between the vaccinated chicken groups. Both IBV-specific antibody isotypes, IgA and IgG, appeared earlier (3 days) and reached significantly higher levels in the SPF chicken group as compared with the commercial-type birds. An analogous situation has been reported for circulating antibodies where passive immunity interferes with the antibody response to vaccines. An inhibitory effect of maternally derived antibodies of young chickens to Newcastle disease vaccine has been reported by Holmes (1979). This has also been reported for IB (Raggi and Lee, 1958; Davelaar and Kouwenhoven, 1977). Passive antibodies affecting local antibody production could be partially explained by capture of the vaccinal antigen by the passively acquired specific IgG. A lower antigen concentration could cause an inhibition of background development of antibody-producing cells.

Higher levels of IgA than IgG were detected in both vaccinated chicken groups (A1 and A2). In agreement with this finding, Baba et al. (1990) found a higher IgA concentration of IgG in the supernatant of in vitro culture of Harderian gland cells obtained from eyedrop immunized chickens.

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