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# OBSERVATIONS ON THE ABILITY OF AVIAN REOVIRUS VACCINMATION OF HENS TO PROTECT THEIR PROGENY AGAINST THE EFFECTS OF CHALLENGE WITH HOMOLOGOUS AND HETEROLOGOUS STRAINS

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## INTRODUCTION

The vaccination of breeder chickens with an attenuated U. Conn S1133 strain Avian reovirus vaccine has been shown to protect their progeny against both naturally occurring avian reovirus infection and against international challenge with virulent S1133 strain (van der Heide, 1975; van der Heide, Kalbac and Hall, 1976; Eidson, Page, Fletcher and Kleven, 1979; Van der Heide andc page, 1980).

Rau, Van der Heide, Kalbac and Girshick (1980) reported that similar Vaccination also provided protection against 5 heterologous avian reovirus isolates, though the degree of protection seemed to relate to the degree of neutralization of the isolates in vitro by S1133 antiserum. Wood and Thornton (1981) and Thornton and wood (1982) reported the failure of a killed reovirus vaccine to provide protection against challenge with virulent S1133 strain virus.

In the study reported here, the ability of avian reovirus vaccines of the S1133 type to protect against both homologous and heterologous challenge was assessed the vaccines being used indirectly to protect progeny via maternally Derived antibody.

## MATERIALS AND METHODS

*Challenge viruses*

The sources of avian reovirus strains S1133, WVU 2937, EK 2286, Reo 25, REO 59 and RI and the preparation from these strains of material of chick inoculation were reported by Wood, Nicholas, Hebert and Thornton (1980). The strains M67 and M126 were obtained from, and isolated by, Mr J. W. Macdonald (MAFF, Lasswade, Scotland). The strain R4 was obtained from, and isolated by, Dr R. C. Jones (University of Liverpool, England). The last 3 strains mentioned were propagated in primary specific pathogen free (SPF) chick kidney (CK) cell cultures to produce material for chick inoculation.

*Vaccines*

The 47th embrounated egg passage of the S1133 strain of avian reovirus, supplied by Dr E. Vielitz (Lohmann GmbH, Cuxhaven, W. Germany), was propagated by growth

on the dropped chorio-allantoic membrane (CAM) of SPF embryonated hen's eggs. Infected portions of the Cam were harvested, ground up and extracted with equal volumes of chloroform and 0.85 per cent saline. The aqueous layer resulting from low speed centrifugation was removed and inactivated with 0.1 per cent formaldehyde at 37 °C for 18 h. The effectiveness of the inactivation was checked by three CAM passages of the inactivated antigen. No signs of virus growth were seen. The inactivated antigen was combined in the ratio 1 to 3 with Freund's incomplete adjuvant and emulsified to make a vaccine.

A live vaccine, prepared from a derivative of the S1133 strain which had received 207 embryonated egg passages (207 EP), was supplied by MR B. Robinson (Duphar Veterinary, Southampton, England). This vaccine had a titre of $10^4$ $EID_{50}$ per 0.1 ml of vaccinal inoculums.

*Serology*

Agar gel precipitin (AGP) tests were carried out, with the S1133 strain being used to prepare antigen, as describe by Wood, *et al*. (1980). Virus neutralization (VN) tests were carried out in microtitre plates, using a constant amount of virus and varying serum dilutions. The S1133 strain of virus was used. Test and control serum samples were inactivated at 56 °C for 30 mins. Serial dilutions of antiserum at 2-fold intervals over the range 1 in 2 to 1in 2048 were made in phosphate buffered saline in a microtitre plate with a capstan diluter set to leave 0.025 ml serum per well. To each well was added 0.05 ml of virus containing 200 $TCID_{50}$ and 0.2 ml of secondary chick embryo fibroblasts, to give 70 000 cells per well. The medium in which the cells were suspended consisted of Eagle,s M.E.M. containing 5 per cent (vol per vol) newborn calf serum and 1 per cent (vol per vol) of 7.5 per cent (wt per vol) sodium bicarbonate solution. All wells were examined for cytopathic effects (CPE) after 5 days' incubation and the serum titre was expressed as the reciprocal of the last serum dilution at which no CPE could be seen.

*Chickens*

Sixty white Leghorn SPF in-lay hens and 4 cocks were obtained from a commercial supplier. These were divided into 2 groups of 30 hens and 2 cocks each. One of the groups was vaccinated twice, with a 5 week interval, with inactivated reovirus oil emulsion vaccine. After a further 12 weeks, the same group was vaccinated with one dose in 0.1 ml of the 207 EP live avian reovirus vaccine. All 3 vaccinations were by subcutaneous inoculation. The other group of birds was retained as unvaccinated controls. Eggs were collected from the vaccinated breeder group between 11 and 29 weeks after their third vaccination and hatched to provide chicks with maternally derived antibody to avian reovirus. Eggs from the unvaccinated breeder group were hatched to provide non-immune controls. Both breeder groups were bled at intervals throughout the experiment for serological tests.

*Experimental Procedure*

Groups of 1-day-old maternally immune and fully susceptible chicks were challenged with serial 100-fold dilutions from undiluted to $10^{-8}$ of the avian reovirus strains. Each virus dilution was inoculated into the right foot pad of 5 chicks, the volume of inoculums being 0.1 ml per chick; thus for each virus strain, 25 maternally immune and 25 susceptible chicks were challenged. Each group of 25 was housed separately in negative pressure isolators. The birds were kept for 7 days and were examined for lesions around the hock joint. Clinical signs were recorded, together with any deaths. Thus, each challenged virus was titrated in parallel in susceptible and immune chicks, the results being expressed as the difference between the two titres so obtained.

## RESULTS

*Serology*

In the vaccinated breeder flock, the proportion of birds in which antibody was detected in the AGP test rose to a maximum of 87 per cent 3 weeks after the second inactivated vaccine treatment. From this it fell away to a fairly constant value of between 27 per cent and 43 per cent for the rest of the experiment. The proportion was 27 per cent just before egg collection for hatching began and was 41 per cent at its conclusion. Although not all of the dams gave positive AGP reaction on all occasions, particularly following the initial peak, those that were positive varied during the experiment, suggesting that antibody was present in most or all of the group at approximately the threshold for detection by the AGP test. This suggestion was supported by evidence from the VN tests on the same sera. The higher sensitivity of this test was reflected in the fact that all of the dam gave positive titres on all but one occasion. Individual bird's VN titres varied between 1 in 2 and 1 in 2048 with the mean titres towards the higher end of this range. During the egg collection period, the mean titre varied between 1 in 776 and 1 in 315. Thus, taking the evidence from both serological tests, it would seem that all of the chicks hatched from the vaccinated flock were likely to have had similar amounts of maternally derived antibody. The unvaccinated flock stayed negative for reovirus antibodies throughout the experiment.

*Effect of Maternal Antibody*

Table 1 shows the differences in titre of challenge virus in the maternally immune birds compared with the susceptible controls for the 9 challenge strains. The titres were calculated by the Spearman–Kärber method (Finney, 1978) and expressed as the median dose required to cause death or mid or upper leg lesions, the values given in the Table were obtained by subtracting the logarithm of the titre obtained for each challenge virus when titrated in

**TABLE 1**
**DIFFRENCES BETWEEN TITRES OF CHALLENGE STRAINS, EXPRESSED AS THE MEDIAN DOSE TO CAUSE DEATH OR MID OR UPPER LEG LESIONS, IN MATERNALLY IMMUNE AND FULLY SUSCEPTIBLE 1 DAY OLD BIRDS**

| *Challenge strain* | *Difference between titre** | *Significnce* |
|---|---|---|
| S1133 | $\geq 10^{5.3}$ | $P < 0.001$ |
| EK2286 | $\geq 10^{5}$ | $P < 0.001$ |
| WVU2937 | $\geq 10^{4.8}$ | $P < 0.001$ |
| REO59 | $\geq 10^{0.6}$ | N.S. |
| R1 | $10^{2.4}$ | $P < 0.001$ |
| REO25 | $10^{2.4}$ | $P < 0.01$ |
| R4 | $10^{2}$ | $P < 0.01$ |
| M67 | $10^{3.2}$ | $P < 0.001$ |
| M126 | $10^{4.1}$ | $P < 0.001$ |

*Titre in control birds minus titre in maternally immune birds.

†Parents flock vaccinated with killed and live U.conn S1133 strain.

≥ indicates no deaths or lesions in maternally immune birds.

maternally immune birds from that obtained when titrated in susceptible controls. Four of these challenge strains caused no deaths or mid or upper leg lesions even with the undiluted challenge in the maternally immune birds, so the titer reductions are smallest probable estimates and might have been shown to be greater had the inoculum contained more virus. The maternally derived antibody to S1133 protected against death and lesions caused by all 9 strains, though the degree of cross–protection varied from strain to strain, the differences being significant at the $P < 0.01$ or $P < 0.001$ level in 8 of the strains and insignificant for the Reo 59, which had very little effect in the fully susceptible birds.

## DISCUSSION

Previous work with parental vaccination (Wood and Thornton, 1981; Thornton and Wood, 1982) Failed to demonstrate protection of the progeny. In the present work, double vaccination with inactivated vaccine followed by live vaccine was used in an attempt to stimulate adequate immunity. Good protection was conferred on the progeny against challenge by the homologous S1133 strains. However, the degree of protection provided against other challenge viruses varied considerably from strain to strain.

When attempts were made to relate the degree of protection, as assessed by difference in titre of challenge between control and immune birds, to in vitro neutralization of the same strain by S1133 antiserum, as reported by Wood *et al*., (1980) the results were, in some cases, similar.

The EK 2286, which was allocated to the same serotype as the S1133 by in vitro neutralization, did not show dissimilar protection by the in vivo method. However, the RI, again allocated to the S1133 serotype in vitro, showed far less protection by maternal immunity while the WVU 2937, of different serotype in vitro, again did not give dissimilar protection to that with S1133 by the in vivo method .

These results show that cross-neutralization in vitro and cross-protection may not always be closely related phenomena, although there are some similarities. Rau *et al* .(1980), working with an S1133 stain vaccine in breeders and heterologous challenge strains in their offspring, reported a correlation between lack of neutralization in vitro and lack of protection by maternal immunity . They reported less than full protection against oral challenge at one day of age with the Reo 25 strain and virtually full protection against the WVU 2937 strain. These results agree with our findings for both strains using the in vivo method, although our in vitro findings for WVU 2937 differ somewhat, perhaps confirming the statement of Rau *et al*. (1980) concerning the inherent variability in the degree of neutralization between a given reovirus and antiserum.

Certain of the mid or upper leg lesions reported in this work were similar to those found in early clinical viral arthritis and, where comparable, the findings were similar to those or Rau el al. (1980), who looked at early lesions of viral arthritis by histological means. Given these statements, it is probably reasonable to extrapolate the findings of this work to protection against full clinical viral arthritis.

The rapid decline in sensitivity of chicks to clinical viral arthritis with increasing age at infection (Wood and Thornton, 1981) means that protection by parental vaccination should be a very effective procedure, in that the maternal antibody should be present in the offspring at highest concentration when they are most susceptible; when the antibody concentration has fallen, so will have susceptibility. However, the variation in degree of cross-protection suggests that vaccines prepared from 2 or more differing antigenic components may be more effective in terms of breadth of protection.

## SUMMARY

Avian reovirus vaccines of the S1133 strain, used indirectly in the dams of challenged chicks, were found to confer protection against clinical signs and/or deaths resulting from the use of both homologous and heterologous challenge strains. There was some protection against every strain used, although this did vary in degree. It was difficult to relate this variation in protection to the differences in in vitro neutralization of the strains by S1133 antiserum, though there were some similarities.

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