

chapter 4

Results

1. Comparison of various treated antigens for the detection of agglutinating antibody

Four antigens, autoclaved antigen, formalinized antigen, HCl-treated antigen and living-cell antigen were compared with regard to their efficiency in detecting agglutinating antibody. Forty percent of the antisera samples collected from ducks vaccinated with mineral-oil and corn-oil adjuvanted vaccine were used at the following intervals 0, 15, and 30 days into the postvaccination period. The antibody titers were determined by the tube agglutination test.

On the 15th day of the postvaccination period, thirty-nine antisera samples from ducks vaccinated with MDC vaccine were detected. The results were as follows: 3 samples (1:8), 1 sample (1:16), 3 samples (1:32), 3 samples (1:128) and 23 samples (1:256). Similar results were also obtained from ducks vaccinated with CDC vaccine (table 9). The agglutinating antibody varied from 0 to 1:256 with an autoclaved antigen. No antibody titer was detected when using formalinized, HCl-treated, or living antigen.

2. Comparison of antibody titer as measured by the tube agglutination method with microtitration method

The microtitration method was developed for assaying antibody titer. Antisera samples from ducks which had received two injections of Livestock Department vaccine, and from a control ducks which had not been vaccinated at 15, 30, 45, 60, 90, and 120 days postvaccination were used. Fig 1, table 10 shows the antibody titers of serum samples measured by both the tube agglutination and microtitration methods. In the vaccinated ducks the tube agglutination titer was 1 : 45 and that of the microtitration titer was 1 : 26 at 15 days post-vaccination. However, the difference was not statistically significant ($p > 0.05$).

3. Determination of antibody titer

Mineral-oil-adjuvanted vaccine Fig.2 and table 11 show the antibody titers of serum as measured by the tube agglutination (TA) test and indirect hemagglutination (IHA) test as well as the percentage of survival. The maximum TA titer was 1 : 125 while that of the IHA titer 1 : 268 at 15 days postvaccination. At 30, 60 and 90 days postvaccination the TA titer was 1 : 58, 1 : 16, and 1 : 5, respectively while the IHA titer was 1 : 164, 1 : 17, and 1 : 22 respectively. Some correlation was observed between the TA and IHA titers. Sera from the unvaccinated ducks exhibited a very low antibody titer as measured by both tests (TA and

IHA Test). The difference in GMT, as measured by the TA test, between vaccinated and unvaccinated ducks was statistically significant ($p < 0.05$) at 15-60 days postvaccination, while the difference in GMT, as measured by the IHA Test, between vaccinated and unvaccinated ducks was statistically significant at 15-90 days postvaccination. The antibody titers (TA, IHA titers) did not correlate with the level of protection afforded. Fig.3 and table 12 show the antibody titers and rate of survival among ducks vaccinated twice with the mineral-oil-adjuvanted vaccine. After the second immunization, the TA titer was lower than it was after the first vaccination, although a high level of antibody was obtained by means of the IHA test. At 15, 30, 90, and 135 days after the second immunization the TA titer was 1 : 50, 1 : 22, 1 : 11, and 1 : 12 respectively, while the IHA titer was 1 : 256, 1 : 215, 1 : 256, and 1 : 2 respectively. No correlation was observed between the TA and IHA titers. the antibody titers (TA, IHA) did not correspond with the level of protection afforded. The IHA test yielded a higher antibody titer than the TA test.

Corn-oil-adjuvanted vaccine Fig.4 and table 11 show the antibody titers of ducks vaccinated once with corn-oil-adjuvanted vaccine, and the percentage of survival. The antibody titer of the serum was measured by TA and IHA tests. The TA titer was 1 : 44 while the IHA titer was 1 : 80 at 15 days postvaccination. At 30, 60 and 90 days postvaccination, the TA titer was 1 : 15, 1 : 10 and 1 : 5 respectively, while

the IHA titer was 1 : 36, 1 : 14 and 1 : 7, respectively. Some correlation between the TA and IHA titer was observed. However, there was no correlation between TA, IHA titers and the level of protection.

Fig.5 and table 12 show the antibody titers of ducks vaccinated twice with corn-oil-adjuvanted vaccine, and the percentage of survival. At 15 days after the second immunization, the TA titer was lower than that obtained after the first vaccination, although a higher antibody titer was obtained by means of the IHA test. At 15,30,90, and 135 days after the second immunization, the TA titer was 1 : 19, 1 : 5, 1 : 8, and 1 : 6, while the IHA titer was 1 : 146, 1 : 25, 1 : 139, and, 1 : 8 respectively. No correlation was seen between the TA and IHA tests. However no correlation was seen between the antibody titers and the level of protection. The IHA test also yielded a higher antibody titer than the TA test.

Aluminium hydroxide - absorbed vaccine Fig.6 and table 11 show the antibody titers, as measured by the TA and IHA tests, of sera from ducks vaccinated once with aluminium hydroxide-absorbed vaccine, and the percentage of survival. A low antibody titer (1 : 48) was obtained at 15 days postvaccination as measured by the TA Test, whereas the IHA titer was 1 : 161. At 30,60,and 90 days postvaccination the TA titer was 1 : 15, 1 : 9,and 1 : 4 respectively,while the IHA titer was 1 : 43, 1 : 17, and 1 : 16, respectively. Some

correlation was seen between the TA and IHA titers. However, no correlation was seen between the TA, and IHA titer and the level of protection.

Fig.7 and table 12 show the antibody titers of ducks vaccinated twice with the aluminium-hydroxide-absorbed vaccine, and the percentage of survival. A lower antibody titer was obtained at 15 days after the second vaccination, as measured by both the TA and the IHA tests. At 15,30,90, and 135 days after second vaccination the TA titer was 1:20, 1 : 14, 1: 5, and 1 : 5, respectively, whereas the IHA titer was 1 : 128, 1 : 63, 1 : 71, 1 : 26, respectively. No correlation was seen between the TA and IHA titers. However no correlation was seen between the antibody titers and the protection .

Formalin -killed whole- cell vaccine Fig.8 and table 11 show the antibody titers of ducks vaccinated once with the formalin-killed whole-cell vaccine and the percentage of survival. The maximum antibody titer of the serum, as measured by the TA and IHA tests, was 1 : 82, 1 : 21, respectively at 15 days postvaccination, No correlation was seen between the TA and IHA titers. The higher titer was obtained by means of the TA test. At 30,60, and 90 days post-vaccination, the TA titer was 1 : 18, 1 : 14, and 1 : 8, respectively, while the IHA titer was 1 : 19, 1 : 22, 1 : 11, respectively. There was no correlation between the antibody titers the level of protection.

Fig.9 and table 12 show the antibody titers of ducks vaccinated twice with the formalin- killed whole- cell vaccine and the percentage of survival. As measured by the TA test, a lower antibody titer was obtained (1 : 28) 15 days after the second vaccination, while the IHA test yielded a high antibody titer (1 : 104). At 30,90, and 135 days after the second vaccination the TA titer was 1 : 16, 1 : 6, and 1 : 6 respectively, while the IHA titer was 1 : 9, 1 : 98, and 1 : 25.

Livestock Department vaccine Fig.10 and table 11 show the antibody titers of ducks vaccinated once with Livestock Department vaccine and the percentage of survival. the maximum antibody titer of the serum, as measured by the TA and IHA tests, was 1 : 51, and 1 : 16, respectively at 15 days postvaccination. The higher titer was obtained with the TA test. At 30,60, and 90 days postvaccination the TA titer was 1 : 12, 1 : 10, and 1 : 2, respectively, while the IHA titer was 1 : 16, 1 : 2, and 1 : 5. Some correlation was observed between the TA and IHA titers. However, no correlation was observed between the TA and IHA titers and the levels of protection.

Fig.11 and Table 12 show the antibody titers of ducks vaccinated twice with the Livestock Department vaccine, and the percentage of survival. As measured by the TA test a lower antibody titer, was obtained (1 : 26) 15 days after

the second vaccination, although the IHA test yielded the higher antibody titer (1 : 88). At 30, 90, and 135 days after the second vaccination the TA titer was 1 : 15, 1 : 10, and 1 : 6, respectively, whereas the IHA titer was 1 : 7, 1 : 19, and 1 : 2. No correlation was observed between the TA and IHA titers. There was, however, no correlation between the antibody titers and the level of protection.

4. Correlation between antibody titer and the level of protection against challenge. Table 11 shows the antibody titers of ducks vaccinated once with various vaccines, as measured by the TA and IHA tests and the level of protection against challenge. The vaccinated ducks as well as the control group were sampled and challenged. Some of the control group died within 48 hours. The percentage of survival among the control group was 10 %, 36.3 %, 14.2 %, and 66.6 %, respectively at 15, 30, 60, and 90 days post-vaccination. A high percentage of survival-i.e. 36.3 % and 66.6 % was noted. With the MDC and FDC vaccines there was a 100 % rate of survival throughout the experiment. With the CDC vaccine there was a lower percent of survival (90 %, and 70 %) at 15, 30 days postvaccination, although this increased to 100 % after intervals of 60 and 90 days. At the end of the experiment a group of ADC vaccine experienced a 66.6 % drop in the rate of survival. With DCL vaccine there was a 66.6 % survival at 60 days postvaccination, which is increasing to 100 % by the end of the experiment. Most of

the vaccines provided a high degree of immunity at 15-60 days postvaccination, as measured by the percentage of survival and degree of potency in accordance with the procedure followed by Heddleston (Table 13).

The potency of these vaccines at 15-60 days postvaccination was over 60 % ; at 90 days postvaccination, however, the potency had begun to decline (<60%), indicating that the vaccine might have been unsatisfactory. With regard to the CDC vaccine, there was an abnormally high rate of survival, a phenomenon for which no explanation has been found. As measured by the percentage of survival and the calculations relating to potency at 60 days postvaccination, the MDC, ADC, FDC, and DCL was 85.8%, 85.8%, 85.8%, and 52.4%, respectively.

The antibody titer did not clearly indicate the immune status of ducks that were vaccinated. No apparent correlation was observed between the TA and IHA tests and protection. Table 12 shows the antibody titers of ducks vaccinated twice with various vaccines, as measured by the TA, IHA tests and the level of protection afforded against challenge. The percentage of survival in the control group was 40 % , 100 % , and 14.2 % , respectively at 30, 90, and 135 days after the second vaccination. A high percentage of survival was obtained at 90 days after the second vaccination. All of the vaccines provided excellent immunity at 30-135 days after the second vaccination as measured by survival

rate and potency (both expressed in terms of a percentage). DCL vaccine gave 100 % survival through the experiment (5 1/2 months). MDC gave 75 % survival whereas the ADC vaccine gave only 50 % survival and a lower of 35.8 % potency at the end of the experiment. With the CDC and FDC vaccines there was a 80 % rate of survival at the end of the experiment. No correlation was seen between the TA, and IHA titer and protection (Table 14).

As measured by the percentage of potency, the results showed that MDC, CDC, FDC, and DCL vaccine produce a level of immunity lasting for upto 5 1/2 months. The percentage of potency by the MDC, CDC, FDC, and DCL vaccine was 60.8%, 65.8%, 65.8%, and 85.8%, respectively.

5. Immune response in male and female ducks

Fig.12,13 the antisera of ducks vaccinated with mineral-oil and corn-oil-adjuvanted vaccine, two injections were used.

The ratio of male to female ducks in both groups was 3:2. The antibody titers were determined by using the tube agglutination test. The antisera of ducks were at random selected from 40-50 % of each group. The antisera both male and female ducks were then titered, but there was

no significant difference between the titers of either gender.

6. Antibody titer after challenge

Table 15 and 16 show the mean TA antibody titer before and after challenge in ducks vaccinated once and twice with various types of vaccines. With regard to the mean antibody titers in ducks vaccinated once after challenge, there was no significant difference between them and the mean antibody titers before challenge ; these findings applied to all groups of vaccines at different times. At 60 days post-vaccination, as regards the MDC vaccine groups there was no significant difference ($p > 0.05$) between the titers before challenge (16.81) and those after challenge (17.66).

At 30 days postvaccination the titer in the MDC vaccine group after challenge (26.25) was lower than before challenge (58.98)- a significant difference ($p < 0.05$); these finding also applied to all groups of vaccination different time.

At 60 days postvaccination the titer in the DCL vaccine group after challenge (90.50) was higher than the titer before (10.55) - again a significant difference ($p < 0.05$). The same phenomenon being observed in ducks vaccinated twice with all types of vaccines at an interval of 135 days after the second vaccination. At 135 days after the second vaccination the antibody titer with MDC vaccine after

challenge (203.18) was higher than it was before challenge (12.69)- a significant difference ($p < 0.05$), it was found, however, that after varying intervals of time, there was no significant difference between the antibody titer before and after challenge.