CHAPTER V

DISCUSSION

Single vaccination

Within 2 months after the first vaccination, although the antibody level in the oil vaccine (BEI-DPM) was significantly higher than in the other two vaccines (DP-L and BEI-DP), the protective immunity of inactivated vaccine both with and without oil was lower than in the attenuated vaccine. These results do not agree with those of Butterfield and Dardiri, who found that duck plague virus, inactivated with 1-acetylaziridine, afforded a greater level of protection than live chicken-embryo-adapted vaccine (6). In their work, however, the virus used for the purpose of inactivation designated as Holland attenuated virus, was not of the virulent kind, thus the two inactivated vaccines cannot be compared on an equal basis.

The study by Delta-Porta, A.J. showed that using 500 times less virus in the attenuated vaccine against Bovine Ephemeral Fever than in the inactivated vaccine, similar or slightly lower antibody levels were attained, although most animals resisted challenge (64). His work seems to correlate with the present study.

Two months after vaccination, the outbreak occurred. It is believed that some infectious particles that had not been completely inactivated may have spread to the farm. All the ducks that had

been vaccinated one time and survived, including the control group that had come into contact with the infection, develop high level of neutralizing antibody (NI $\geq$ 2.8). Certainly, the increase in titer was due to infection. However, during the third month after the first vaccination, when these ducks were challenged again, not all birds that were superinfected (reinfected) died. Only attenuated vaccine and inactivated vaccine without oil gave 100% protection, whereas the rest provided protection only 50% of the cases. In the same way, surviving, unvaccinated ducks (2 ducks), whose antibody titers were followed until the end of the project, also resisted challenge. It used to be thought that a duck that survived an attack of duck plague was immune to infection a second time (65) but our findings show that this is not always so with birds that are carrier of duck plague. One possible hypothesis that might explain this rather uncommon occurrence is that some ducks that survive superinfection may already have developed sufficient immunity after vaccination and/or natural infection, whereas the others that succumb to the second challenge may only have built up local immunity and thus may have been unable to mount a rapid immune response when they are reinfected. These results coincide with the findings of Burgress and Yuill, who concluded that it is of epizootiological value to know that the birds surviving an initial duck plague infection are not necessarily immune to death from a second duck plague virus infection (60). The immune mechanisms involved in superinfection by duck plague virus are still unclear.

Double vaccination

During to the experiment, the second vaccination was followed by an outbreak of the disease. Because the 2nd vaccination also induced development of antibody (59), one hesitates to claim that the increase in titer is a result of vaccination or infection. Reviewing the reports of other investigators, it is difficult to reach a definite conclusion as to the cause of the increase in antibody titer.

Sattaporn stated that the antibody titer to attenuated vaccine reached its highest level one week following the 2nd vaccination (NI $\leq$ 2.5) (67) while Toth observed that the 5th week after the 2nd vaccination, the NI value was about 1.7 (59). In the present experiment, the highest antibody occurred at the first month (NI $\cong$ 2.8) although no data were obtained for the 1st, 2nd and 3rd weeks. Thus, it may be assumed that infection, as well as vaccination was a possible cause of the rise in titers.

On the other hand, at least 3 observations support the contention that infection is preferable to the vaccination.

1. Toth (59) and Sattaporn (67) found that the second vaccination induced the highest titer within 1 month but the NI value did not exceed 2.5, which that is lower than the levels observed during our experiment.

2. Dardiri et al (68) studied the response of ducks to experimental infection with duck plague virus. The NI level during the 24 to 38 day postinoculation period ranged from 2.0-4.0.

3. In a previous study by Toth (59), the NI level after the 2nd vaccination declined to 0.7 within 4 months, whereas the NI level after challenge-virus inoculation decreased from 4.0 to 3.1 within 3 months.

Burgress and others have reported that healthy waterfowl can be persistently infected with duck plague virus and may shed the virus for at least four years (65). A characteristic feature of herpesvirus infection in animals is the ability of the virus to remain latent in the host until certain stimuli cause an increase in virus replication (69). These two cases may explain why the antibody titer after the second vaccination tended not to decline over the period examined.

With regard to ducks vaccinated once or twice with various vaccines, the losses among these ducks due to the outbreak varied whereas the mortality rate among unvaccinated ducks was almost 100%. Both vaccinated and unvaccinated ducks had an equal chance to combat any outbreak. Thus, it is believed that the efficacy of various vaccines should also affect the different rates of survival. Consequently, the level of protective immunity within 6 months of the 2nd vaccination may be a result of the immunity developed from both the vaccine and natural infection.

Again, the level of protective immunity of the attenuated vaccine was higher than that afforded by both inactivated vaccines, although the antibody titers of these 3 vaccines were not statistically different. This is the first report on revaccination with inactivated duck plague vaccine. As already indicated, given

the superiority of modified-live vaccine when compared with inactivated vaccine, it is clearly best to choose the attenuated vaccine since it seems to develop and maintain immunogenicity.

Triple vaccination

From figure 6, it may be observed that when the NI of ducks given double and triple injections of various vaccines were compared at the same time, only oil vaccine (BEI-DPM) induced any development. As for the other two vaccines (DP-L, BEI-DP), there was no development at all. These findings were the same with regard to the first vaccination for these 3 groups of vaccine. Thus, the antibody titer after the 3rd vaccination actually comes from the vaccine. The actual way in which the adjuvant increases the antibody level is only vaguely understood; it may be said, however, that adjuvant may decrease the distribution and elimination of antigen in order to be kept in the body for a longer period (70).

There is an interesting outcome with regard to the level of protective immunity in ducks vaccinated three times that it is lower than with those vaccinated twice. This was the case with all vaccines, especially the attenuated vaccine. Because there is no evidence with regard to the efficacy of triple vaccination, the experiment should be repeated and if the results are the same, it would seems to indicate that one should be careful when vaccinating more than twice. One possible explanation for this unusual phenomenon is that the ducks may develop immunotolerance or unresponsiveness to duck plague virus. Burgress found that transovarial birds were tolerant to duck plague virus and when they

were infected, they may have been unable to respond immunogenically to the infection (66). The mechanism of immunologic tolerance may function as the efferent limb of the immune response. When there are specific antibodies or sensitized T-cells present, these two components cannot approach the antigen because there is a blocking antibody present or because the antigen-antibody complex has already enclosed the antigenic determinant (70).

As we have observed in this and other studies (33), serum from ducks surviving an immunity challenge have a high antibody level. It is also noted in this and a previous studies (66) that, following a challenge inoculation of vaccinated ducks, some anamnestic antibody response should be encountered.

NI and the protective immunity

The study reveals that there is a lack of correlation between the level of neutralizing antibody and the level of protective immunity. The observations made during in the present experiment support this finding.

1. The protection afforded ducks vaccinated once with attenuated vaccine is very high, even though these ducks have a very low negative NI.

2. The mortality rate among unvaccinated ducks is much greater than among single vaccinated ducks, although in terms of these two groups both with regard to the individual and the group, the average NI are equally high.

3. In the first vaccination, the antibody titer of attenuated vaccine is significantly lower than with oil vaccine,

while the level of protective immunity afforded by the former group is higher.

The present study corresponds to other reports (4,5,6,59,61). The experiments described above may indicate that the resistance acquired is probably not based on the formation of specific antibody. It is well-known that a nonantibody defense mechanism exists. Interference phenomenon and cell-mediated immunity may be responsible for such protection (4,7,57,59,63).

β neutralization

Each serum was test by both ∝ and β neutralization tests. There is a very good rate of correlation between titers in these two tests (r=0.9556). The β method has advantages when a lot of samples need to be tested because it requires less equipment and reagent. In addition,the ∝ method is limited in that the virus titer is not high enough in cell cultures to detect a difference. Blore studied the antibody level against the avian infectious bronchitis virus in broiler-breeder chickens and found that the β method was able to detect differences in flock antibody titers that the ∝ method could not (71). The author felt that if the virus titer had been higher, the ∝ method would have been much more sensitive (71). Thus the constant virus microneutralization test (β method) is as useful as the method for detecting and quantifying antibody to duck plague virus.

With regard to titers which are deemed significant, this study shows that serum diluted 1:5.6 or higher imply infection (equivalent to NI 1.75 by the ∝ method). The level is a little lower than the titer in a previous study by Lin (1:8) (55).

However, the virus concentration in the present experiment is 100 $TCID_{50}$ while Lin used only 10 $TCID_{50}$ in his test, so the two significant titers are comparable in that the virus titer appears to have an inverse relationship with the antibody titer (71).

Indirect hemagglutination

This is the first report to use the IHA test for the assaying of duck plague antibody. According to the results, the factors governing the final sensitivity of formaldehyde fixed sheep cells for estimating the level of antibody are the concentration of duck plague antigen for sensitizing the cells and the concentration of sensitized cells. The conditions-the time of exposure of the tanned cells to the antigen-seem to make no difference. Other variations which can cause a difference in titer are the concentration of tannic acid, as well as the time and temperature of the tanning and sensitizing procedure (72).

The correlation between titers determined by the IHA and neutralization tests ($\propto$ method) is 0.7253; hence the IHA test can be used to determine the antibody titer to duck plague virus. One method of improving the correlation is to purify the antigen. Loon AM. et al purified herpes virus by sucrose gradient centrifugation (73). Hamper B. et al used chromatography technique to prepare herpes simplex virus antigen to detect antibody by means of the ELISA method (74).

The fixing agent also exerts some influence in terms of correlation. Nyerges G. et al determined the tetanus antitoxin titer by means of the seroneutralization test in mice as well as by

the IHA test using reagents prepared from either native or preserved cell. The results in terms of both in vivo and in vitro values, concurred to a high degree when native erythrocytes were used (75).

A comparison of the IHA and ∝ neutralization tests shows that serum diluted 1:128 is equivalent to NI 1.75. Based on the significant level obtained, it can be said that the IHA test is quite a sensitive method for evaluating the antibody titer. However, the specificity of the IHA test should be elucidated after incorporating a definitive test for specificity because the antigen used is only a crude one so the presence of cross reaction with antibody to other heterologous virus may be occurred.

The IHA test has several advantages. It requires only 3 hours to complete, is fairly simple to perform and requires inexpensive equipment and reagents (53,73). Thus, once the test has been improved, it will be sufficiently sensitive and specific for practical, diagnostic purposes for dectecting duck plague antibody.

Further Study

The study of the efficacy of inactivated duck plague vaccine with various adjuvants may possibly foster further research into the study other methods of preventing duck plague infection. In addition, since there have been no studies on the immune response in the cell system of duck-cellular mediated immunity, this needs to be investigated in order to explain the role played by protection during the postvaccination phase.