CHAPTER V

DISCUSSION

Experiment I : Growth Curve of P. multocida : CU strain

The growth of P.multocida : CU strain in BHI broth was better than that in tryptose broth with thiamine, shaking condition got higher yield than static condition and incubation at 41.5 °C which is the body temperature of avian was not prevailed for this avian isolated strain at 37 °C. These growth characteristics of the CU strain was mostly resemble with the 8:A strain reported by Pongsopida et al.(107).

The purpose of this experiment was selection for the best conditions and suitable harvesting time for vaccine preparation. The results have been assessed and the incubation at 37 °C without agitation in BHI broth for 6-9 hr has been chosen. Although it seems likely that shaken condition attained slightly higher viable cells, during the period of harvesting (6 - 9 hr) the viable cells in shaking flask still increased rapidly and afforded exceedingly in some experiments (Table 6). This circumstance was so difficult to get precise the amount of viable cells in each vaccine preparation.

There were a few reasons to select the late log phase (6 - 9 hr) as the suitable period for harvesting. In many previous reports the 16 - 20 hr old BHI broth cultures incubated at 37 °C have been used as the live vaccine (5, 88, 90, 92). Heddleston and Rhoades (17) have reported that growth of P.multocida in broth reached maximum in 16 - 24 hr In contrast with the study of Juangpanich et al. (108) which has found that the duration of log phase was only 7 - 9 1/2 hr This contrary was concluded by Pongsopida et al. (107) that the duration of the log phase depended on the inoculum size. Starting with the low amount of bacteria about 10^3 cells/ml would take 24 hr to

reach the stationery phase but about the 10^6 cells/ml would take only 6 hr This finding was consistent with our experiment that standardized inoculum yielding about 10^6 cells/ml would peak in 6 - 9 hr after incubation at 37 °C in BHI broth both shaking and static conditions.

In addition, study with certain other bacteria have indicated that vaccination of animals with the organisms from young culture may afford better protection against challenge exposure (109). Similar result was obtained in live vaccine of P.haemolytica. The culture in the log phase (6 - hr culture) developed a maximal capsular size and induced a higher protection than the culture in the stationary phase (20 to 22 hr culture) which had a minimal capsular size (110). However it has been reported that the P.multocida developed a high toxin in late log phase (111) thus further study about the correlation of culture age virulence and efficacy of this live vaccine should be designed.

Experiment II : Virulence of the CU Strain in Various Ages of Ducks

The results demonstrated that the CU strain of P.multocida was virulent for ducks by the S/C route in high dose. This indicates that the organism is not virtually avirulent for all host species. In addition, the result showed that older ducks were more resistant to exposure as an evidence that ducks inoculated with 1.0 ml of the stock culture of 1.6×10^9 CFU/ml were all died in 1 wk of age then decreased in the mortality rate when the age increased until in 4 wk of age only 1 of 20 ducks inoculated with 1.67×10^9 CFU died. It was quite conceived that at the age of vaccination (6wk) this vaccine strain would not be harmful to ducks.

Experiment III Immunological Response.

Protective Immunity

Only 5% death (1 of 20 ducks) was observed in 4 wk of age but unexpectedly, as high as 30% and 20% death occurred in the S/C vaccinated group , after the first and the second vaccination in the age of 6 wk and 10 wk This finding indicated that old ducks became more susceptible to the CU strain similarly to the report in turkeys (95). It has been stated that the virulence of P.multocida depended on age, host species, and contact condition (17). Study in other poultry found that the CU strain was moderately virulent for coturnix quail by the oral route, highly virulent for bobwhite by stick-wing and avirulent for quinea fowl (8). The result of our study showed that this organism was virulent for ducks by the S/C route which was similar as in the bob white. Furthermore it has been found that the susceptibility to the CU strain vaccine in turkeys appears to vary more and probably was influenced by stress factors such as environmental temperature (112). Presumably, the reason for our resuty could be that each duck had to be handled in the S/C and the oral vaccination which would create the stress condition especially in S/C vaccinated ducks thus the severe losses following vaccination (30% and 20%) occurred in this group whereas only 2% and no death occurred in the oral and in the drinking water vaccinated group respectiverly.

It has been generally accepted that turkey must be given approximately 10^8 organisms of this vaccinal P.multocida orally in order to develop a sufficient level of immunity (88, 90). Dose as hight as 2.26×10^9 or 5.29×10^9 organisms per turkey has been used without the detrimental effects in previous study (74, 89). Thus,

before this study began, dose of vaccination per duck was designed in range of not less than 1 x 10^8 organisms to not more than 5 x 10^9 organisms.

Dose of vaccination used in this experiment approximately was 1 x 10^9, 4.4 x 10^8 and 2.0 x 10^8 viable cells per duck in the first vaccination, in the second vaccination one month apart, in the second vaccination two months apart respectively. In spite of the difference in each dose of vaccination, it has been presumed that an effective immune respouse could still be comparable. This relying bases on the previous report by Bierier (89) that vaccination in dose of 1.13 x 10^8 viable cells per average turkey appeared to be equally as effective as vaccination in dose of 2.26 x 10^9 viable cells with reference to an effective immune response.

For the convenience in field use and the precision of dose of vaccine the lyophilized form may be available. Since it has been found that the freshly cultured vaccine was as effective as the lyophilized vaccine in turkeys. Moreover, turkeys vaccinated with the latter were less depressed than those vaccinated with the former (91). However study in duck should be done further.

The level of protective immunity was expressed in term of the percentage of protection which was calculated by substracting the percent survival of vaccinated group from the percent survival of unvaccinated group that exposed to the same challenged dose. The high level of more than 60% protection was cited to be a satisfied protective immunity. Thus it was concluded that the single vaccination could not provide an adequate immunity in all groups

except the S/C vaccinated group. However a quite low immunity was observed in 1 and 2 wk postvaccination (-15% and 56% protection). Especially in 1 wk postvaccination the percent survival of this vaccinated group was lower than that of the unvaccinated group. Comparison to the other two vaccinated groups which an evidence of immunity has been observed since 1 wk postvaccination. In addition, data in in turkey showed that vaccination with the CU vaccine via wing-web puneture or drinking water provided the same onset of immunity as early as 4 days after the first vaccination (91, 83). Thus the plausible reason to explain this data should be that the S/C vaccinated group has still been weak from the attack of vaccination rather than the onset of immunity was more than 1 wk .

The data suggestsed that double vaccination is superior to single vaccination in stimulation an effective immunity. Similar results was obtained in chicken (93) and turkey (91, 95). Double vaccination by S/C route appeared to be equally as effective as vaccination by oral route with reference to a high level of immunity. For double vaccination of 1 month interval, a high level of protective immunity (not less than 80% protection) persisted throughout 1 to 8 wk postvaccination except in drinking water vaccinated group which the immunity gradually waned on 2, 4 and 8 wk postvaccination. An apparent conflict was observed that double vaccination of 2 months interval this group becomingly showed higher immunity than the others. It was so difficult to explain what influenced this variation. One possible reason was that the 1 : 20 dilution of vaccine in drinking water was not adequate to accomplish regular consuming in this group. Some ducks were unable to consume an adequate amount of vaccine during a single period. Perhaps the administration vaccine in higher dilution (more than 1 : 20) for the two consecutive days would be more

advantageous. Futhermore the amount of viable bacteria in the drinking water should be investigated although the nonchlorinated water was used in this experiment . Perhaps increasing dose of vaccine or mixing some enhancing substances into the drinking water as skimmed milk would afford an adequate viable cells. We quite conceive that the immune response of this group will be as good as that of the oral vaccinated group if a good vaccine administration is performed.

In comparison of double vaccination of 1 month interval and double vaccination of 2 months interval, it seems likely that the former should be more reasonable than the latter for S/C vaccination which was contrary to the oral and drinking water vaccination. This relying bases on the comparison of the degree of protection in 4 wk after the second vaccination between Table 8 and 9 and considering the percent protection in 4 wk and 8 wk after single vaccination. There was no difference in an effective immune response between ducks vaccinated twice 1 month and 2 months apart in S/C vaccination. However a high level of immunity has still remain at 4 wk (88% protection) then dropped to 60% on 8 wk after the first vaccination. To avoid the immunocompetitive effect, thus the second vaccination in 2 month apart seems to be more suitable for this group. Considerably, the level of protection of the other two groups was very low since 4 wk after the first vaccination thus the second vaccination in 1 month interval would be quite reasonable.

In conclusion, for the single vaccination could not provid an adequate immunity except the S/C vaccination. For double vaccination the S/C vaccination gave high protection as hight as the oral vaccination and gave higher protection than the other. However

the S/C vaccination induced high mortality and the oral vaccination was so difficult in practical thus the drinking water vaccination seems to be the most available if the administering method was improved to accomplish that each duck was able to consume an adequate amount of vaccine to provide an effective immune response.

Antibody Response.

The results suggested that the S/C vaccination induced higher TA and PHA titers than the oral and the drinking water vaccination especially the GMT detecting by the CU strain sonicated antigen in these latter 2 groups mostly were lower than 2.00. It was probable that vaccination by oral or drinking water could not establish enough invasion in order to provide a high systemic humoral immune response as in the S/C vaccination. Since some bacteria had loss in feces as reported by Coates et al. (88) and some remained behind in the digestive tract as study in mice by Flossmann et al. (113). However on view of these, it became a good benefit to ducks vaccinated by oral and drinking water route that these ducks would expose the vaccinal bacteria continuously for a time and certainly the remaining bacteria would stimulate a long period of local immunity. These coincide with the finding of Dua and Maheswaran (94) who stated that vaccination of turkeys by administering the live CU strain vaccine in drinking water induced local antibodies in the tracheal secretion up to 42 days after single vaccination. It has been reported that oral vaccination of turkeys with the CU strain resulted in local as well as systemic dissemination of the organisms, it persisted in lung and spleen up to the fourth week after vaccination (114). Deplorably , no detailed study about the dissemination details of the S/C vaccination with this vaccine was reproted .

There was a fluctuation of antibody levels. Two hypothesis for explanation are as following.

1) The organisms grow and disseminate in ducks when the level of antibody increases, the bacteria drop in the amount and disseminate to a low blood supplying organ of duck as a latent period. When the level of antibody decreases it become grow up and reach the blood that will stimulate the antibody response repeatedly in cycle. This relying bases on the findings that the CU strain strain persisted for a long period in vaccinated turkey (114, 115) and medication of turkeys with systemic antibiotics reduces immune responses (116).

2. The autoclaved antigen is crude heat labile antigen and the sonicated antigen is crude protein soluble antigen. These antigens might contain many kinds or many antigenic determinants which will elicit many specific antibodies for each antigenic determinant which these antibodies will peak on maximum level in different time.

This experiment found uncommonly that the antigens prepared by the heterologous 8 : A strain tended to give higher antibody titers than those from the homologous CU strain . This result may be due in part to the difference between the amount of hyaluronic acid in capsules of two strain. Carter (117) noted that the presence of hyaluronic acid particularly in serogroup A strain inhibited the reaction between antigen and antisera in the PHA test. After treating these strains with hyaluronidase he was able to demonstrate considerably higher PHA titers. Other study of Alexander and Soltys (102) dealing with the difference in determination of agglutinating titers with the autoclaved antigen between the strain 1 : A and 5:A has cited to the effect of the hyaluronic acid in the difference of antigenicity between two strains .

With regard to the unvaccinated ducks enhanced the TA titers as comparable with ducks before vaccination. Although the level of antibody titers could not represent protective immunity, the rise of antibody titers indicated that the control ducks may obtain the natural exposure or expose to the low number of vaccinal bacteria by inhaling the fecies of the vaccinated ducks. Study of Coates et al. (88) have found that turkey received live CU strain vaccine in drinking water would excrete the bacteria in feces for 1 wk following vaccination. This finding was in contrast with the report by Bierer and Derieux (87) that transmission to nonvaccinated turkeys in cohabitation among turkeys vaccinated with this live vaccine by administering in drinking water, was not easily accomplished.

Again, the TA titers of unvaccinated ducks were surprisingly higher than those of some vaccinated ducks as shown in Fig. 4, 5, 6. This abnormality can not, however, be explained.

Another point of view , the fluctuation of TA titers and the raising of TA titers of unvaccinated ducks may be interpreted that the TA titers is a result of non-specific antigen-antibody reaction. However PHA titers showed a normal pattern of antibody response Thus it is more reasonable to employ the PHA test in determination of antibody levels .

The study revealed that there was a lack of correlation between the TA and PHA antibody and the level of protective immunity. These finding were consistent with previous reports of Heddleston and Watko (118), and Bhasin and Biberstein (119). They concluded that neither the agglutination nor the passive hemagglutination reaction gave indications of the immune status of vaccinated birds where they were

subsequently exposed to virulent P.multocida . However, the study of Pongsopida et al. (120) found a correlations between resistance to challenge and the TA titer detecting by the 8:A strain autoclaved antigen. Another study of Dua and Maheswaran (74) reported a significant degree of correlation between antibody against the sonicated Ag of the P 1059 strain of P.multocida by means of the PHA test and the level of protection. This discrepancy to our trial may be due to the difference in vaccine type for the former and the difference in the organism strain used for antigen for the latter.

To date, the nature of immunity induced by various fowl cholera vaccines remains poorly understood. Schlink (115) as well as Dua and Maheswaran (74) concluded that both cell - mediated immunity (CMI) and humoral - mediated immunity (HMI) played important role in protection of the CU strain vaccine against fowl cholera in turkeys. Protection studies in cattle (121) and mice (122, 123) indicated that HMI was important in protecting vaccinated animal against P.multocida infection. In chickens and turkeys unlikely in mammals, studies of Baba et al. (124, 125) indicated that CMI plays a more important role than HMI in protecting against systemic pasteurellosis. The results of antibody response in our trials of ducks was in agreement with the reports of Baba et al. (124, 125). That HMI may be participating in some unknown way to encourage the CMI but not play a significant role throughout the defense mechanism since the antibody levels could not indicate the immune status of ducks.