## 5. DISCUSSION AND CONCLUSION

### 5.1 Discussion

In this study, three diagnostic methods compared respectively, embryonated eggs, MDCK cells and RT-PCR to find out minimum detectable avian influenza virus concentration. The selected test were similar with WHO recommend test method (WHO, 2002) only available reference describe virus isolation and identification that is recommend the test method out of these three methods. But carrying out this study found out. The lowest of minimum detectable concentration detected by using embryonated eggs and MDCK cells ($HA\times10^{-7}$, $10^{-6}$). Furthermore, the highest of minimum detectable concentration was received by using RT-PCR ($HA\times10^{-2}$). The samples use to work from cloacal swab and lung organs from apparently healthy chicken. WHO recommend selection of the sample (WHO, 2002). The result of minimum detectable avian influenza virus concentration by using two types of sample is cloacal swabs and lung organs ($HA\times10^{-5}$, $10^{-4}$).

The high sensitivity of virus isolation by using embryonated eggs (Table2.) similarly of the result with MDCK cell method (Table3.) which similar result of sample from cloacal swabs and lung organs. In addition, compared with RT-PCR test the result show the lowest of sensitivity at the highest of virus concentration. (Table 4.). Comparison of the detectability level between methods; compared virus isolation and identification using embryonated eggs and MDCK cells was not difference the result (p=0.594) between this method. Embryonated egg compared with RT-PCR method and MDCK cells compared with RT-PCR method there were difference of the result (p=0.001, 0.001) respectively. Among embryonated egg, MDCK cells and RT-PCR their were difference of the result (p=0.001). For cloacal swab sample compared with lung organs sample was not difference the result (p=0.219).

Specimens are inoculated into allantoic cavity of 9 -11 day embryonated chicken eggs. High yields of virus can be harvested after 3 days of incubation and use for the routine diagnosis of influenza infection (WHO 2005d). For conventional culture: various cell lines are utilized to isolation influenza viruses. some author recommend the use of trypsin to aid virus entry into the cell line (WHO 2005d). Virus isolation by using MDCK cells cultures is a conventional culture (Petric Martin, et al., 2006)

Compared to other reported assays (OIE and National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy.). Virus isolation in specific-pathogen-free (SPF) embryonated eggs or cell cultures is traditionally considered the method of choice for the detection and identification of avian influenza (AI) viruses. However, its value is limited because it is time-consuming and not cost-effective. AI is a highly contagious disease, able to spread in a susceptible population in a short period of time. Therefore, the prompt identification of an infected flock is crucial for control and eradication purposes.

Comparison among three diagnostic methods. For the results of virus isolation and identification by using embryonated eggs and Mardin Darby Canine Kidney cells (MDCK) culture received better results than RT-PCR in the present investigation.

Result of the present study showed the negative result for the first passage and the positive result for the second passage in case of cloacal swabs and lung samples following the methods of embryonated eggs and MDCK cell cultures. Conversely following the RT-PCR method received negative results it may due to the virus detection from the suspension. When compared these three methods RT-PCR was time saving method than other 2 methods MDCK and embryonated eggs. As well WHO has recommended RT-PCR is the most sensitive method (WHO, 2005) But leaving the rapidity and sensitivity of the RT-PCR these 2 method of MDCK and embryonated eggs were able to detect virus at the lowest concentration($HA10^{-5}$to $10^{-8}$) but the RT-PCR was unable to detect virus at the same lowest concentration ($HA10^{-5}$ to $10^{-8}$). On the other hand, the RT-PCR did not perform well for this method in this study. Three factors may have influenced the results of this assay. First, preparation

and inoculation of antigen were not optimized by the number of samples that were inconclusive due to loss of antigens during processing. Second, store the antigen and suspension of experimental study were not appropriate for this procedure. The last factor the total amount of virus particle were not enough due to influenced the result. Additional work is needed to optimize this test method, which could be rapid method for diagnosis of avian influenza virus infection. On the other hand, molecular diagnostic techniques play a more and more prominent role in laboratory diagnosis of influenza. Direct rapid tests have also become an important tool for investigating influenza disease. In addition, suspected case from HI test should apply RT-PCR for further viral identification. The result from combined test is more efficiency.

Finally, in this study, the virus isolation and identification using embryonated eggs and MDCK cells culture should be select the appropriate laboratories, which require most infrastructure and can be performed by staff with limited knowledge of virology. Furthermore, viral culture however remains important especially for reference laboratories since it is cheap, sensitive and enables characterisation of viruses. Furthermore unlike molecular testing it is "unbiased" and can detect the unexpected new strain.

## 5.2 Conclusion

Methods used for influenza virus isolation and identification in birds should be specific enough to allow detection of antigenically and genetically different influenza subtypes. For the virus isolation and identification, embryonated eggs, MDCK cells and RT-PCR are widely used to detect influenza viruses directly in specimens collected from animal species susceptible to influenza virus infection. Among them, The RT-PCR is the highest virus detectable concentration. For the virus isolation and identification using embryonated eggs and MDCK cells yield similar minimum detectable virus concentration, which both of the method can detect at the lowest concentration of virus. However, there was a statistically significant difference among 3 different methods but there was not statistically significant difference between two types of samples of the cloacal swabs and the lung organs. It was not different between two sample types of virus isolation and identification.

This assay would be highly useful as a diagnostic tool to help identify of H5 Avian Influenza A virus isolate from poultry specimens. and control influenza epidemics. We can thus conclude that virological diagnosis for influenza has value for the individual laboratory, epidemiological investigations and infection control. The appropriate selection of a particular test is determinded by the test characteristics and the specific diagnostic need.