Distribution of Highly Pathogenic (H5N1) Avian Influenza Virus in Meat from Experimentally Infected Chicken and Ducks

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ABSTRACT

Avian influenza caused by H5N1 is one of the most important Zoonotic disease and is becoming great threat to poultry industry as well as to humans. Contaminated poultry meat may play role as reservoirs and sources of transmission for avian influenza virus. However, very little is known regarding the distribution of highly pathogenic (H5N1) avian influenza virus in poultry meat in tropical countries. The aim of this work was to study distribution of highly pathogenic (H5N1) avian influenza virus in meat from experimentally infected chicken and ducks. Three chicken and ducks were artificially infected with Highly Pathogenic Avian Influenza (H5N1) virus isolates (A/duck/Tripura/103597/2008 and A/duck/Tripura/02CA10/2011) obtained from the 'Avian influenza virus repository' of ICAR- National Institute of High Security Animal Diseases, Bhopal (NIHSAD). Different meat samples from the infected birds were collected. Presence of virus in all meat samples was confirmed by egg inoculation followed by haemagglutination test prior to use for the experiments. In the present study, chicken meat samples were found to have the highest viral load. The study also revealed that the initial viral load of both the isolates in chicken meat samples was considerably higher than that in duck meat samples. The study gives insights into the role of poultry meat in avian influenza virus transmission and helps in formulating avian influenza control strategies.

Keywords: HPAI; Meat-borne H5N1; meat viral load

Introduction:

Indian poultry industry has emerged as the world’s second largest market and has been growing at around 8-10% annually over the last three decades. India ranks fifth in world egg production and sixth in the poultry meat production. Frequent HPAI AIV outbreaks in the last 7 years reduced our meat exporting due to lack of surveillance of AIV in poultry meat. Even though there is no exact report regarding human cases because of infected meat consumption there is a high risk of disease transmission by this route particularly during the handling and transport of the infected products. Food-borne transmission of infection through poultry products may only occur if there is viable virus in the commodity and the concentration of the virus is sufficient to infect the given host that is exposed to the source of infection (Beato et al., 2008). Highly pathogenic AI virus has been isolated from poultry meat (Swayne and Beck, 2005) and thus infection of poultry with these viruses poses a risk of spread and transmission through movement and trade of meat and its products. For example, the incursion of H5N1 HPAI in backyard chickens in Germany in 2007 was linked to exposure to contaminated duck meat and offal from commercial ducks with a subclinical infection with HPAI H5N1 (Harder et al., 2009). AIV can survive for several days in poultry carcasses at ambient temperatures (Animal Health Australia, 2008). Tissue derived from infected birds can become a source of environmental contamination (Stallknecht and Brown, 2009). In addition, contaminated tissue can be a source of direct infection for other animals or humans because it can become a target for scavenging wildlife (Webster et al., 1992), food for domestic animals and humans or because its disposal requires human handling (Songserm et al.,
In view of the importance of the meat derived from avian influenza virus infection in infecting the humans as well as potential source of viral contamination of the environment, a study was carried out to determine the viral spread in different tissues commonly used for meat from the experimentally infected ducks and chickens.

Materials and Method

The Virus:

Highly Pathogenic Avian Influenza (H5N1) virus isolates (A/duck/Tripura/103597/2008 and A/duck/Tripura/02CA10/2011) obtained from the "Avian influenza virus repository" ICAR-NIHSAD, Bhopal were used in this study. The two isolates were selected because of their clade variations that indicated variation in their origin and to observe whether there was any difference in the spread of the virus amongst the different tissues. In order to simulate the actual possibility of infected meat being available in the market chain, three chicken and ducks were artificially infected with two different virus isolates and the different meat samples from the infected birds were collected. The presence of virus in meat samples was confirmed by real time reverse transcriptase polymerase chain reaction (RRT-PCR) prior to use for the experiments. In order to estimate the viral load in the different tissues, EID$_{50}$/g of each meat sample was calculated as per Reed and Muench (1938) method (Table 1 and 2). Both the virus isolates were amplified by ECE inoculation and the allantoic fluid containing virus was collected and pooled. The HA titre of allantoic fluid was found to be 28. EID$_{50}$/g of the virus isolates (A/duck/Tripura/103597/2008 and A/duck/India/02CA10/2011) was estimated to be 10$^{7.43}$/ml and 10$^{7.17}$/ml, respectively.

Experimental inoculation and Sample collection:

Six SPF chickens of 4 weeks of age reared in SPF unit of the institute and six ducks tested and found to be sero-negative against H5 and H9 avian influenza virus antibodies were used in the study. The chickens and ducks were divided into two groups, each group consisting of 3 birds, and transferred to different bird isolators at the animal house facility of the ICAR-NIHSAD, Bhopal, Bhopal. Two different groups of both chicken and duck were inoculated intra nasally with 100 µl each of 10$^{7.43}$/ml and 10$^{7.17}$/ml of A/duck/Tripura/103597/2008 and A/duck/India/02CA10/2011 isolates, respectively. After infection, the birds were kept under observation till death or up to one week in case the birds were not dead, which were then sacrificed. Dead birds were brought to the laboratory for post-mortem examination which was performed inside Class II BSC. The samples required for the present study, viz., liver, gizzard, thigh muscle and breast muscle were collected in sterile polypropylene containers and immediately stored at -80°C till further processing. Presence of virus in all meat samples was confirmed by egg inoculation followed by haemagglutination test as described below prior to use for the experiments.

Hemagglutination (HA) Test:

Twenty-five µl of 1X PBS was dispensed into each well of a plastic 'V' bottomed microtitre plate (MS Greiner, Germany). In the first well of the first row, 25 µl of harvested allantoic fluid was dispensed and two fold serial dilutions of virus were made up to 12th well. Further, 25 µl PBS was dispensed in each well and finally, 25 µl of 1% (v/v) chicken RBCs were dispensed in all the wells. Likewise, allantoic fluid was dispensed into different rows and the dilutions were made as mentioned. The last row of the microtitre plate was kept as RBC control, which was prepared by dispensing 50 µl of PBS and 25 µl of 1% chicken RBCs. The plate was gently tapped for mixing and incubated at the room temperature (25°C) for 30 min in a biosafety cabinet. The HA titer was determined by tilting the plate and observing the presence or absence of tear shaped streaming of the RBCs against the RBC control. The reciprocal of the highest dilution giving complete HA

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Highly Pathogenic (H5N1) avian influenza virus in meat

Estimation of EID_{50} in meat samples:

For determining the initial viral load in the infected samples, the 50 % egg infective dose (EID_{50}) was calculated in one gram of meat sample using 9-11 days old embryonated chicken eggs. Live embryonated chicken eggs were selected by candling and used for calculation of EID_{50}. Tissue homogenate (10%) was prepared by taking one gram of the tissue in 10 ml of antibiotic treated phosphate buffered saline and processed using sterile pestle and mortar. Tissue suspension so prepared was transferred into 1.8ml sterile micro-centrifuge tubes and centrifuged at 8000 rpm for 5min, after which the supernatant containing the virus was transferred to 1.8 ml sterile micro-centrifuge tubes and stored at 4°C. A 10 fold serial dilution (10^{-1} to 10^{-10}) of the virus suspension was prepared. For each dilution, five eggs were inoculated with 100 µl each of the suspension through amnio-allantoic cavity and sealing was done with fevicol. Eggs were incubated at 37°C under 65% humidity. The mortality in the eggs was recorded for the next 5 days. After 5 days of incubation, the allantoic fluid harvested from live,dead embryos was subjected to HA test to ascertain the presence of the virus. The EID_{50} was calculated as per the method described by Reed and Muench (1938).

Results and Discussion

The estimation of viral persistence in meat is significant as consumption of infected meat has been linked with HPAI disease outbreaks in backyard poultry (Harder et al., 2009). Wild bird carcasses from animals that have died out of avian influenza pose a danger of virus transmission to susceptible birds and other animals through predation or cannibalism of infected carcasses (Swaye et al., 2008). There are several reports of HPAI virus infections following consumption of infected poultry meat by chickens (Swaye and Beck, 2005), consumption of infected duck carcasses by poultry (Harder et al., 2009), consumption of infected chickens by cats (Kuiken et al., 2004), consumption of infected duck carcasses by dogs (Songserm et al., 2006) and consumption of infected poultry by tigers and leopards (Keawcharoen et al., 2004). Hence, contaminated meat can be a potential source of virus transmission to many animal species. The two isolates selected in the present study belonged to two different clades. Isolate A. Duck/Tripura /03597/2008 belonged to clade 2.2.1, whereas, isolate A. Duck/Tripura /03597/2008 belonged to clade 2.3.2 (Nagarajan et al., 2012).

In the present study, the chickens which were inoculated with both the virus isolates died within 48 h, however, none of the ducks inoculated with the two virus isolates died upto a period of one week, and were sacrificed for harvesting the meat samples. In case of chicken samples, the liver and gizzard samples were found to have the highest viral load. The study also revealed that the initial viral load of both the isolates in chicken meat samples was considerably higher than that in duck meat samples. The fact that both the virus isolates failed to cause mortality in the ducks indicated that the systematic viral spread in the ducks was much lower than those in the chickens, wherein not only the chicken died within 48 hrs, but also showed a very high viral presence in all the tissues. There have been several reports, which have corroborated the observations of the present study regarding the difference in the effect of H5N1 avian influenza virus in different bird species (Beato et al., 2012). The variation in the viral load in different tissues of the two species of poultry could be attributed to the nature of the systemic spread of the virus amongst the various parts of the body, since H5N1 viruses have been known to proliferate faster in the chicken organs as compared to the ducks. This observation was supported by a study conducted by Beato et al. (2012) who showed that at zero day, meat samples collected from chickens showed the highest virus titer, followed by turkey, then duck. At zero day, virus titers in duck meat were (log10 1.9-2.7 g), turkey meat (log10 3.1-4.5 g) and chicken meat (log10 3.9-5.1 g). Amongst the two different muscles in the present study, the thigh muscle was shown to have higher viral load as comparing to breast muscle in both the species. Swayne and Beck (2005) reported about the variation in the viral load among breast and thigh muscle, shown log10 2.2-2.7/ g and log10 2.6-3.2 g, respectively. Other study shows that brain, lung, kidney, and thigh muscles shown higher titer (6.0 to 6.7 log10 ELD_{50}/gm of tissue), whereas, breast muscle shown comparatively less viral titer (5.3 to 5.5 log10 ELD_{50}/ gm of tissue) (Tumpy et al., 2002).

The present study gave an insight into the presence of highly pathogenic avian influenza virus in the infected poultry and considering the large quantity of poultry meat being produced worldwide, inactivation of the avian influenza virus after the slaughter or during the processing, transport and storage of meat is an unfeasible proposition, owing to post processing cross contamination of the meat and the presence of viruses in the meat, particularly at lower temperatures. It therefore, reemphasizes the need to keep a constant vigil during poultry production stage so that the meat from avian influenza infected birds does not enter into the food chain at the consumer level.

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