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Epidemiology and characterization of avian influenza in smallholder poultry in Mozambique

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ABSTRACT

Livestock constitutes an invaluable asset for the rural poor in the developing world, as a source of protein as well as of income, and its importance for global development, poverty alleviation and food security was recently acknowledged in the World Development Report. Poultry production is seen as a way of stimulating economic growth in rural and peri-urban areas and to increase protein consumption. An outbreak of infectious disease affecting the poultry could have disastrous consequences for rural communities depending on their chickens. The aim of this study was to get an indication of the prevalence of Avian Influenza in Mozambique and to characterize the different strains that circulates in the poultry and, on a personal note, get increased understanding of how infectious diseases are affecting livestock production in developing countries and get experience of carrying out veterinary work under significantly different conditions.

This study was performed as a Minor Field Study (MFS) in collaboration between SLU, Eduardo Mondlane University (EMU) and the Central Veterinary Laboratory of the Directorate of Animal Sciences (CVL)

During eight weeks we collected blood samples and cloacal swabs from rural poultry in different villages around Maputo in the south of Mozambique. We also collected data from the households through a questionnaire. Initial laboratory analyses focused on antibody detection, using commercial ELISA kits. Further analyses, to detect and characterize virus, are being done in Sweden using PCR and HI-test.

Out of 457 samples 16 were ELISA positive but there were some indication that the prevalence is higher in the north.

INTRODUCTION

Livestock constitutes an invaluable asset for the rural poor in the developing world, as a source of protein as well as of income, and its importance for global development, poverty alleviation and food security was recently acknowledged in the World Development Report (www.worldbank.org).

The development of the poultry industry is a good example of agricultural success. Today it is the largest income producing agricultural commodity in many regions around the world. Poultry production is seen as a way of stimulating economic growth in rural and peri-urban areas and to increase protein consumption. In low-income countries most people would benefit from eating more meat and eggs and in Africa the demand for animal protein products are rapidly growing. In many African countries, commercial farming has started after independence (Garcês A, 2008).

Village chickens require the lowest capital investment of any livestock species and they have short production cycle. Goats and cattle require herders to stay with them during the day so in households headed by widows, children or grandparents chicken represents the easiest species to raise for sale and home consumption.

Chicken meat and eggs provide a readily available, high-quality source of proteins and vitamins (Ahlers C, 2009).

AVIAN INFLUENZA

In 1878 an Italian scientist, Edoardo Perroncito, described an outbreak of an infectious disease affecting domestic birds in farms near Turin, Italy. This is believed to be the first reported case of HPAI or “fowl plague” (Perroncito, 1878). During the last 50 years, there have been 24 outbreaks of HPAI in the world (counting the Asian H5N1 HPAI virus as one single epizootic) the first being in Scotland 1959. The frequency of outbreaks and the number of affected birds is however increasing. The currently circulating highly pathogenic H5N1 was first isolated in China 1996. It drew public attention in 1997 when it crossed the species barrier and infected 18 humans, killing 6 (WHO). In 2003 the same virus caused sudden outbreaks in poultry populations in several countries in Southeast Asia and then it just escalated. “The emergence of HPAI H5N1 virus in Southeast Asia and its spread across Asia and into Europe and Africa is unprecedented in the virological era” (Capua and Alexander, 2009). So far more than 500 million birds have died or been destructed. Between 2003-2009, 442 human cases have been reported to WHO, with 262 deaths (www.who.int).

Avian influenza is a highly contagious viral disease affecting several species of birds. It is caused by various serotypes of influenza A virus of the Orthomyxoviridae family. The Orthomyxoviridae family includes Influenzavirus A-C, Thogotovirus and Isavirus. Influenza A virus is the only one in the Orthomyxoviridae family that is known to infect birds. Type A influenza can be divided into subtypes based on the relationship between the glycoproteins on the surface, haemagglutinin (H1-H16) and neuraminidase (N1-N9). Each virus has one haemagglutinin and one neuraminidase antigen in any combination (Capua and Alexander, 2009). Exemples are H1N1 (current “swine flu”), H3N8 (equine influenza), H5N1, H5N7 etc.

Influenza viruses have segmented negative-sense single stranded RNA genomes. It replicates in the nucleus of infected cells. Like other RNA viruses, influenza A virus display a high mutation rate and since the genome consists of eight different segments of RNA which act like mini-chromosomes, genetic mixing or recombination (reassortment) can occur if the host is infected by two different strains of influenza. Reassortment is responsible for some of the major human pandemics in the history of the influenza virus (Zohari, 2009).

The AI viruses in poultry are divided in two groups, or pathotypes, based on their ability to cause disease. Low pathogenic avian influenza (LPAI) viruses cause mild disease which may be undetected whereas highly pathogenic avian influenza (HPAI) viruses spread rapidly and cause serious disease with high mortality rates (up to 100% within 48 hours). The symptoms of AI include respiratory tract disease, decrease in egg production, or a multiorgan systemic disease. HPAI is caused by some haemagglutinin subtypes, H5 and H7, which exhibit certain molecular characteristics, more specifically, multiple basic amino acids at their proteins cleavage sites, which LPAI viruses are lacking. This mutation allows protein to be cleaved by different proteases which makes it possible for the viruses to multiply systemically.

So far, the subtypes H5 and H7 are the only ones that have caused HPAI but far from all of them are virulent. It seems to be a mutation from LPAI that occurs when poultry is infected by wild birds. Most HPAI viruses have been the result of spontaneous duplication of purine triplet but this is not the only mechanism by which they can arise. What causes the mutation from LPAI to HPAI are not known but it would be reasonable to assume that the more LPAI circulating in the poultry, the greater the risk of mutation. Therefore it is of great importance to try to limit the spread of LPAI.

Influenza virus is shed in faeces and discharge from infected birds. It can survive for weeks in a pile of droppings but is destroyed by direct sunlight. Poultry living outside is most likely to be exposed. Migrating birds are a major source of infection but transmission also occurs with people, vehicles, feed, equipment etc (Capua and Alexander, 2009).

Symptoms

Clinical signs of AI are observed mainly in chickens and turkeys. Ducks and geese may carry the virus without showing any symptoms. The incubation period is usually 2-7 days and the symptoms range from subclinical to sudden death. Morbidity in HPAI is high, up to 100 %, in LPAI it varies.

LPAI: The severity of the clinical signs depends on several factors like age, secondary infections and bird species. Depression, ruffled feathers, anorexia, respiratory distress and swelling of the infraorbital sinuses are common symptoms. In young birds or with certain strains of LPAI, mortality can be significant.

HPAI: In most cases the infection is fulminant. Sudden deaths, anorexia, drop in egg production, mild to severe respiratory distress and/or nervous signs like tremors and incoordination is often seen. There might also be respiratory discharge and swelling of periorbital tissues and sinuses, Mortality may be as high as 100 %.

Differential diagnosis

Newcastle disease can not be distinguished from AI without laboratory testing. The symptoms of ND include ruffled feathers, lethargy, marked decrease in egg production, respiratory signs, greenish diarrhoea and nervous signs like tremor and paralysis of wings and legs. Other differential diagnoses are:

LPAI : any disease which cause similar symptoms e.g.: Infectious Bronchitis, which in young birds causes depression, sneezing, nasal discharge and coughing. Infectious Coryza can give symptoms like facial swelling, ocular and nasal discharge and dyspnoea. Mycoplasmosis causes poor production and chronic respiratory signs including ocular discharge and coughing.

HPAI. The main infectious diseases similar to HPAI are: Infectious laryngotracheitis which in its acute form induces severe respiratory distress and high mortality. Infectious bursal disease in young chickens causes diarrhoea, dehydration, nervous signs, anorexia, ruffled feathers and increased mortality. Acute Fowl Cholera can cause high mortality, lethargy, neurological signs and discharge from nose and mouth. Differential diagnosis to HPAI could also be any

other circumstance causing a sudden high mortality e.g poisoning or botulism (Engström, 2004).

Avian influenza in wild birds-the natural reservoir

Almost all subtypes and combination of AI have been found in wild birds. Especially aquatic birds are considered to be a natural reservoir for AI. Nearly 60 percent of the wild species infected with H5N1 virus are birds with affinities to wetland habitats. Waterfowl (ducks, geese, swans) are natural hosts for LPAI viruses and they are the only group of wild birds where it can be found year round. Many bird species travel long distances between their breeding ground and non breeding areas. As natural hosts for AI viruses, the seasonal movements of these species can play an important role in spreading LPAI and might also have played a role in the spread of the H5N1 virus. Areas with big problems with AI are usually located in migratory routs (www.fao.org).

Avian influenza in mammals

There have been several reports of mammals infected by avian influenza. For example during 1979 and 1980 there was an outbreak among harbour seals around Cape Cod Peninsula in the USA, killing about 20 % of the population and there has been reports of fatal infection in both domestic cats and zoo felids after feeding on infected chickens during the 2003-2004 HPAI H5N1 outbreak (Capua and Alexander, 2009). In 1984 in Sweden, H10N4, previously only seen in birds, was isolated from farmed mink during an outbreak of respiratory disease (Klingeborn et al., 1985). Around 100 000 animals became infected and 3000 died (www.sva.se). Pigs are considered a risk to humans in their role as mixing vessels. Since they are susceptible to both human and avian viruses there is a constant chance they might “create” a new pandemic virus (Capua and Alexander, 2009). This year’s pandemic H1N1 is a mix of swine-, avian- and human influenza genes. This so called triple reassortment has however circulated for some time in pigs (Peiris et al, 2009).

Diagnosis

Early detection of HPAI is essential for rapid control of the disease. Even if only LPAI is suspected it is important to know which type since the subtypes H5 and H7 are progenitors of HPAI. EU has a diagnostic manual for avian influenza in order to ensure uniform procedures. Analyses are performed step by step in order to achieve detection, subtyping and pathotyping. Two main diagnostic pathways can be used. The conventional pathway includes virus isolation in eggs and HA test and HI test for subtyping. To evaluate the pathogenicity, chickens are inoculated with the isolate. OIE defines HPAI as any AI with a 75 percent or greater mortality rate. ELISA can be used in screening as a complement to HA/HI. The molecular pathway includes several PCR assays performed stepwise. Firstly, samples are screened by a real-time Matrix PCR. Positive samples are then subjected to H5 and H7 specific PCR. Finally, sequencing is done to identify pathogenic characteristics of the virus and to trace it epidemiologically.

Elisa-test

Enzyme-Linked Immunosorbent Assay (ELISA) - is used to detect antibodies against avian influenza.

Viral antigen is coated on 96-well plates. During incubation, antibodies specific to AI forms a complex with the antigens. After washing away unbound material from the wells, a conjugate is added which binds to any attached antibody in the wells. Unbound conjugate is washed away and enzyme substrate is added. Subsequent colour development is directly related to the amount of antibody against AI present in the test sample. There are commercial assays that are based on this method.

HA/HI-test

Haemagglutination test (HA test) – this method is based on the reaction between haemagglutination activity of the virus and red blood cells. Samples are diluted with PBS across a micro titre plate and then a red blood cell suspension is added. After incubation the plate is read. If virus is present, a fine layer of red blood cells will line the bottom of the wells. The HA titre of the viral suspension is the highest viral dilution that causes agglutination. To perform HI test and characterise the virus subtype, a 4-HAU antigen solution is used. Common HA test cannot distinguish between AI and NDV. HI test is then necessary to tell them apart.

Haemagglutination Inhibition Test (HI test) - is based on a reaction between the virus and a specific antiserum; it binds to the epitopes that are responsible for haemagglutination, thus these epitopes will not bind to red blood cells which will sediment in the shape of a button. In wells of a plastic micro titre plate, antiserum and antigen solution is added and diluted. After incubation red blood cell suspension is added and after a second incubation period the plate is read.

PCR

Polymerase chain reaction (PCR) – is a molecular method. If a sample contains just a few copies of an intact RNA/DNA strand it can be amplified, as long as a sequence of at least part of the gene of interest is known. Two chosen or created primers have sequences complementing primer binding sites at the end of the target gene. *Taq* polymerase then synthesizes complementary strands. It is also possible to start from RNA, like in the case of influenza, if it is first transcribed into cDNA. There are a number of published PCR assays for avian influenza and other viral diseases.

Prevention

Vaccination against HPAI has been used in several countries but mostly to stop ongoing outbreaks. Other ways to prevent outbreaks is to keep birds inside and larger facilities should not be placed in migratory routes. Very strict biosecurity can limit spread in commercial farms. Avoid feeding birds outside to prevent attracting wild birds, transports of live birds should be limited and precaution should be taken to avoid spreading of disease.

LPAI should also be fought to prevent spreading and the risk of mutation.

When it comes to rural poultry this doesn't really apply, they can't keep their poultry inside. What can be done are the little things like keeping the poultry house clean and have separate night housing for different species. Dispose of sick and dead animals correctly. Keep new birds separate from the flock for 2 weeks. If possible, provide supplementary feed to promote good health. Inform poultry traders about the risks and how to take precautions.

THE STUDY

In Mozambique, as in many other African countries, poultry are kept everywhere. Medium sized to small-scale production and village or backyard poultry adds up to over 20 million poultry. The biosecurity standard varies from reasonable to low. The most common is family poultry, which consists of the extensive (or scavenging) system where all birds move freely within the village, feeding on what is available and mixing with other animals or small scale intensive poultry production where the birds are fenced in and fed. It is quite common to house the poultry at night to protect them from predators. The type of poultry house varies widely depending on the area. It can just be a barrel or a small house made of mud or straw. Most birds are sold alive throughout the country, on markets or by traders, which constitutes a risk of spreading disease. Two thirds of the country is infested with tsetse fly, which limits the cattle production and makes the poultry production even more important. Unfortunately, the poultry industry has serious problems with management, nutritional deficiencies and diseases. The most important known diseases are Newcastle disease, Fowlpox, Fowl cholera and parasites. Two thirds of Mozambicans live in absolute poverty, surviving on less than 0.25 USD per day and like other sub-Saharan African countries, Mozambique has been seriously affected by AIDS (Anjos, 2007). An outbreak of infectious disease affecting the poultry could have disastrous consequences for rural communities depending on their chickens. Avian influenza (AI) and Newcastle disease (ND) are considered two of the most devastating diseases of poultry throughout the world. Newcastle will be dealt with in another degree project (Perttula, 2010), and the focus of this work will be AI.

Mozambique has come a long way after almost two decades of war (ended in 1992) but there is still a lack of sufficient infrastructure for necessary epidemiological studies and surveillance and even if the presence of highly pathogenic avian influenza (HPAI) is unlikely, the prevalence of AI is unknown. So far, 13 countries in Africa (Zimbabwe, 2005, Burkina faso, Cameroon, Côte D'Ivoire, Djibouti, Egypt, Niger, Nigeria, South Africa, Sudan, Ghana, 2006, Benin, Togo, 2007) have officially reported cases of HPAI to the World Organization for Animal Health (OIE). There is a risk of further spread in Africa and it could occur from the legal or illegal movement of poultry or poultry products, and/or the interaction of domestic poultry with infected wild bird populations. Free-range flocks are a high risk group since they are constantly exposed to wild birds.

The aims of this study were to get a first estimate on the prevalence of AI in poultry in Mozambique and to partially characterize any influenza viruses detected in the study population. It was also a way to get increased understanding of how infectious diseases are affecting livestock production in developing

countries and get experience of carrying out veterinary work under significantly different conditions.

Study region and study population

Samples were collected, consisting of blood samples for serology and cloacal swabs for virus isolation and PCR. The samples were taken from chickens, ducks and turkeys and collected in villages in the Michungulene and Magude region and previously collected samples from Limpopo national park were also included in the study.



Figure 1. Map of Mozambique (www.mapsofworld.com). The sampling areas are within the circle.

Each village was considered as one epidemiological unit since the poultry were mostly kept as free-range and had the opportunity to mix. From each village 27-35 birds from 7-9 households was randomly selected for sampling. This sampling strategy allowed us to detect infection within each village with a confidence of 95%, assuming a prevalence of 10-15%. It also allowed us to estimate the seroprevalence in the two regions with a 5% precision and a 90% confidence interval.

Sampling

After permission was given from the chief of each village, samples were collected. From each bird 0,5-2 ml blood was collected from the brachial vein using a 21 gauge needle and a 2 ml syringe. The syringes were positioned horizontally for 60 minutes to allow the blood to clot then the serum was transferred into test tubes and put in a cooler.

Two cloacal swabs were taken from each bird, one for virus isolation and one for PCR. The swabs were put in tubes with PBS antibiotic solution and put in cooler. Back at the laboratory they were put in a freezer that kept -70 °C.



Figure 2. Blood sampling

Laboratory analysis

Initial laboratory analyses was carried out at the CVL, and focused on antibody detection using commercial ELISA kits (FlockCheck Avian Influenza Antibody Test Kit, IDEXX Laboratories, Inc, USA) according to manufactures instructions. Confirmation of positive samples was attempted using HI-test. The equipment was not always of very high standard and there was a lack of multipipettes in the right size, making some of the analyses a bit difficult. There was also a lack of proper antigen to perform HI-test for other subtypes than H7. Further analyses, PCR and sequencing, will eventually be done in Sweden to detect and characterize virus.

At CVL we made our own Red Blood Cell suspension for HI: 5 ml blood was collected from a chicken, it was mixed 50/50 with anticoagulant and then centrifuged. The anticoagulant was discarded and then the red blood cells were washed 3 times in 10 ml PBS solution, centrifuged after each washing. The

supernatant was removed with a pipette, leaving the packed red blood cells. The packed red blood cells were then mixed with PBS to a 1 % solution. To avoid non-specific reaction when doing HI on sera from other species than chicken, the serum from ducks and turkeys was pre-treated with a 2 % RBC suspension.

Data collection

Data on the poultry population (number of chicken, ducks, geese, etc), disease history, and vaccination status was collected from each village and each selected household through a questionnaire. The complete questionnaire can be found in Appendix 1.

RESULTS

Prevalence

In the Michungelene area only 1 of 65 samples was ELISA-positive for avian influenza. In Magude, 2 out of 172 was positive and in Limpopo province, 13 out of 220 samples. All the samples were H7 negative in HI test.

The questionnaire (Appendix 1)

The questionnaire was answered by 35 households. It showed that the average number of chickens per household were 8, 5. Sixty-six % also had other poultry. Most of the households had a poultry house of some kind where they locked the poultry in at night. Seventy-seven % of the households had lost poultry within the last 3 months. The biggest problem was diseases, predators and thieves. Only 14 % vaccinated their poultry and only against ND. Most owners used traditional remedies to treat diseases and buried the birds if they died.

	Number who answered yes	percentage
Do you have a poultry house?	27	77%
Do you own other poultry?	23	66%
Do you have other animals?	22	63%
Do you have problems with disease?	23	66%
Do you have problems with theft?	14	40%
Do you have problems with predators?	17	49%
Do you have problems with feed?	7	20%

Has any of your poultry died in the last 3 months?	27	77%
Do you vaccinate your poultry?	5	14%
Do you use any kind of treatment if your birds are sick?	20	57%

Table 1. Summary of some of the questions in the questionnaire

Other observations

Most of the poultry were in good condition. Some were a bit thin. A lot of them had ectoparasites. A few suffered from what appeared to be poxvirus (nodules and scabs on the featherless parts of the body).

Two neighbouring, small scale broiler farms in the Magude region, suffered from an outbreak of some kind. Many chickens had died during the last 14 days. Most of the surviving birds were sick and one died during sampling. The symptoms were mainly respiratory distress with periorbital swelling; some had yellowish diarrhoea and petechial haemorrhages under the wings. They had all been vaccinated against ND.



Figure 3. Chicken in respiratory distress with periorbital swelling

DISCUSSION

The prevalence of AI in the areas around Maputo was just around 3,5 %, according to our limited study, but during our last week people at CVL started to analyze samples from Zambezia, which is in the north of Mozambique, and the

preliminary results pointed at a prevalence around 10-12 %. The north is a resting area for a large amount of migratory birds which could explain the higher prevalence but our low number of positive samples could also be due to how our samples were handled. The timeframe between when the sample was taken until the sera was separated from the blood clot was sometimes very long and the ELISA kits we used are made for chickens but our samples were collected from chickens, turkeys and ducks. It was sometimes difficult due to logistical problems and shortage of proper equipment to perform this study in an ideal systemic way. We sometimes had to keep the samples in our fridge over the weekend because the laboratory closed so early.

It was interesting to find AI in Mozambique and they were surprised at CVL because they really didn't believe it was present in the country. The presence of LPAI could be a factor in the high mortality in the backyard poultry. AI is probably maintained since free-range flocks are constantly exposed to wild birds. If the prevalence is higher in areas up north, near known migratory routes, it does constitute a risk of further spread of LPAI to other parts of the country with the constant movement of poultry and poultry products. Most birds are sold live throughout the country, on markets or by traders. Some poultry probably comes from neighboring countries, the control at the borders are not very thorough, which also increases the risk of HPAI since several of Mozambique's neighboring countries have reported HPAI. And of course when LPAI is present there is always a risk that it mutates into HPAI. It would be useful to elaborate a strategy for surveillance with repeated testing of the domestic poultry and to perform some epidemiological studies.

Future aspects

It would be interesting to perform a larger study including different areas of the country. Some of the plants used as traditional remedies would also be interesting to take a closer look at. In retrospect I would also have liked to have had the question where people had bought their poultry in the questionnaire. Important would also be to characterize the found viruses in more detail. Future work in the laboratory in Sweden will sort this in the near future.

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www.fao.org

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www.who.com

www.worldbank.org

Appendix 1

The questionnaire

The questionnaire was answered by 35 households. It contained the following questions:

Do you have a poultry house? : 27 of the households had a poultry house of some kind where they locked the poultry in at night

Number of chickens? : Ranged between 1-30, Average 8,5

Do you own other poultry? : 23 of the households had other poultry as well. Ducks being the most common but some also had turkeys or pigeons,

Do you have other animals? : 22 of the households also owned other animals. The most common was goats; other animals were pigs, cattle and guinea pigs (raised for food)

Do you have problems with?:

Disease? : 23 of the households experienced problems with disease, many mentioned ND.

Theft? : 14 had problems with people stealing their poultry

Predators? : 17 had problems with predators, mostly birds of prey taking small chickens but some also had problems with snakes or dogs.

Feed? : All birds moved freely during the day, scavenging but most households also gave them harvest residue, like corn, and household waste as additional feed. 7 of the households experienced difficulties with feeding their poultry.

Has any of your poultry died in the last 3 months? : 27 (!) of the households had had deaths in the last 3 months.

Do you vaccinate your poultry? : 5 owners vaccinated their poultry (2 of them raised broilers) against ND.

Do you use any kind of treatment if your birds are sick? : 20 owners tried some kind of treatment

Medicine like aspirin 1, unknown medicine 1

2 gave them soapwater

7 gave their poultry Potassium permanganate mixed with water

1 gave barch from the Mafura tree and leaves from Lantana trees squashed in water and 1 gave leaves from the Seringa tree

1 gave finely grained garlic mixed with water

3 gave some kind of antibiotics, sulfonamid 1, alfatrim 1 and unknown kind 1

3 gave aloe vera squashed in water

What do you do with your dead poultry? :

9 owners answered that they threw them away

4 owners ate them

1 didn't know

3 did nothing

15 buried them

1 owner burned the carcasses

1 said throw away or give it to someone else to eat

1 gave the dead birds away for someone else to eat