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Fakulteten för Veterinärmedicin och husdjursvetenskap
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Epidemiology and Characterization of Newcastle Disease in Smallholder Poultry in Mozambique

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ABSTRACT

The aims of this study were to estimate the prevalence of Newcastle disease in rural poultry in Maputo district in Mozambique and to get an insight in rural family poultry production and management. The study included two different parts; a serology study of the prevalence of Newcastle disease by enzyme linked immunosorbent assay (ELISA) and a small survey about management by a questionnaire. Samples were collected from three different areas/villages in Maputo district; Michanulane, Mafavuka 2 and Magude. Chickens, ducks and turkeys were included in the study. Blood samples were collected for serology and cloacal swabs were collected for polymerase chain reaction (PCR) and virus isolation. From 237 serum samples 106 were positive for antibodies against Newcastle disease virus, this gives a prevalence of 45%. If all vaccinated birds are excluded (12 birds) the prevalence is 44%. The highest prevalence was in turkeys (71%) followed by ducks (44%) and chickens (41%). When it comes to the questionnaire about production and management many of the families thought that their biggest problems were diseases and predators, but many also had problems with theft and sufficient feed to the poultry. Most of the households we visited didn't vaccinate against Newcastle disease even though many of them were aware of the disease and many said that they had problems with the disease. The high seroprevalence of Newcastle disease in this study supports the general opinion that Newcastle disease is widely spread and one of the biggest constraints to rural poultry production. This was a small study that only took place in three different areas in Maputo province and therefore a bigger study covering more areas throughout the country and a more thorough questionnaire about production and management would be necessary to get a more exact picture about the prevalence and other possible problems. At the same time it is important not to forget that other factors than Newcastle disease have an impact on the poultry production and that education about poultry management is important to increase the production.

INTRODUCTON

Aims

The aims of this thesis were to estimate the prevalence of Newcastle disease (ND) and to look into management and production of family poultry in three different rural areas in Maputo province in Mozambique, and to look at the vaccination status for ND, what the owner experienced as the main problems with the poultry production and if they did anything when the animals became ill.

The study included

1) A study of seroprevalence of Newcastle disease in rural poultry by enzyme linked immunosorbent assay on serum (ELISA).

2) A small survey about production and management through a questionnaire to the owner of the poultry. This questionnaire included questions about poultry population, management and if there were any specific problems, disease history and vaccination status.

Background

The project was a Minor Field Study (MFS) that was partly financed by Swedish International Development Cooperation Agency and took place in both Sweden and Mozambique. The study was performed in collaboration with Swedish University of Agricultural Sciences (SLU) and National Veterinary Institute (SVA) in Uppsala, Sweden, and the Veterinary Faculty of Eduardo Mondlane University (EMU) and the Central Veterinary Laboratory of the Directorate of Animal Science (CVL) in Maputo, Mozambique.

Rural poultry in developing countries

In many developing countries rural or village poultry are the most common and most important livestock in rural areas. Most of these village poultry belongs to the poorest families, usually women and children, for whom they have an important role both economically and socially. It is a livestock that is quite easy to gain and to keep, they don't demand a big capital investment and mostly they survive on what the surroundings can offer and furthermore they have a short production cycle. Poultry meat and egg are very important sources of nutrition and an important income for these families, and therefore important for the rural development. The income from the poultry can help the family with every day things like education, health care and clothes. Family poultry can also be the first step to get other livestock like cows and goats (Ahlers et al. 2009, Copland & Alders, 2005).

In general the standard of husbandry of the village poultry is low. Many families don't have any kind of housing for the poultry to protect them from the weather and predators. Feeding of the poultry is another thing that often are insufficient and the poultry have to survive on leftovers like maize bran and household waste or whatever they can find in the surroundings. They occasionally have to walk long distances, sometimes up to a few kilometres, to find feed and water. The lack of sufficient food and water are an important constrict for higher production. The

feed problems are usually greater during the dry season when the harvests are poor and there are not as much of worms and insects as in the rain season. It is also the season when the highest mortality is reported due to lack of feed and the increased risk of disease (Ahlers, 2009, dos Anjos, 2007, Mavale, 2001).

Other problems that the village poultry have to face and that cause great loss for the families are infectious diseases, and predators like snakes, birds of pray but also domestic animals like dogs. Theft is another problem that occurs frequently (Ahlers, 2009, dos Anjos, 2007, Mavale, 2001).

Village poultry compared to commercial poultry and other livestock

There are some important advantages with family poultry compared to commercial poultry and commercial poultry production. The family poultry don't need big investments, extensive technical skills or technical supplies to start or to maintain compared to commercial poultry production. Meat and egg from family poultry doesn't need a cold chain before it reaches the consumer, the family. The family can prepare one bird, which is enough for the family meal, and does not need a fridge for storing the leftovers (Copland, 2009).

Family poultry also have some advantages comparing to other livestock, like cows and goats. Family chickens don't need much feed to survive and can therefore live where other animals cannot. Another advantage is that family poultry are better survivors in natural disasters like flood, tsunamis and fires and can quickly come back to a productive state (Copland, 2009). They are also quite cheap to get and don't require high labour input from the owner to be managed and protected. Family poultry are therefore very suitable for female-headed household with children where time and economy are a limitation (Ahlers, 2009, Mavale, 2001). Female-headed households have increased in Mozambique, but also in many other countries, because of civil war or/and an increase in HIV/AIDS (Copland, 2009).

Village poultry in Mozambique

Around 75% of the population in Mozambique lives in rural areas with agriculture as their main source of income (Cambasa, 2009). According to the Mozambican Veterinary Authority and Trabalho de Inquerito Agricola (TIA) there are between 16-20 million poultry in Mozambique. This represents approximately 63% of the livestock production in the country. The biggest part consists of poultry (88%) followed by ducks (9%). Other species are turkeys, geese and guinea fowl (dos Anjos, 2007).

There are three different types of poultry production systems in Mozambique; medium sized commercial, small scale commercial and village or family poultry. The most common of these forms of poultry production in Mozambique is free ranging village or family poultry with a flock size of 1-50 birds, but most commonly between 5-15 birds. It is quite common that families with village poultry also have other animals like goats, pigs and cows (Ahlers, 2009, dos Anjos, 2007).

Family poultry mostly consist of Landim, a local breed that is well adapted to local conditions. It is often multi coloured and sometimes crossed with exotic breeds (dos Anjos, 2007). They have good hatching and mother ability and the hens lay, brood, hatch and look after the young chickens. They are usually well adapted to the harsh surroundings and are more resistant towards disease and poor husbandry conditions compared to commercial chicken breeds. A village chicken flock consists of birds of different sex and age groups. Since many of the diseases affect only one specific age group the effect of the diseases are not as devastating as if the flock would have been the same age. They also have better flight skills, which make them more likely to escape predators (Ahlers, 2009). Their productivity in growth rate and egg production is quite low, but at the same time the input from the owner to achieve this are none or very little. The hens usually start laying egg when they are 24-30 weeks old (Ahlers, 2009, dos Anjos, 2007, Harun, 2001). The highest production is under the harvest season from April to July while the lowest production is from July to December because of the winds in July and August and the rain in November and December (Harun, 2001). They give 2-4 clutches per year with an average of 10-13 eggs per clutch. Hatchability is around 70-90% but it varies with the season, but only 20-50% will reach adulthood (after two months only around five chickens have survived). Around 85% of the death takes place during the first three weeks of life (Ahlers, 2009, dos Anjos, 2007, Harun, 2001).

In Mozambique there are no official marketing of scavenging chickens or eggs, and the trade with rural chickens is an old tradition. Chickens are used as food for the family or are sold alive, the eggs are usually saved for breeding. Chickens can be sold within the village to neighbours or they can be taken to small local markets. Sometimes informal traders will travel around the villages and buy or exchange chickens for goods and when he has enough chickens he will take them to the city and sell them at poultry markets (dos Anjos, 2007).

Poultry housing is one thing that varies extensively in Mozambique and many other developing countries where village poultry are common. There are mainly two types of poultry houses, one that is built on the ground and one that are built above the ground. The houses are usually built from what is available for example mud, rocks, bricks, straw, bamboo, reeds, sheet metal or pieces of plastic. The houses on the ground are usually small and dark, and are not the healthiest environment for young poultry but they are good in that they conserve heat during the cold season. The elevated houses are situated about one meter above ground with some kind of small ladder or stick that give the poultry excess to the house. These houses are easy to keep clean and protect the poultry from predators, not snakes, but are not suitable for hens with chickens. Poultry that are not offered any housing takes shelter wherever they will find it, e.g. bushes, threes and under grain storage huts. Birds that don't have any housing are more likely to be exposed for predators, infections and theft (dos Anjos, 2007, Harum, 2001).

In Mozambique, as in many other developing countries, ND is endemic and a major problem in the village poultry production. It is one of the biggest causes to impaired production and increased mortality (Ahlers, 2009, Mavale, 2001). In Mozambique and other countries where ND is common there have usually been one or two outbreaks a year but in recent years there are reports from the farmers about outbreaks of the disease all year around. This is probably because the birds

are infected with different serotypes of the virus and immunity against one serotype doesn't protect against other serotypes. (Harun, 2001). Even if there are outbreaks all year around in Mozambique most outbreaks occur from January to March (May) and from July to September (October). Some believe that the seasonal outbreaks are due to seasonal changes in the age of the birds, shortage of feed at some periods of the year, climate stress, incidence of other infections and village chicken market activity (Mavale, 2001). Spradbrow (2001) believes that the seasonal conditions only influences the outbreaks indirectly and that it is the movement of the birds that are the main reason for the seasonal outbreaks. The marketing of rural chickens is generally livelier during the dry season. This is probably because the flocks are usually larger and the farmers know that the mortality increase toward the end of this season. It is also a time when the family need money to buy food as the accessibility of crop products declines (Mavale, 2001).

The mortality rate can be as high as 50-100% (Bagnol, 2001). An effective control of ND is therefore essential to reduce the mortality and increase the productivity, and thereby increase the income of the families. It has been estimated that control of ND alone could increase the household income from the village poultry by 40-60%. If ND control is combined with other improvements the income could increase by 80% compared to pre-ND control levels (Ahlers, 2009).

Newcastle disease virus

Newcastle disease (ND) is an acute and highly contagious virus infection that can affect most bird species. The disease is endemic in many parts of the world and causes big economical losses due to high mortality and reduced production. In rural areas the disease can kill up to 80% of unprotected poultry and is thereby one of the biggest constraints to village poultry production and a considerable restrict of rural development (Alexander, 2004).

History

The first outbreaks of Newcastle disease as a defined viral infection was in 1926 in Java, Indonesia, and in Newcastle-upon-Tyne, England, from where it gained its name. There are however earlier reports of similar disease outbreaks. Like the death of all the chickens in the Western Isles of Scotland in 1896, which are believed to be due to NDV. From 1930s it has been clear that almost identical viruses can cause less severe infections or subclinical disease (Alexander, 2004).

Aetiology

Newcastle disease is caused by a negative single stranded, non-segmented RNA virus belonging to the Paramyxoviridae family. So far nine serotypes of avian paramyxoviruses has been found, APMV-1 to APMV-9. Five of these serotypes can cause disease in poultry; APMV-1, APMV-2, APMV-3, APMV-6 and APMV-7. Of these APMV-1 is the most pathogenic serotype and is also referred to as Newcastle disease virus (NDV) (Caupa, 2009). The serotypes are usually classified into three groups depending on how virulent they are when inoculated in chicken embryo and chickens, velogenic (virulent), mesogenic (moderately virulent) and lentogenic (low virulence) (Kahn, 2005). Even if it is uncommon

there have been reports that that viruses of low virulence can mutate and become high virulence (Caupa, 2009).

Hosts

It has been showed that many different species can be infected with NDV. It is believed that all bird species are most likely at risk to be infected, but the effects of the disease varies very much with different species i.e. chickens are most sensitive whereas ducks and gees are least sensitive (Caupa, 2009).

Virulent NDV strains have been found in all types of domestic poultry, from pigeons to ostriches, but also in wild birds, caged pet birds and racing and show pigeons. Even if NDV is found quite commonly in wild birds like migratory feral waterfowl and other aquatic birds, it is generally a low virulent isolate for chickens and similar to viruses of the "asymptomatic enteric" pathotype. Sporadically virulent viruses have been found in wild birds but this is usually at the same time that the NDV has been present in domestic poultry in the same area. Even if it is not common for migratory wild bird to be infected with a high virulent serotype of NDV there is still a risk that they introduce an infection into a new area. A more considerable risk is the spread within an area where the disease have already occurred in domestic poultry (Caupa, 2009).

Virulent NDV isolates have been obtained from captive caged birds. The infection is most likely to originate at the holding station before export, most probably because of enzootic NDV at the holding stations or of spread from nearby poultry, e.g. backyard chicken flocks. In 1991 in USA there were some outbreaks of severe ND in pet birds. The infection was believed to come to the area through illegal importations. It has been established that infected parrots and parakeets can excrete virulent NDV for more than a year and they can therefore be important in spreading the disease to new areas (Caupa, 2009).

Clinical signs

The clinical signs in birds infected with ND virus vary greatly from very high morbidity and mortality (up to 100 %) to asymptomatic carriers. The severity of an infection is dependent on factors like the virulence and tropism of the virus, host species, age of host, immune status, other diseases and environmental conditions (Kahn, 2005).

The onset of the disease is often rapid and the first signs are usually seen throughout the flock within 3-5 days, but can vary from 2-15 days. Young birds are usually more susceptible for infection but the disease causes heavy losses in birds of all ages. Depending on where the virus has its predilection site you can observe symptoms from the respiratory tract, digestive system or nervous system (picture1) Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhoea, depression and inappetence, partial or complete drop in egg production and an increased production of deformed eggs (Kahn, 2005).

Depending the clinical signs and course of disease strains of NDV have been grouped into four different pathotypes (Alexander, 2004, Engström, 2004).

Viscerotropic velogenic

It is the most virulent form and appears suddenly and spreads rapidly. Symptoms that can be seen are obvious depression, inappetence, substantial drop in egg production, increased respiration, a profuse greenish-yellow diarrhoea that rapidly leads to dehydration and collapse, swollen heads and cyanotic combs. Mortality can be up to 90% and infected birds usually die within one or two days. Birds that survive the initial phase often develop nervous signs. Sometimes birds die peracutely without previous clinical signs.

Neuroptopic velogenic

Acute signs from the respiratory tract and nervous system dominate. Sudden depression, inappetence and drop in egg production are seen together with coughing and other signs from the respiratory tract, followed by nervous signs within a few days. Mortality is usually around 10-20% for adult birds but can be higher for young birds.

Mesogenic

Coughing and other signs from the respiratory tracts dominate. Other symptoms are depression, loss of weight and decreased egg production for up to three weeks. Signs from the nervous system can develop late in the disease. Mortality is around 10%.

Lentogenic

Are often subclinical but mild respiratory signs and a small drop in egg production can be seen. No nervous signs and mortality is usually negligible.

Picture 1. Sick chicken with acute respiratory distress.

Transmission and spread

Newcastle disease is very contagious and is easily spread from one bird to another. The infection is usually transmitted by direct contact with sick birds or unaffected birds carrying the virus. Even vaccinated birds that are clinically healthy can excrete virulent virus after they have been exposed. Virus can also be transmitted indirectly by people, other animals, equipment, vehicles, contaminated poultry products, feed and water (Caupa, 2009).

The infection takes place by inhalation or ingestion of the virus or by contact with mucous membranes, specially the conjunctiva. Infected birds shed virus in aerosol, respiratory discharge and faeces. Infected birds start to excrete virus during the incubations period and continue to excrete virus for a varying but limited time during convalescence (Caupa, 2009).

MATERIALS AND METHODS

Study region and study population

Two villages outside Maputo, Michangulene and Mafavuka 2, and the area around the city Magude were chosen as our study regions. Our local supervisor in collaboration with people from CVL chose these areas with considerations of logistical possibilities and accessibility to accommodation and a cold chain for the samples.

The study population consisted mostly of free ranging family poultry that have the possibility to mix with other birds and animals. One exception was two small-scale meat producers were the chickens were kept in enclosures containing around 200 birds per enclosure. These enclosures were made of chicken net so they still could have contact with free ranging chickens outside the enclosure. Samples were collected from young and old birds, both from healthy and from clinically affected birds when this was possible. The only clinical affected chickens seen were at the small-scale meat producers were many birds had died recently. Samples were also collected from a few birds that were supposed to be vaccinated.

Each village was considered as one epidemiological unit assuming that the poultry in each village were kept as free ranging and had the possibility to mix. To estimate the seroprevalence of Newcastle disease with a precision of 5% and a confidence interval of 95% and with an unknown prevalence of the disease the total number of samples were calculated to 270. We collected samples from 240 birds in total (table 1) but due to some practical problems we could only collect samples from three different villages and the number of birds selected from each family depended of how many birds the family had and how many birds they were able to catch (not everybody had a chicken house were they could lock the birds in). Number of birds from each family ranged from 1 to 11, in one family we collected samples from 19 birds but some of the birds came from their neighbour.

Table 1. Number of serum samples from each village and from different species.

	Number of samples			
Village	Total	Ducks	Chickens	Turkeys
All villages	237	69	154	14
Michangulane	33	19	14	-
Mafavuka 2	32	23	9	-
Magude	172	27	131	14

Sample collection

Blood samples for serology and cloacal swabs for polymerase chain reaction (PCR) and virus isolation were taken from each bird. It was not always possible to store the samples according to the protocols. Most of the materials like syringes, needles, cotton swabs, antibiotic solution and different kind of plastic tubes were brought from Sweden.

Blood sampling

Between 0,5-2 ml of blood was collected from the brachial vein. In the first 68 samples taken from Michangulane and Mafavuka 2 the blood was transferred from the syringes to 2,5 ml plastic tubes with red stoppers. Each tube was marked with a bird number that could be linked to species, village and household. These plastic tubes were kept in a cool box while sampling. Because of holidays these samples had to stay in the fridge for three to four days before we could separate the serum from the blood through centrifugation. The serum was stored in 1,5 ml plastic tubes in the freezer. The serum tubes were marked with the same number as the blood tube. We had some problems in separating the serum and because of this we only managed to get serum from 237 samples. This was probably because of the long storage time in the fridge before separation.

In the rest of the blood samples that were taken from Magude the blood was left in the syringes in an approximately 45° angle in room temperature to clot. The syringes were marked with a bird number like the plastic tubes from previous sampling. The serum was thereafter transferred to 1,4 ml plastic tubes marked as the syringes and stored in the fridge for 3-4 days before they could be stored in the freezer. Some samples were centrifuged before they were put in the freezer.

Cloacal swabs

Two cloacal samples were collected with sterile cotton swabs from almost every bird; there are one bird from which we don't have any cloacal swabs and four birds from which we only have one swab from. Each swab were stored in 2 ml cryo vials containing 0,5 ml antibiotic solution. These cryo vials were marked with the same bird number as the blood samples. The antibiotic solution was made of 10 ml Phosphate Buffered Saline supplemented with Mg/Ca (PBS), 2 ml 90°°° U/ml penicillin, 2 ml mg/ml streptomycin, 2,25 mg/ml gentamicin and 45°°° U/ml nystain. The mixed antibiotic solution was kept in the fridge until sampling. Both the antibiotics and PBS were brought from Sweden.

The swabs were kept in a cool box during sampling. After that they were kept in a fridge for 3-4 days before the swabs could be removed. Some of the samples were stored in -22°C for one or two weeks before they were put in -80°C.

Laboratory analyses

ELISA tests were done at CVL with IDEXX FlockCheck Newcastle disease virus antibody test kits. Most of the equipment that were used to perform the analyses were provided by CVL. This includes pipettes, disposable pipette tips, 96-wall plate reader and distilled water. Plastic tubes with stoppers and 96 tubes tube holders that were used to dilute the samples were brought from Sweden.

The test procedure followed, with a few exceptions, the IDEXX FlockCheck manual from 2007 that came with the test kits. The samples were diluted 1:500 either by diluting 1 µl of sample with 500 µl of sample diluent or by diluting 0,5 µl of sample with 250 µl of sample diluent. Samples were not tested in duplicates. Emptying and washing of the plates was done manually by turning the plate up-side down and then the wells were washed 3-5 times with approximately 300 µl of distilled water. The absorbance was measured at 630 nm instead of 650 nm.

Data collection

A survey about the poultry population, disease history and vaccination status was also included in this project. The survey was made in form of a questionnaire to the households. The questionnaire was designed and translated to Portuguese in collaboration with our local supervisor. The interviews were done with help from our local supervisor or from the staff from CVL who translated the questions to the farmers. Many of them neither spoke English or Portuguese but local languages like Shangane. Every household from where we collected samples were included in the survey. The questionnaire contained 12 questions and was designed to try to keep the answers quite short. The complete questionnaire can be found in appendix 1.

RESULTS

Prevalence

From 237 serum samples collected from the three different areas and 35 different household 106 samples were positive for antibodies against Newcastle disease.

This gives a seroprevalence of 45%. If all the birds that were vaccinated against NDV are excluded from the survey the prevalence is 44% (12 birds excluded).

*Table 2. Seroprevalence of NDV in unvaccinated birds in different villages and different species. *Vaccinated birds included.*

	Seroprevalence			
Village	Total	Ducks	Chickens	Turkeys
All villages	44 %	44 %	41 %	71 %
Michangulane	45 %	53 %	36 %	-
Mafavuka 2	40 %	30 %	67 %	-
Magude	44 %	50 %	39 %	71 %
All villages*	45 %	42 %	45 %	71 %
Magude*	45 %	41 %	52 %	71 %

Questionnaire and observations

All 35 households answered the questionnaire. In one of the households it wasn't the owner but a neighbour who answered the questionnaire so he didn't know the answers to all the questions. The questionnaire translated to English can be fond in appendix 1.

Question 1-3, general information about the family. These questions included name of the owner and which area and village they live in. Eight families come from Michangulene, six families from Mafavuka 2 and 21 families from Magude.

Question 4, chicken house or not. 27 of the households had some kind of shelter for their poultry. These were mostly used to close the birds in for the night for protection.

Question 5, number of chickens. Number of chickens varied between 1-30. The mean number was 9 chickens (9,3) and the median 9 as well. There was one family who didn't have any chickens but had other poultry. Two of the households had had 200 chickens (the small-scale meat producers) but because of disease and sales they now had 5 and 25.

Question 6, other poultry. 23 of the households had other poultry as well. Mostly ducks but also geese, turkeys and pigeons. These were mostly mixed with the chickens.

Question 7, other animals. 22 families had other animals. Goats were most common but some of families also had cows, pigs and guinea pigs (as feed for the families). The family with the least animals had only three chickens while the household with most livestock had 10 chickens, 30 ducks, 10 sheep, 20 goats, 400 cows and 20 pigs, but this was an exception compared to most of the families.

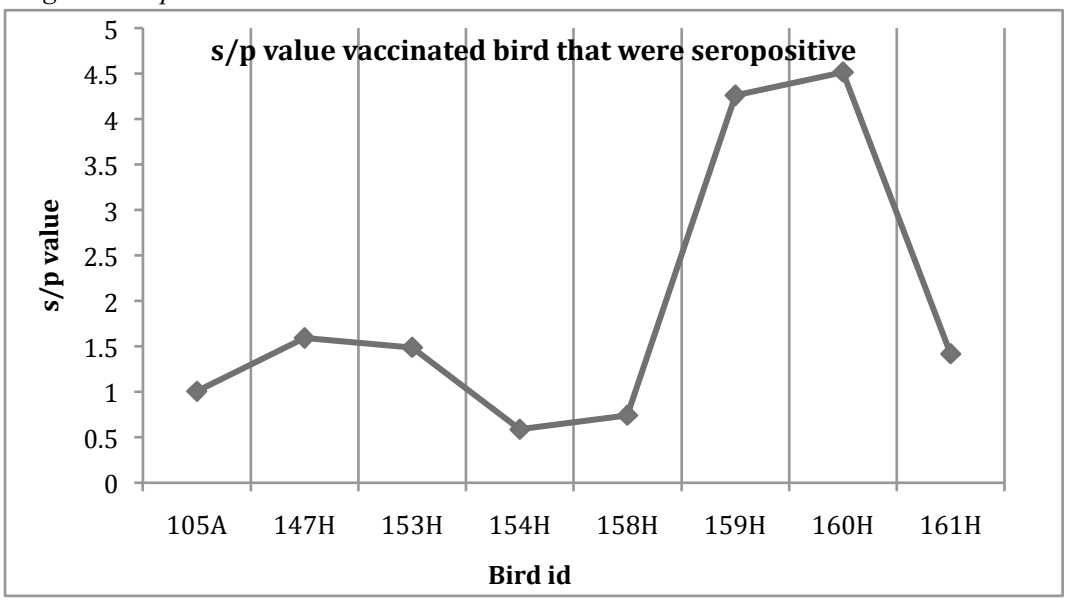
Question 8, problems they have. We specifically asked if they had problems with disease, theft, predators and feeding the animals. They could choose all suitable options. 23 of the households think they have problems with disease and many of them believe it is NDV. 14 of the families think that theft is a problem and 17 think that they have problems with predators, mostly birds of pray but even snakes and dogs. 7 of the households think that they have problem feeding the birds most of the time but there are also some families that think they only have problem feeding the poultry during some periods. Most of the families that feed the birds gave them maize bran and other household wastes.

Question 9, *mortality the last three months*. In 27 of the households there had been deaths but many of the families couldn't say exactly how many birds that had died. Most number of chickens that had died was 70 and 50, and this was at the small-scale meat producers. Among the other families the highest number was 37 birds. The highest mortality was among younger chickens.

Question 10, vaccination status. Four of the households had vaccinated some of their poultry against NDV. One of these had only vaccinated the chickens but not the other poultry and one had only vaccinated the ducks. Two of these households were the small-scale broiler producers and they had also vaccinated their chickens against Infectious bursal disease (IBD). Three of the households had vaccinated against NDV last year but not this year. One family didn't know if the bird were vaccinated or not. One family vaccinated the birds after the sampling.

In total 12 birds had been vaccinated against ND and of these 8 were positive for antibodies against NDV. The s/p value varied between 0,589-4,514 (diagram 1) and s/p values >0,200 are counted as positive. It is impossible to say if these birds are positive due to vaccination or natural infection. Maybe a high s/p value indicates a natural infection but to be sure you have to test birds that are vaccinated to get a reference value.

Diagram 1. s/p value in vaccinated birds.



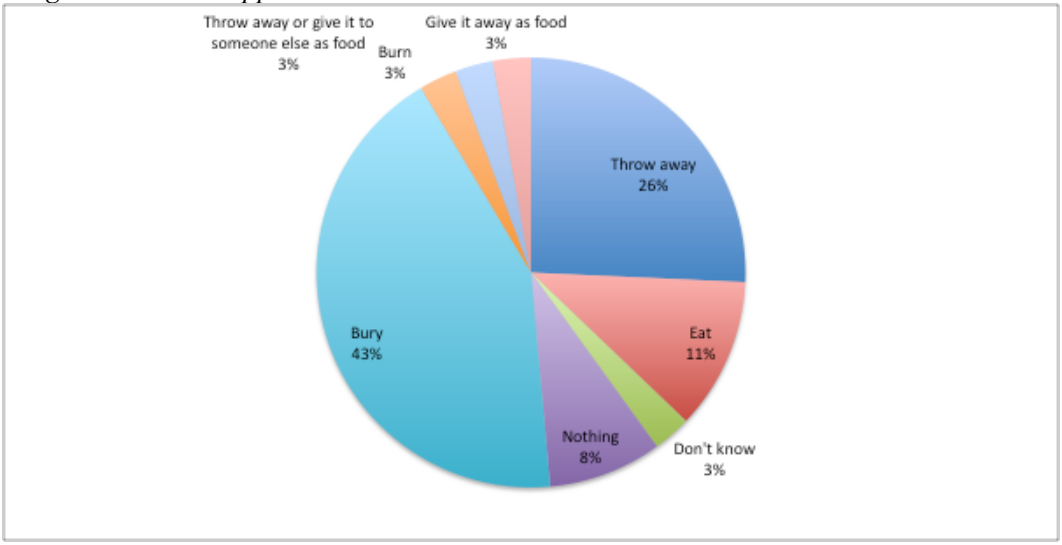
Question 11, treatment of sick birds. 20 of the households treated the birds when they became sick. The treatments varied a lot between the households (table 3).

Table 3, treatment.

Treatment	Number of households
Potassium permanganate with water	7
Aloevera mixed with water	3
Antibiotic	3
Leaf or bark mixed with water	2
Garlic mixed with water	1
Medicine like aspirin	1
Unknown medicine	1
Soap water	1
Washing detergent with water	1

Question 12, what happens with the dead bird (diagram 2). Most of the families buried the dead birds, which is good considering bio security and spread of diseases.

Diagram 2. What happens with the dead birds



Most of the birds that we collected samples from where healthy looking. The most common problem we saw were ectoparasites like ticks and lice. There were also chickens that were infected with chicken pox. Some of the birds had diarrhoea and were thinner than the others. Three of the chickens with obvious diarrhoea were positive for antibodies against NDV. The s/p value for these birds were 1,550, 0,311 and 0,370. Two chickens died during the sampling or soon after sampling. One was at the small-scale broiler producers where they had problems with sick birds. Almost all of the few birds that were still alive were sick with serious symptoms from the respiratory tract, petechial bleedings under the wings and some were swollen around the head. These birds were supposed to be vaccinated against NDV but not all of them were seropositive for NDV according to ELISA.

Picture 2. Ectoparasites around the eyes

Picture 3. Chicken pox

DISCUSSION

Prevalence

To our knowledge there are very few studies that have been done about the seroprevalence of NDV in Mozambique but accessible information indicates that the disease is widespread and that it is one of the biggest constraints to the production of rural chickens (Dias, 2001). In this study 44% of the unvaccinated poultry were positive for antibodies against NDV (41% if turkeys are excluded), which supports the general apprehensions about the extension of the disease. Because a comparison of the seroprevalence found in this study to other studies is difficult only what was found from the analysis process, sample collection and storage of the samples that can have affected the results will be discussed.

In the manual of the IDEXX FlockCheck it says that you are supposed to test chicken serum in the kits. We have tested serum from chickens, ducks and turkeys and it's difficult to say if serum from other bird species than chickens can be used for these test kits without getting inaccurate results. From the IDEXX product information it says that turkeys have an own test kit for NDV. The seroprevalence in chickens and ducks don't distinguish so much, 44% for the ducks and 41% for the chickens, but than a little bit more than twice the number of chickens were tested. The turkeys that were tested with these test kits had a seroprevalence of 71%, which is much higher compared to ducks and chickens, and that can be an indication that these test kits are not suitable for testing serum from turkeys. But than only 14 turkeys were tested, which are to few.

Because some technical and logistical problems the handling and storage of the samples were not always optimal, which can have had an influence on the test results. We didn't have access to the lab at weekends and the samples collected in Michangulane and Mafavuka 2 were collected the day before a weekend and therefore couldn't be handled until after the weekend. This means that both the cloacal swabs and blood samples were kept in the fridge for 3-4 days before we could separate the serum and put it in the freezer and remove the cloacal swabs from the suspension and put the suspension in -80°C. For some of the blood

samples it was difficult to make a good separation of the serum and some of the samples had to be centrifuged two or three times before we got any serum. A long storage time makes the blood haemolyse and this can make the separation more difficult. Long storage can also make the blood samples fatty and both haemolysed and fatty blood can affect the ELISA results. The blood samples from Magude were handled a little bit better. These samples were separated within a few hours through natural coagulation and not through centrifugation and this made it difficult to get serum samples without any erythrocytes. The serum samples were than kept in the fridge for 1-4 days before we were back to the lab and could put them in the freezer. Some of the samples were centrifuged before they were put in the freezer because they still contained erythrocytes. The insufficient separation of some of the serum samples can have affected the serum through haemolyse and thereby affected the ELISA analyses. The cloacal swabs from Magude were first kept in the fridge for 1-4 days and after that kept in a normal freezer for 3-11 days before they were put in -80°C. If more studies are to be done it's important to plan the sampling so that you can handle and store the samples according to the protocol.

The equipment at the laboratory was not always optimal for ELISA analyses. We had no multi channel pipettes that exceeded 300 µl and it is not known when the pipettes had last been calibrated. That the pipettes are accurate in volume is very important for the results.

It is important to keep in mind that we only collected samples from three different areas in Maputo province and that other areas, both inside and outside Maputo province, can have a different picture. It is therefore important to do further studies throughout the country. If more studies are to be done there are some things that have to be considered. One important thing is to plan the field trips so that the samples can be handled as correctly as possible to minimize possible sources of errors at analyses. If you want to compare the seroprevalence between different bird species is important to test the same number of different species, and not like in this case were we only collected samples from 14 turkeys. This can be interesting because different species are different susceptible for the virus.

There have been a few bigger vaccination campaigns in the country in the last years and it would be interesting to know if there was a difference in the prevalence of NDV before and after vaccination and how big the difference in that case was. This is unfortunately something that we will probably never know but it is maybe something to have in mind if there is another campaign to come.

Questionnaire and other observations

The questionnaire was used to get an insight in family poultry production in rural areas and possible problems. To make a more thoroughly survey a more detailed questionnaire had to be necessary but we wanted to keep the questionnaire as simple as possible because we don't speak the native language and more misunderstandings usually occur when you have an interpreter.

One thing that was good was that most of the families had some kind of shelter for their poultry. This is an important protection against predators and rough weather (Harun, 2001). Most of the families only used the poultry house during

night time and during the days the poultry were scavenging freely around the village. This way of keeping the poultry is quite easy because it doesn't demand much work and the owner don't have to worry so much about feed to the animals. At the same time it is more unsafe, it increases the risk of predation, theft and the spread of diseases from both wild birds and other livestock. If the owners were to hold the poultry in enclosures all the time it would be necessary with more education about poultry management.

Most of the families thought that the biggest problem was diseases and 27 of the families have had problems with deaths the last three months. The highest mortality was among the young chickens, which is not a surprise because they are more susceptible for diseases and more sensitive to poor management. Many of the households mentioned Newcastle disease as a main problem. This shows that the farmers are aware of the disease and it is likely because of the vaccination campaigns that have been carried out in some parts of the country the last few years. Even if many are aware of ND only four households had vaccinated some of their poultry against ND this year. What the reasons for this may be would be interesting to know, if it is economical or other reasons. It is good that the farmers are attentive about ND but it is important that other diseases are not forgotten. One disease that is important to have in mind is avian influenza, which can have the same symptoms as an infection with a velogenic serotype of NDV in its highly pathogenic form. To decrease the deaths among the poultry it is important to improve the management. A good poultry house protects the animals from things like theft, predation and extreme weather. Sufficient and good quality feed and vaccination gives a better immune status and a better resistance against disease. But to achieve this more education and support to the households are needed.

It was interesting to see what kind of different treatments owners used for their animals when they became sick. Most popular was treatment with potassium permanganate. Our local supervisor hadn't heard about this treatment before so from where they have learned this is unknown. But according to some articles potassium permanganate can be used as a disinfectant to inactivate bacteria, viruses and protozoa (EPA, 1999) and it has been used in fish pounds to treat some fish pathogens (Lazur, 1992). A good thing is that antibiotics were not used in many households, but the bad thing was that the antibiotics that were mentioned were broad-spectrum antibiotics (different sulpha preparations), which is bad bearing in mind the increased resistance to antibiotics.

What happens with the birds that die from a disease or out of itself is important for the control of infectious diseases. Most of the households buried the birds and one family burned the birds. These are good ways when trying to prevent the spread of diseases. But around 1/3 of the families just threw the birds away or did nothing and this is not to prefer because healthy birds can easily come in contact with them directly or indirectly and by that means spread the disease.

The bird that died and was supposed to be vaccinated against NDV wasn't positive for NDV. This indicates that the vaccination hasn't worked properly. The other bird that died was a young bird that looked healthy. This was also negative for NDV.

Conclusion

Newcastle disease is probably one of the biggest problem and constraints when it comes to rural poultry production in Mozambique and many other developing countries. Many of the poorest families are economically and socially dependent of their village poultry and a high mortality among the poultry, due to disease or other factors, is especially hard for these families and can even be devastating. It is therefore important to gain more knowledge about the prevalence and impact of the disease. This was a small study that only covered three areas in Maputo province in the southern part of Mozambique and was conducted to get an indication about the seroprevalence of NDV and other possible problems rearing rural poultry production in the country. A bigger study covering more areas throughout the whole country and a more thorough questionnaire about the poultry production would be necessary to get a more exact picture of the prevalence and other potential problems. Even if NDV probably is one of the biggest threats against poultry production it is important not to forget other factors that also have a great impact on both the production and health of the poultry. It is essential to educate the farmers about good management and husbandry of their poultry so that both the production and well being of the poultry can be increased.

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APPENDICES

Questionnaire for the farmer

The questionnaire contained the following questions for the farmers:

1. Name?

2. Name of the district?

3. Name of the village?

4. Poultry house?

5. Number of chickens?

6. Other poultry?

7. Other animals?

8. What kind of problems do you have with the management of the poultry? *(The farmer could choose several of these options)*

    a) Disease?

    b) Theft?

    c) Predators?

    d) Feeding?

    e) Other?

9. How many of your poultry have died the last three months?

10. Do you vaccinate your poultry?

11. Do you treat your poultry when they are sick?

12. What do you do when the poultry dies by it self?

The geographic coordinates were taken with a GPS from some of the households in the different villages.