Efficiency of the I-2 Newcastle Disease Vaccine produced at the Ministry of Livestock, Development and Fisheries in Dar-es-Salaam

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Key words
NewCastle Disease Virus (NDV), I-2 Vaccine, Tanzania, Free range chickens, Flock immunity, Horizontal transmission, Efficiency vaccine

Abstract

In Tanzania the I-2 Vaccine is produced at the Ministry of Livestock, Development and Fisheries in Dar-es-Salaam. The horizontal transmission under laboratory conditions and the efficiency in the field is evaluated. The results suggest that more than 80% of the chickens in the contact group produce a sufficient antibody titer against ND, but apparently the titer level in the contact group decreases at a faster rate than that of vaccinated group, which has protective antibody titers for at least 4 months.

In the field flocks were sampled at different times after vaccination. Some of them had protective antibody titers against NDV and other flocks were not protected, while vaccination was only between 1 and 3 months ago. Also one flock with a average titer of 8.67 was found, probably due to a field infection. It was found that more than 40% of the flocks in the field have titers below the protective level within 3 months after vaccination.

Introduction

Village chickens play a very important role in the lives of rural people in Tanzania and other developing countries. They are very widespread and are kept by about 70% of the households in Tanzania this households have together 26.594.000 chickens. Chickens can be used for consumption (chickens provide 30% of the protein intake) or for sale of eggs and meat (Permin 2002).
With the sale of chickens, the farmer can provide his family in education, health and clothes. Chickens also have cultural meaning. They are used during rituals, traditional healings, and as gifts to respected guests. Village chickens are mostly held by women and poor people of the village. They are especially important in female-headed households and those households where women are caring for sick or disabled family.
The results of the production of meat and eggs from chickens are low, the mean production of a chicken is 40 eggs a year. A broiler has a weight between one and two kilograms and they grow five grams a day (Mtambo 2002). Holding chickens hardly needs any investment and the costs are low, so it is still cost effective. However, with just small changes in management, such as better health control and better disease prevention, an increase in production could be achieved, so there will be more eggs and meat available for consumption. An increase in the amount of chicken eaten is important especially for children because most children do not receive enough animal protein in their diet at the moment. The average intake of children is 0,7 up to 1 kilogram chicken meat and 13-16 eggs a year per person. To compare: in Europe is the intake 16 kilograms of meat and 200 eggs a year (Msami, 2000, Bagnol 2005, Copland and Alders 2005, Alders 2001, Mtambo 2002).

The Newcastle disease virus is endemic in poultry populations in Africa and Asia. The virus is the greatest constrain on the production of village poultry. Chickens infected with ND show different clinical symptoms, including respiratory, reproductive, digestible signs and also nervous signs are associated with NDV.
The virus causes economical losses in Tanzania where the mortality can reach up to 90%. And due to ND infection 50% of the chickens die before they reach two months of age. Also ND infections may cause decreased egg production (Mtambo 2002). Sometimes the whole flock will be lost when they are infected with NDV.
The virus is also known because of its zoonotic properties. Symptoms in humans can be a conjunctivitis that develops after exposure within 24 hours. Recovery takes place within a week. Other symptoms can be fever, chills, headache, pharyngitis, depressed appetite, photophobia, and general apathy, but this are symptoms that occur rarely. Most cases of NDV are reported in laboratory workers (Alders 2001, Adwar 2008, Msami and Young 2005, Swayne 2003).

Vaccination is the most important way to control NDV. The available heat-labile vaccines like La Sota, which are used in the commercial poultry branch, are very unpractical for use in village chickens. These vaccines are not available in small amounts, which would cause large expenses for the rural poultry keeper. Another major problem is that the vaccines need a ‘cold chain’: they have to remain cold from manufacturing process to administration. The bad infrastructure and lack of refrigerators make this impossible for the rural poultry keepers. To avoid the problem with the cold chain, much research is being done to design ‘thermostable’ vaccines. These vaccines could offer a solution to the problems.

The first thermostable vaccine produced was the NDV4-HR vaccine. This was proven to be effective in Cameroon, Ghana, South Africa, Tanzania, Zambia and many countries in Southeast Asia (Alders and Spradbrow, 2001). However, this is a

commercial vaccine, only produced under commercial ownership, which leads to the fact that it only comes in large amounts and costs foreign exchange. That makes it unsuitable for use in small flocks of village chickens in rural areas.

The Australian Centre for International Agricultural Research (ACIAR), which is working together with developing countries to prevent Newcastle Disease, developed another thermostable vaccine: the I-2 strain. This strain is avirulent and is able to spread from vaccinated to unvaccinated poultry. This is a very useful property in village chickens (Bensink and Spradbrow 1999). This vaccine is free of commercial ownership, which is the main reason for the low cost of this vaccine. Another advantage is that it can be manufactured in Tanzania itself, and in smaller amounts (Alders and Spradbrow 2001, Msami 2005). Although it is thermostable, it is still important to keep the vaccines away from sunlight, excess heat and cold and frequent shifts in temperature, to ensure that their activity will remain. It is a biological product and therefore sensitive for these factors.
The vaccine can be produced in freeze-dried or in liquid form. Although freeze-dried vaccines have advantages (less storage space, longer storage possible), in Tanzania the vaccine is produced in its liquid form. This vaccine is easy to produce, cheaper, and no specialized equipment is needed (Young, 2002).
The vaccine is colorless and this makes it difficult to determine whether the vaccination has been administered entirely . For that reason there is the possibility to produce a colored vaccine. This will hopefully help to increase the efficiency of vaccine administration (Wambura 2009).
The strain I-2 vaccine is the most used vaccine at the moment in Tanzania. The vaccine can be administrated intranasally, ocularly (eye-drop) or orally (for example coated with I-2 vaccine oiled rice). Vaccination by eye-drop results in the highest titer development. The mean protection that can be reached in the field is 80% (Alders 2005).
As mentioned earlier, the flocks in Tanzania are small; they only contain between the 5 and 20 chickens per household. Within a flock all age groups are represented, there are twice as much chicks as growers and adults. These different age categories have different immunological states (Bell 2004). Little is known about when the farmers have to vaccinate their flock for a good protection against NDV. Currently chickens a vaccinated every 3 or 4 months by a local ND vaccinator who comes to each village to vaccinate the chickens.

Aim of the study

The aim of the study is to establish the efficiency of the I-2 vaccin produced at the Ministery of Livestock, Development and Fisheries in Dar-es-Salaam in Tanzania. Two strategies are used to obtain the results.

1. The horizontal transmission of the I-2 vaccine in chickens under laboratory conditions is established
2. The flock immunity at different times after vaccination with the I-2 vaccine in the field is determined.

Data collection

Horizontal transmission

Information is gained about the immunological status of vaccinated and unvaccinated chickens that have been in contact with each other. For some days after vaccination there is excretion of the vaccine in the feces of the vaccinated chickens (Illango 2008). The question is whether chickens in both groups will produce a HI titer of 3 or above so that there is a protective immunity against ND.

At day 0 blood is taken from 40 chickens that are older than 20 weeks old. 20 of them are white chickens and 20 of them are black chickens. The white chickens were vaccinated with the I-2 vaccine and placed in the cage with the black chickens. In this way they stay in contact with each other after vaccination.
For the vaccination the I-2 vaccine batch 81 is used. This vaccine is produced by the Ministry of livestock, development and fisheries in August 2009 and has a EID of 10^7.
After 18, 45, 62 and 120 days blood is taken from the white and the black chickens .
The serum is tested with the haemagglutination inhibition test (HI) test to determine the antibody titer of NDV and the titers of both groups are analyzed.

Flock immunity in the field

Information about the immunological status of the flock in village chickens is gained through use of a survey. This study took place in the surrounding of Iringa.

In the surrounding areas of Iringa 6 villages have been visited. Blood is taken from 20 flocks and from each flock between the 2 and 15 chickens are sampled.

Village	Flock	Number of chickens	Number of samples taken	Vaccinated … months ago
Mkimbizi 1	1.	6	4	3
Mkimbizi 1	2	30	6	2
Mkimbizi 1	3	50	4	2
Mkimbizi 1	4	50	3	1
Kehesa 2	5	5	2	5
Kehesa 2	6	30	6	3
Kehesa 2	7	7	4	1
Tangazo 3	8	10	6	5
Tangazo 3	9	7	5	3
Tangazo 3	10	20	2	1
Etemba 4	11	16	9	3
Etemba 4	12	11	10	3
Etemba 4	13	11	9	3
Denbosko 5	14	500	14	2
Kitulan 6	15	14	5	3
Kitulan 6	16	21	9	3
Kitulan 6	17	7	6	3
Kitulan 6	18	6	6	3
Kitulan 6	19	19	15	3
Kitulan 6	20	6	5	3

Tab. 1 Experimental group Village number in combination with flock number are corresponding with the numbers in annex 2

Blood samples are collected from village chickens that have been vaccinated by eye-drop method. A drop of 30µl with a virus titer of 10^7 EID50 to each chicken (Young 2002). In some cases the owner vaccinated the chickens, in other cases a vaccinator administered the vaccine. The 'wet' I-2 vaccine has been used (the liquid form), because this is the cheapest in production and thus the best option for use in Tanzania. This vaccine is produced at the Ministry of Livestock, Development and Fisheries in

Dar-es-Salaam and it costs the farmer about 30 TSH per chicken. One bottle of vaccine (100 doses) costs 2000 TSH (US$ 1,35) (Young 2002).
The blood samples are taken from the flocks that are recently vaccinated (between 5 and 1 month ago).

Blood samples (1% body weight) are collected from the wing vein, with a 23 G needle. A new needle is used for each chicken. Blood is collected in a 3 ml syringe all of them were marked with village, flock and chicken number.
The samples were preserved in a cool box with icepacks. At the laboratory the samples were placed in the incubator (37 ºC) for 3 hours, so that the serum separates from the red blood cells. After that the serum was poured of from the coagulum and placed into a centrifuge tube and placed in the fridge (5 ºC) for 1 to 5 hours until it was processed.

Serum samples are used for the haemagglutination inhibition (HI) test to determine the antibody titer of NDV. Serum samples were mixed with NDV antigen resulting in binding between NDV antibodies in the serum and the Newcastle disease virus. The binding sites on the virus particles are no longer free for binding and haemagglutination is therefore inhibited. The red blood cells roll to the bottom of the V-bottom walls of micro well plates and form a distinct dot, whereas in case of haemagglutination the bottom is covered with erythrocytes.

The expected result is a decrease in antibody titers of the individual chickens and thusly also a decrease in the immunity of the flock in the period between the vaccination and the sampling of blood increase. For a good immunity of the flock it is required that 80% of the chickens in the flock have a good antibody titer against NDV. A HI titer of 3 or above is considered positive and protective against NDV (Allan 1974, Rehmani 1996, Alders 2002). When the results are beneath this 80% revaccination of the flock is desired.

Materials and Methods

For the materials and methods the Laboratory manual of M. Young and R. Alders is used as ruler guide.

Bleeding of the chickens

Blood is taken from chickens to prepare a 10% red blood cell suspension, or to gain blood samples for the experiment.

Materials:

- Chicken
- Injection needle (23G, 5 ml syringes)
- 70% alcohol
- Alsever's solution
- Tubes

Methods:

Blood is taken from the brachial vein. First the wing is disinfected with 70% alcohol.
The amount of blood taken should not be more than 1 % of body weight of the chicken.
Blood used for the RBC preparation, should be collected in a tube containing Alsever's solution. If the blood is used for serum samples, no anticoagulans is used.

Preparation of Phosphate Buffered Saline (PBS)

Materials:

- Disodium hydrogen orthophosphate(anhydrous) 0,92 g/L
- Potassium dihydrogen orthophosphate 0,20 g/L
- Potassium chloride 0,20 g/L
- Sodium chloride 8,00 g/L
- Distilled water up to 1L
- HCL
- Balance
- Bottle of 1L
- pH meter (Wagtech 3505)
- Autoclave sterilisator (Astell Scientific)

Methods:

The amounts of salts are measured and placed in the bottle. A volume of 800 ml of distilled water is added to the salts, and placed on a hot plate to dissolve the salts. The mixture is cooled to room temperature and more distilled water is added to reach a total amount of 1L of PBS. The pH is measured. pH should be 7.2, if necessary, pH can be adjusted with HCl.
After this, the PBS is placed in the autoclave at 121ºC for 15 minutes.
The PBS is stored at 4-8 ºC.

Preparation of Alsever's solution

Materials:

- Sodium Citrate 8.0 g/L
- D-glucose (dextrose) 20.5 g/L
- Sodium chloride NaCl 4.2 g/L
- Distilled water up to 1L
- Citric acid
- Balance
- pH meter (Wagtech 3505)
- Sterilisator (Astell Scientific)
- Bottle of 1L

Methods:

The amounts of salts are measured and placed into the bottle. Distilled water is added until 1L is reached. The pH is measured. pH should be 6.1, it can be lowered by using a 10% citric acid solution.After this, the alsever's solution is placed in the autoclave at 121ºC for 15 minutes. It should be stored at 4 ºC.

Preparation of Washed Red Blood Cell Suspension

Materials:

- centrifuge tube 10 ml.
- Alserver's solution
- Centrifuge (Centurion Scientific LTD K3 system)
- PBS
- Capillary centrifuge (Hawskey England microhematocrite centrifuge)

Methods:

Blood is taken from 2 to 3 chickens into a centrifuge tube, already containing Alserver's Solution. This is gently mixed. The blood is centrifuged at 150 x 10 RPM for 5 minutes. The supernatant is discarded and the tube is refilled with PBS and centrifuged again at 150 x 10 RPM for 5 minutes, then the supernatant is discarded. This step is repeated one more time. One last round of centrifuging is done without adding PBS and afterwards the supernatant is discarded. The hematocrite is determined and the RBC is diluted to a 10% solution, by adding PBS.

Testing of antigen

Materials:

- 96 wells V-shaped microtiter plate
- ND Antigen
- 10% RBC
- PBS
- Pipettor (Transferpette-8, Eppendorf Research)
- Pipettor tips

Methods:

25 µl of PBS is added to each well. 25 µl of antigen is added to the first wells of the first 4 rows. This is mixed, and tenfold dilutions are made by transferring 25 µl of the fluid to the next well. After the last well, 25 µl is discharged.
The red blood cell suspension is diluted to 1% and 25uL of this is added to each well.
After 45 minutes the results can be read.
The required HA unit is 2^2 (=4). To find this, the HA titer is divided by 4.
The required volume for antigen suspension is calculated. Antigen is diluted using PBS. This antigen suspension was used for the HI testing of the serum.

Back titration

Materials:

- 96 wells plate
- ND Antigen
- 10% RBC
- PBS
- Pipettor (Transferpette-8, Eppendorf Research)
- Pipettor tips

Methods:

The HI test is used to confirm the 4 HA unit.

HI testing

Materials:

- 96 wells plate
- ND Antigen
- 10% RBC
- PBS
- Pipettor (Transferpette-8, Eppendorf Research)
- Pipettor tips

Methods:

25 µl of PBS is added to each well. 25uL of sample sera is added to the first and last well of each row (the last well contains the control sera). The antigen is then added to each well except the control wells. RBC suspension is diluted to 1% and then 25uL of this dilution is added to each well of the plate. The plate is shaken by tapping with the hand on the side of the plate for a couple of times. After that the plate was placed on the table. After 45 minutes the results can be read.

Results

Results Horizontal transmission

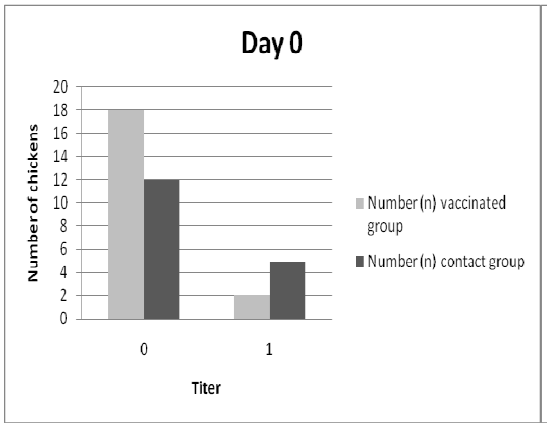

Fig 1.1

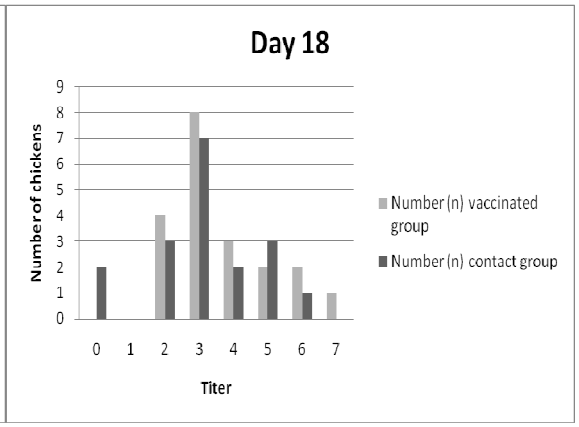

Fig 1.2

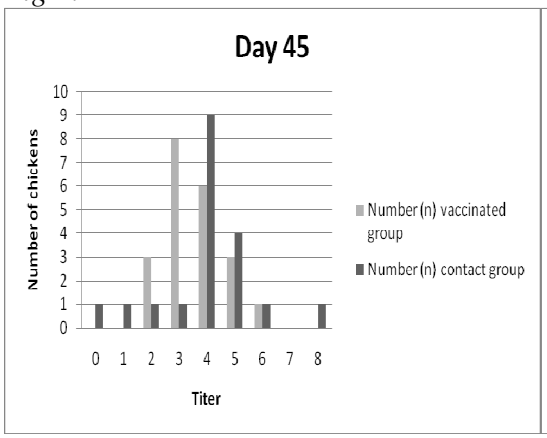

Fig 1.3

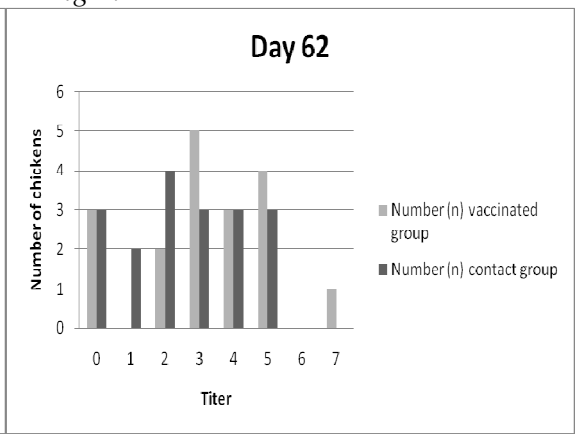

Fig 1.4

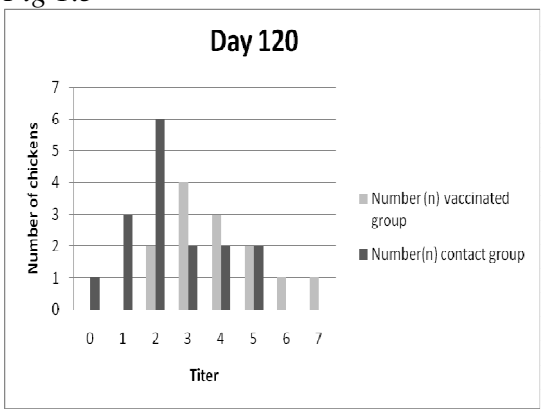

Fig 1.5

Figure 1 shows the titers of the chickens at different times after vaccination. In figure 1.1 is shown that there was no antibody titer at day 0. After day 0 the titers rises to protective amounts.

Results vaccinated Group (white chickens)	Number of samples	Prevalence of protective titers	Average of protective titers HI titer of ≥ 3	Average titer
Day 0	20	0 %	0	0.10
Day 18	20	80 %	4.06	3.65
Day 45	21*[1]	86 %	3.83	3.75
Day 62	18 (2†)	72 %	4.15	3.22
Day 120	13 (7†)	85 %	4.27	3.92

Table 2.1 The amount of protection in the vaccinated group.

Results Contact Group (black chickens)	Number of samples	Prevalence of protective titers	Average of protective titers HI titer of ≥ 3	Average titer
Day 0	17 (1†)*[2]	0 %	0	0.29
Day 18	18 (1†)*[3]	72 %	3.58	3.11
Day 45	19 (1†)	84 %	4.56	4.00
Day 62	18 (2†)	50 %	4.00	2.56
Day 120	16 (4†)	38 %	4.00	2.56

Table 2.2 The amount of protection in the contact group.

*[1] Two centrifuge tube were filled with blood of the same chicken, but the second tube wasn't marked

*[2] 2 Samples were lost/ not enough blood for serum

*[3] 1 Sample was lost/ not enough blood for serum

† Cumulative immunity

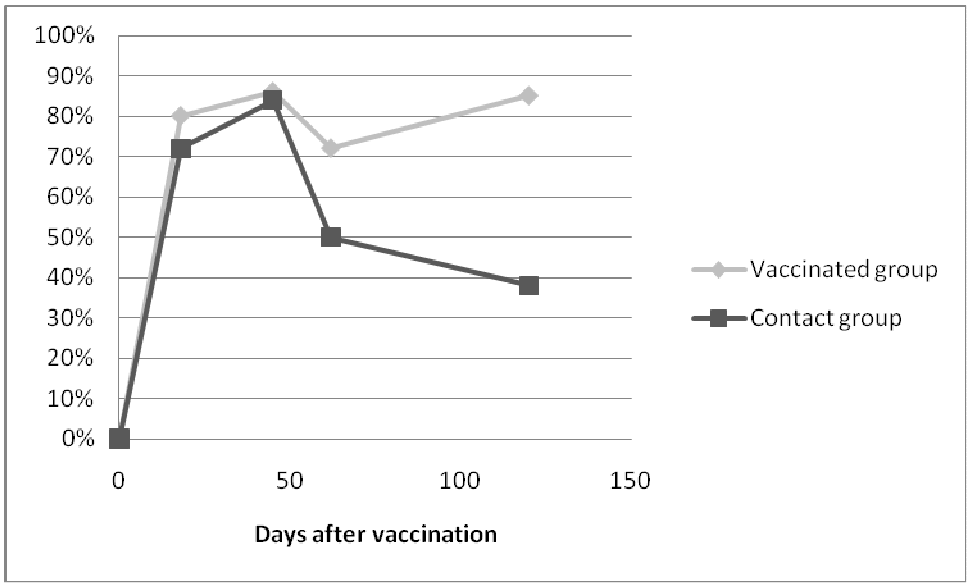

Fig 2 Percentage of the flock with a titer ≥2[3]

After 18 days had 80% of the chickens of the vaccinated group a protective titer against NDV. Of the contact group 72% of the chickens had a protective titer against NDV. This implies that both groups develop protective HI antibody titers against ND and thus contact with vaccinated chickens shedding the vaccine virus will result in immunity in unvaccinated chickens.

On day 45 all the chickens, the vaccinated group and the contact group, reach the necessary 80% that is necessary for a good protection of the flock. Apparently there is effective horizontal transmission of the vaccine (fig 2).

The protection of the contact group seems to decline at a faster rate than that of the vaccinated group.

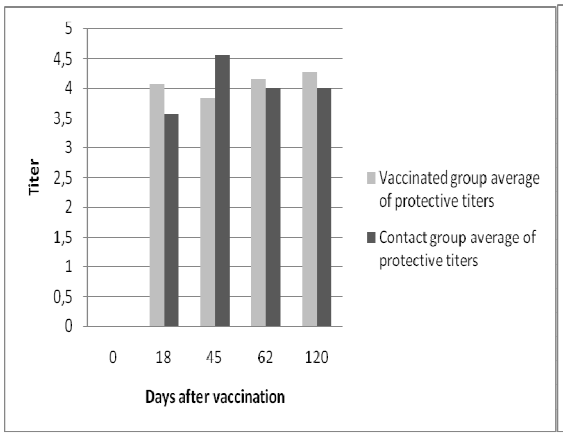

Fig 3.1

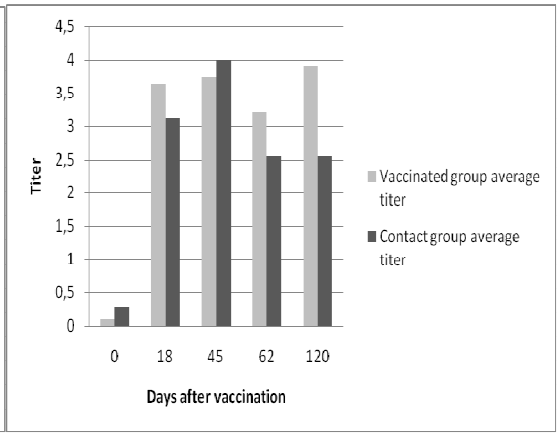

Fig 3.2

a protective titer is a HI titer of 3 or above

The average antibody titer are at protective levels on day 18 in both groups. The titers remains protective till day 45 (fig 3).
It seems that the titers of the contact group do not remain high for 120 days. The titers of the vaccinated group do remain high for at least 120 days, this confirms that chickens vaccinated with a vaccine of a good batch (EID 10^7) have good antibody titers for 4 months.
A marginal note must be placed about this findings due to mortality in both groups: 7 vaccinated chickens and 4 contact chickens died during the testing period of 120 days as given in table 2.1 and 2.2.

Results flock immunity in the field

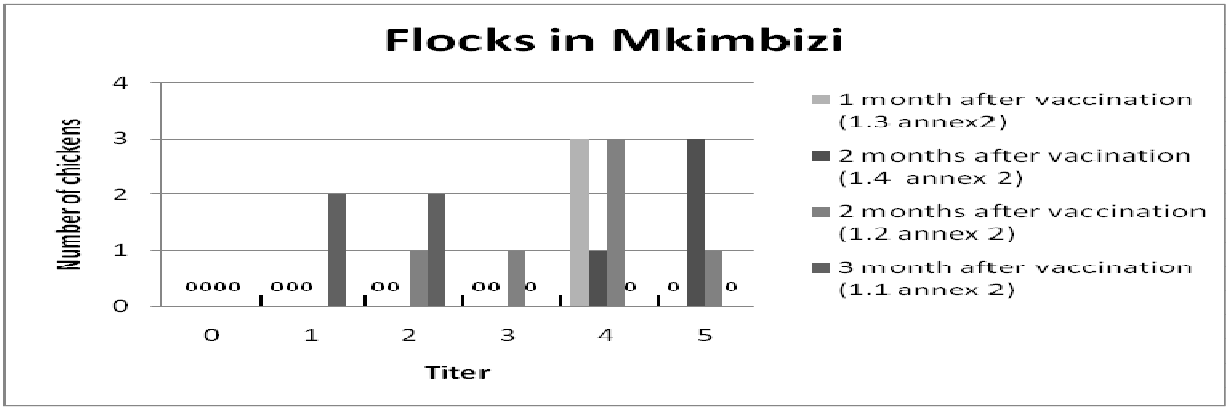

Fig 4.1

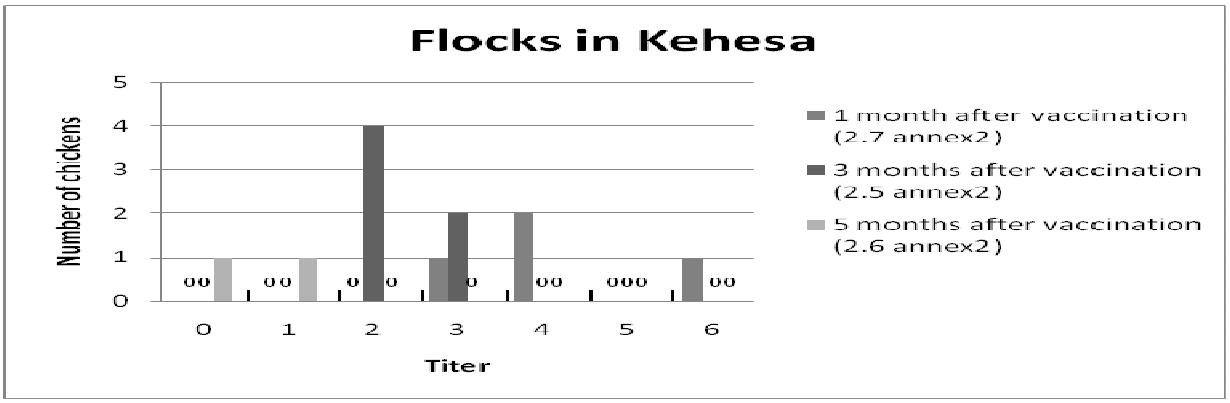

Fig 4.2

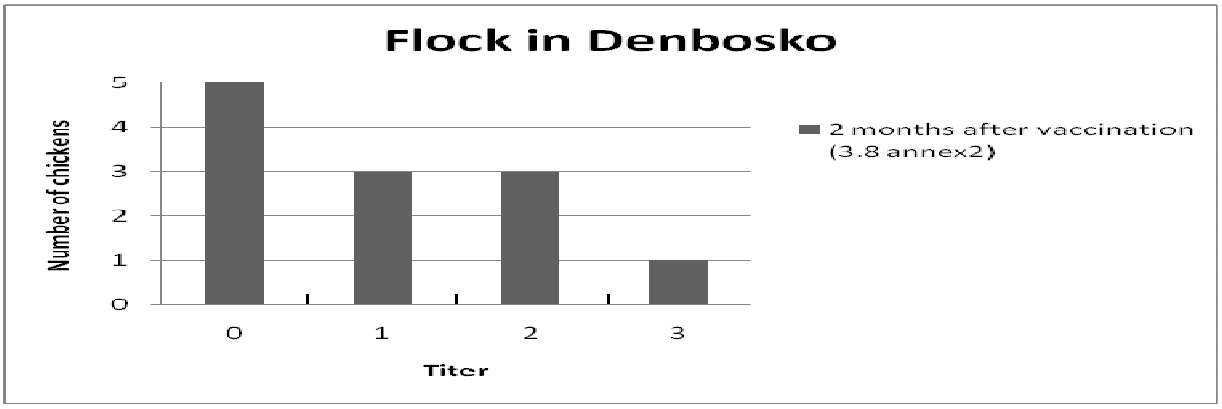

Fig 4.3

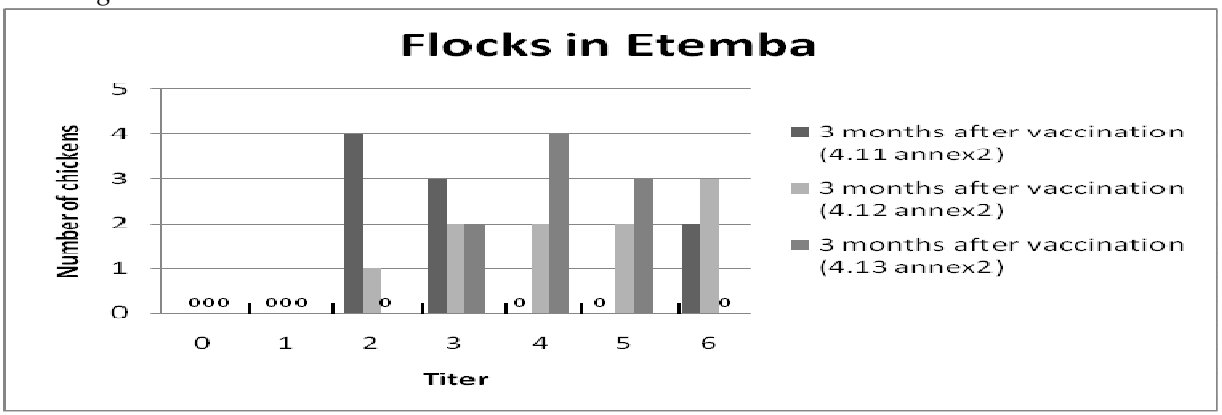

Fig 4.4

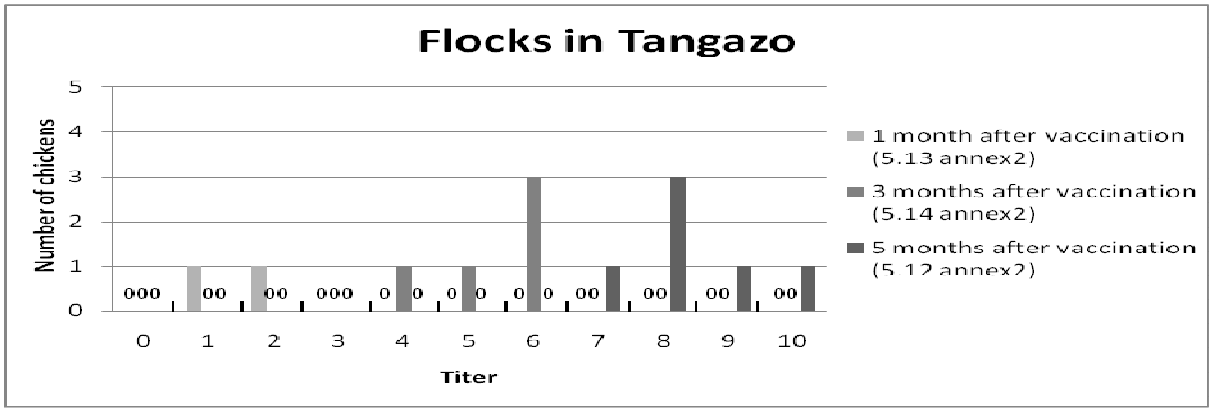

Fig 4.5

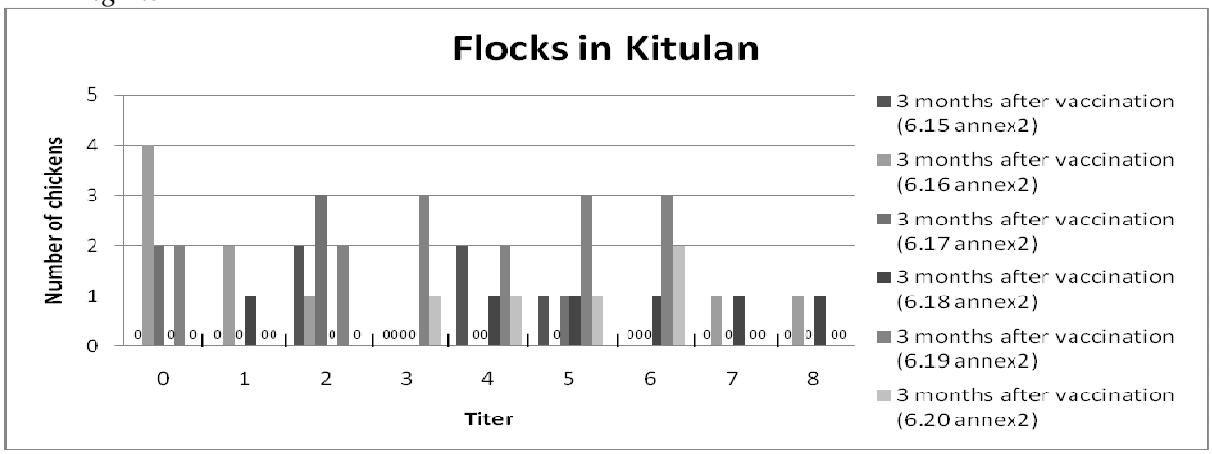

Fig 4.6

In the villages some chickens have a protective titer against ND (titer ≥3) and some chickens have a HI titer under 3. In a flock this pattern can be seen as well. There are also flocks where all the chickens have a protective titer against ND and flocks where all the chickens have a titer under 3. So a difference in immunological status can be seen within a flock or village and between different flocks (fig 4.1-4.6).

Village	Prevalence of protected titers (%)
Mkimbizi	71%
Kehesa	50%
Tangazo	85%
Etembo	82%
Kitulan	59%

Table 3 Protection in the villages
A titer is protective if it is ≥3

Table 3 shows that differences exist in the protection against NDV in the villages. Only the flocks in Tangazo (85% protective titers) and Etemba (82% protective titers) are protected. Poultry in Mkimbizi have a moderate protection as well, but flocks in Kehesa and Kitulan show a poor prevalence of protected titers. So this means that at least two of five villages are not well protected. (Allan 1974, Alders 2002).

Month	Village	Flock number (annex 2)	Prevalence of protected titers	Average protected titers	Average of titers
1	Mkimbizi	1,3	100	4	4
	Kehesa	2,7	100	4,25	4,25
	Tangazo	5,13	0	0	1,5
2	Mkimbizi	1,4	100	4,75	4,75
	Mkimbizi	1,2	83	4	3,67
	Den Bosko	3,8	7	3	0,86
3	Mkimbizi	1,1	0	0	1,5
	Kehesa	2,5	33	3	2,33
	Tangazo	5,14	100	5,4	5,4
	Etemba	4,11	100	4,11	4,11
	Etemba	4,10	90	4,67	4,4
	Etemba	4,9	56	4,2	3,22
	Kitulan	6,20	100	4,8	4,8
	Kitulan	6,18	83	6	5,17
	Kitulan	6,19	73	4,55	3,6
	Kitulan	6,15	60	4,33	3,4
	Kitulan	6,16	22	7,5	2,11
	Kitulan	6,17	17	5	1,83
5	Kehesa	2,6	0	0	0,5
	Tangazo	5,12	100	8,67	8,67

Table 4 Protection against ND of different flocks

In table 4 we can see the protection against ND of the different flocks. The most important finding to point out is the difference in protection. That difference can be seen in the prevalence of protected titers and the average of titers; of the 18 flocks 10 have a good titer, 6 have a very low titer and the rest have moderate protection.
The fifth month there was one flock of chickens with very high titers. In the experiment with horizontal transmission the vaccinated group did not reach such high titers, and also we did not find such high titers in the other flocks during this experiment. Therefore it is likely that the titers found in this flock are caused by a field infection.

Months after vaccination	Total number of chickens	Mean number of chickens in a flock	Number of samples	Mean number of samples in a flock	Prevalence of protected titers	Average of protected titers	Average of titers
1	77	25,70	9	3,0	77,78	4,14	3,56
2	80	40,00	10	5,0	90,00	4,33	4,10
3	154	12,83	85	7,1	67,00	4,74	3,46
5	15	7,50	8	4,0	75,00	8,33	6,37

Table 5 Protection against ND at different intervals after vaccination
A protected titer is a HI titer of 3 or above

In table 5 the prevalence of protective titers after one, two, three and five months after vaccination is shown. Unfortunately there are no results from 4 month after vaccination, because the flocks are vaccinated every three months in Iringa. So only by accident we had two flocks that were vaccinated for the last time five months ago. One of these possibly suffered from a field infection with ND which is suggested by the very high HI titers.
One flock (flock 3.8 see annex 2) is not included in this calculations, because these were not free range chickens.
Based on the results of the vaccinated group in the experiment of the horizontal transmission it may be argued that the prevalence of protective titers and the average titers can be acceptable until at least four months after vaccination.
Effective flock protection for the vaccination interval of at least 3 months may be attained in the field as well, as is demonstrated in the results. Despite it more than 40% of the flocks examined show a immune status below the protective level (table 4 and 5).

Discussion

Horizontal transmission

In the experiment is shown that more than 80% of the chickens in the contact group produce a sufficient antibody titer against ND. The research from Henning and Morton 2008 showed that horizontal transmission was not sufficient to create protective titers in free range chickens. This is a marked difference with our results. This is most likely due to the fact that our contact group were held in close proximity to the vaccinated group. This shows that horizontal transmission can create protective titers in contact chickens if the conditions are correct. So the chickens need to be held in close proximity to one another.

Apparently the titer level in the contact group decreases at a faster rate than that of vaccinated group. This faster decline of titers in the contact group is in line with earlier research (Tran Dinh Tu 1998). This will act to discourage people to vaccinate only a part of their flock.

The faster decrease could be due to the fact that the vaccine dose differs between the vaccinated and the contact group, it is likely that the contact birds receive a smaller dose EID50 than the vaccinated birds.

Another reason could be that there is a change in the characteristics of the antigen in the feces/ nasal discharge of the vaccinated chickens compared with the original vaccine. After vaccination the I-2 vaccine virus multiplies in tissues before excretion, this excreted viable virus, can give immunity to contact chickens (Illango 2008).

Also the route of transmission does matter. If a vaccine is administrated directly into the eye, the harderian gland, is important in developing immunity (Spradbrow 1992, Alders 2002). In the contact group it is more likely that the chickens received the antigen though oral contact. In this case receptors for NDV in the digestive tract are activated and cell mediated and mucosal immunity develop, but this way is less effective than the immunity caused by the harderian gland (Spradbrow 1995). The method of immunity development could be different by a different entry point resulting in the immunity levels have acting different characteristics (Bensink and Spradbrow 1999).

In table 2 and fig 2, 3 it is seen that the titers of the vaccinated group rises again on day 120, the reason may be due to a rapid change in titer levels due to environmental factors which could result in individual chickens having markedly different titers at different periods. This change in titer levels in the flock was also seen in the research of Illango (2005), where the chickens in 4 of the 5 groups show this kind of wave pattern in chickens with a protective antibody titer.

Flock immunity in the field

There is a difference between the immunity of the flocks. Some chickens have a good immunity for months but in others the immunity is very low.

A possible explanation for this is that the vaccinator (somebody who has had training to vaccinate chickens) buys the I-2 vaccine in the district he is working in. The vaccinator visits different villages in the district he is working in to vaccinate chickens. He is allowed to keep the vaccine one week in the fridge so he can work one week day after day with the same vaccine. In this week the vaccine is exposed to different temperatures. Also it is not always possible for the vaccinator to work in the shadow so the vaccine will be exposed to sunlight. This will drop the EID of the vaccine, so the chickens are vaccinated with a lower dose of vaccine at the end of the week. This can have different consequences. It can be that there are less chickens that get infected by the vaccination or the chickens get still infected but have a lower immunological response. The immunity of the flock in the end of the week will be less than the development of the immunity in the beginning of the week due to this fact. This could

be the reason that there are different protection levels of different flocks within the one village (Table 3 and Fig 3.1-3.6).
Of course there are also more general factors that can interfere with vaccination, like method of administration, the time the vaccinator/ owner takes to optimize the method of administration and the health status of the animals. The health status should be tested before vaccination, because the animal needs to be healthy for a successful vaccination. Stress may also have a negative influence on the immune response. Stress factors for chickens in Tanzania are debeaking, transport, high temperatures, start of laying, lack of vitamins and bad hygienically status (Zanella 2007).
Vaccination is usually performed once every 3 months according to the vaccinators schedule. After vaccination some horizontal transmission maybe expected, because of the viable virus in the excrement of vaccinated birds (Illango 2008). This excrement of virus is for some days, chickens introduced in the flock after this period (young chicks or bought chickens) will not develop immunity against ND.
One vaccinated flock that had been vaccinated 5 months ago showed very high titers (average titer of 8.67). The titers of that flock exceeded the titers of all the other chickens in the field, as well as the titers of the chickens vaccinated for the horizontal transmission experiment. Therefore this results are most probably due to a field infection. This emphasizes that ND vaccination is very important.
Very alarming is that more than 40% of the flocks in the field have very disappointing titers 3 months or earlier after vaccination. It is proved that titers can remain protective for at least four months after vaccination with the I-2 vaccine produced in Tanzania like showed in the vaccinated group of the horizontal transmission experiment and in other researches (Mgomezulu 2005).

Recommendations

ND is a very important disease in Tanzania, there are several outbreaks of ND every year (43 in total 2009 and first 5 months of 2010 and 2 have been reported in Iringa), also during this research one field infection was found. This is why vaccination is so important and needs to be done properly and on time.
The results demonstrate indeed that a protective immunity of a flock for a period of at least 3 months may be achieved with the I-2 vaccine produces in Tanzania. However in the field there are differences in protection between flocks and within flocks. With as a result that 40 till 45% of the flocks have not a optimal protection against ND.
To reach a status where every vaccinated flock develops a good prevalence of protective titers it is necessary to optimize the production, transport of the vaccine and vaccination.
Concerning the production of the I-2 vaccine the research of Strijker 2010 show some interesting points.
To optimize ND vaccination more research need to be performed with respect to the transport of the vaccine and vaccination itself. It would be recommended to do research on the development of titers of flocks that are being vaccinated right after opening the bottle compared to the titers of flocks vaccinated halfway the bottle and at the end of a bottle. To obtain these results the route the vaccinator takes also need to be recorded.
In line with this it would interesting to know the EID of the vaccine at these moments, when the bottle is just opened, when it is half full and when the bottle is almost finished.
To gain more information about the effectiveness of the method of vaccination it would be important to know the average titers the different individual vaccinators reach and the titers achieved by every owner who does the vaccination himself.

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Annex 1

Results Horizontal transmission

Vaccinated group		Contact group	
Day 0			
n	Titer	n	Titer
1	2^0	1	2^1
2	2^0	2	2^1
3	2^0	3	2^0
4	2^0	4	2^0
5	2^1	5	2^0
6	2^0	6	2^0
7	2^1	7	2^1
8	2^0	8	2^0
9	2^0	9	2^0
10	2^0	10	2^0
11	2^0	11	2^0
12	2^0	12	2^0
13	2^0	13	2^0
14	2^0	14	2^1
15	2^0	15	2^0
16	2^0	16	2^1
17	2^0	17	2^0
18	2^0		
19	2^0		
20	2^0		

Vaccinated group		Contact group	
Day 18			
n	Titer	n	Titer
1	2^3	1	2^5
2	2^4	2	2^6
3	2^7	3	2^3
4	2^2	4	2^5
5	2^4	5	2^2
6	2^3	6	2^3
7	2^3	7	2^3
8	2^3	8	2^3
9	2^3	9	2^2
10	2^3	10	2^3
11	2^3	11	2^0
12	2^5	12	2^5
13	2^2	13	2^4
14	2^5	14	2^4
15	2^4	15	2^0
16	2^6	16	2^3
17	2^6	17	2^2
18	2^2	18	2^3
19	2^3		
20	2^2		

Vaccinated Group		Contact group	
Day 45		Day 45	
n	Titer	n	Titer
1	2^3	1	2^4
2	2^3	2	2^5
3	2^5	3	2^5
4	2^2	4	2^4
5	2^5	5	2^3
6	2^4	6	2^0
7	2^4	7	2^5
8	2^2	8	2^5
9	2^3	9	2^4
10	2^4	10	2^6
11	2^3	11	2^4
12	2^3	12	2^4
13	2^3	13	2^4
14	2^4	14	2^8
15	2^4	15	2^4
16	2^3	16	2^1
17	2^2	17	2^4
18	2^6	18	2^4
19	2^4	19	2^2
20	2^3		
21	2^5		

Vaccinated group		Contact group	
Day 62		Day 62	
n	Titer	n	Titer
1	2^4	1	2^0
2	2^5	2	2^2
3	2^7	3	2^5
4	2^2	4	2^4
5	2^3	5	2^0
6	2^0	6	2^2
7	2^5	7	2^2
8	2^0	8	2^1
9	2^0	9	2^3
10	2^4	10	2^0
11	2^5	11	2^5
12	2^3	12	2^5
13	2^2	13	2^3
14	2^3	14	2^1
15	2^4	15	2^3
16	2^5	16	2^4
17	2^3	17	2^4
18	2^3	18	2^2

Vaccinated group		Contact group	
Day 120		Day 120	
n	Titer	n	Titer
1	2^3	1	2^4
2	2^7	2	2^3
3	2^3	3	2^4
4	2^5	4	2^1
5	2^2	5	2^2
6	2^3	6	2^3
7	2^5	7	2^2
8	2^4	8	2^5
9	2^2	9	2^2
10	2^4	10	2^1
11	2^6	11	2^2
12	2^3	12	2^0
13	2^4	13	2^2
		14	2^1
		15	2^2
		16	2^5

Annex 2

1. Mkimbizi

1.1 A. M. Nyenza

6 adult chickens
Blood taken of 4 chickens
Vaccination by vaccinator 3 months ago

Sample number	Titer
1.1.1	2^2
1.1.2	2^2
1.1.3	2^1
1.1.4	2^1

1.2 Woman holding the chickens

30 chickens also she has some rabbits housed with the chickens
Blood taken of 6 chickens (het bloed was erg dik, de dieren hadden ook geen water bij 40 graden Celcius)
Vaccination by vaccinator 2 months ago

Sample number	Titer
1.2.1	2^4
1.2.2	2^4
1.2.3	2^3
1.2.4	2^5
1.2.5	2^4
1.2.6	2^2

1.3 Little farm with 100 chickens

Young chickens are being vaccinated
2 or 3 days after hatching
The farmer also has 3 pigs and a cow separated domesticated from the chickens
50 Chickens beneath 2 months of age
Blood taken of 3 chickens
Vaccinated by the owner 1 month ago

Sample number	Titer
1.3.1	2^4
1.3.2	2^4
1.3.3	2^4

1.4 Same farm

50 adult chickens domesticated in another building
Blood taken of 4 adult chickens
Vaccinated by the owner 2 months ago

Sample number	Titer
1.4.1	2^5
1.4.2	2^5
1.4.3	2^5
1.4.4	2^4

2. Kehesa

2.5 Kehesa

30 Adult chickens, also there are some chicks beneath 2 months of age
Blood taken of 6 chickens
Vaccinated by the owner 3 months ago

Sample number	Titer
2.5.1	2^2
2.5.2	2^2
2.5.3	2^2
2.5.4	2^2
2.5.5	2^3
2.5.6	2^3

2.6 Kehesa

There are 2 turkey, 2 goose, 1 rabbit and a pig all housed together with the chickens.
5 Adult chickens
Blood taken of 2 chickens
Vaccinated by vaccinator 5 months ago

Sample number	Titer
2.6.1	2^1
2.6.2	2^0

2.7 Kehesa

7 adult chickens
Blood taken of 4 chickens
Vaccinated by vaccinator 1 month ago

Sample number	Titer
2.7.1	2^4
2.7.2	2^6
2.7.3	2^4
2.7.4	2^3

3. Denbosko

3.8 Awubu Mlowe

500 adult chickens (Divided in 4 groups of 125 chickens separated domesticated)
These chickens aren't free range chickens
Blood taken of 14 chickens
Vaccinated by the owner 2 months ago

Sample number	Titer
3.8.1	2^0
3.8.2	2^1
3.8.3	2^2
3.8.4	2^2
3.8.5	2^1
3.8.6	2^0
3.8.7	2^1
3.8.8	2^0
3.8.9	2^3
3.8.10	2^2
3.8.11	2^0
3.8.12	2^0
3.8.13	2^0
3.8.14	2^0

4. Etemba

4.9 Zakie Kasuva

16 adult chickens
Blood taken of 9 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
4.9.1	2^2
4.9.2	2^3
4.9.3	2^2
4.9.4	2^6
4.9.5	2^2
4.9.6	2^3
4.9.7	2^2
4.9.8	2^3
1.9.9	2^6

4.12 Betrida

11 adult chickens
Blood taken of 10 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
4.10.1	2^3
4.10.2	2^4
4.10.3	2^2
4.10.4	2^6
4.10.5	2^5
4.10.6	2^6
4.10.7	2^6
4.10.8	2^5
4.10.9	2^3
4.10.10	2^4

4.13 Toosk Makitos

11 adult chickens
Also there were some young chickens
Blood taken of 9 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
4.11.1	2^5
4.11.2	2^4
4.11.3	2^3
4.11.4	2^3
4.11.5	2^4
4.11.6	2^5
4.11.7	2^4
4.11.8	2^5
4.11.9	2^4

5. Tangazo

5.12 Romana Utenga

10 adult chickens
Also some young chickens
Blood taken of 6 chickens
Vaccinated by a vaccinator 5 months ago

Sample number	Titer
5.12.1	2^7
5.12.2	2^9
5.12.3	2^9
5.12.4	2^8
5.12.5	2^{10}
5.12.6	2^9

5.13 Shaibu Mbuma

20 adult chickens and also some young chicken, not very healthy chickens
Blood taken of 2 chickens, it was very hard to get blood from this chickens, and we were thinking that it was irresponsible to preside, so we stopped.
Vaccinated by a vaccinator 1 month ago

Sample number	Titer
5.13.1	2^2
5.13.2	2^1

5.14 Tangazo

7 chickens different ages
Blood taken of 5 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
5.14.1	2^6
5.14.2	2^5
5.14.3	2^6
5.14.4	2^6
5.14.5	2^4

6. Kitulan

6.15 Antonino Angogali

A little farm with also some cows and pigs and 2 dogs but all are separated domesticated
14 Adult chickens
Blood taken of 5 Chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
6.15.1	2^5
6.15.2	2^4
6.15.3	2^4
6.15.4	2^2
6.15.5	2^2

6.16 Husin Masamili
21 adult Chickens
Blood taken of 9 Chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
6.16.1	2^7
6.16.2	2^1
6.16.3	2^8
6.16.4	2^0
6.16.5	2^0
6.16.6	2^0
6.16.7	2^1
6.16.8	2^0
6.16.9	2^2

6.17 Kitulan
7 adult Chickens
Blood taken of 6 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
6.17.1	2^0
6.17.2	2^0
6.17.3	2^2
6.17.4	2^5
6.17.5	2^2
6.17.6	2^2

6.18 Kitulan
6 adult chickens
Blood taken of 6 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
6.18.1	2^7
6.18.2	2^6
6.18.3	2^4
6.18.4	2^1
6.18.5	2^8
6.18.6	2^5

6.19 Kitulan
19 adult chickens
Blood taken of 15 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
6.19.1	2^5
6.19.2	2^4
6.19.3	2^5
6.19.4	2^6
6.19.5	2^2
6.19.6	2^0
6.19.7	2^6
6.19.8	2^6
6.19.9	2^5
6.19.10	2^4
6.19.11	2^3
6.19.12	2^3
6.19.13	2^0
6.19.14	2^2
6.19.15	2^3

6.20 Romanus Pambila
6 adult chickens
Also lot of young chickens
Blood taken of 5 chickens
Vaccinated by a vaccinator 3 months ago

Sample number	Titer
6.20.1	2^5
6.20.2	2^4
6.20.3	2^6
6.20.4	2^6
6.20.5	2^3