

**AN INVESTIGATION OF A STRATEGY FOR THE VACCINATION
OF FREE RANGING CHICKENS AGAINST NEWCASTLE
DISEASE IN QWA-QWA, SOUTH AFRICA**

BY

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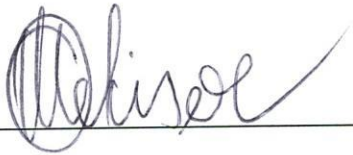
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DECLARATION

I, **Matlhahane Molifi Oriel Thekiso**, hereby declare that this research thesis is my original work and has not been presented for a degree in any other University.

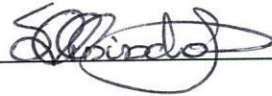


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DEDICATION

This research project is dedicated to the Thekisoie and Semela families,

“Belina Thekisoie and Elizabeth Mosesi”

for your support and inspiration.
(Modimo le badimo ba be le lona)

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ABSTRACT

The primary aim of this study was to develop a strategy to control Newcastle disease in free ranging village chickens using the Nobilis® ND Inkukhu vaccine (Intervet South Africa). The study was conducted at Thibella village in Qwa-Qwa district of the eastern Free State province of South Africa, from April 2001 – October 2002. Three different routes of vaccination (administration via eye-drop, drinking water and feed) were investigated and the most efficient route was determined. Haemagglutination inhibition test was conducted frequently after revaccinations in order to measure the antibody status of village chickens after immunisation against Newcastle disease. The study also sought to determine intervals for re-vaccinations. Trial challenges were conducted to determine the efficacy of the vaccine using a South African isolate of velogenic Newcastle disease virus. A questionnaire survey was also conducted to determine perceptions and knowledge on vaccines by free ranging chicken owners.

The eye-drop vaccination route produced the highest haemagglutination inhibition (HI) titres ranging between 2.7 and 4.4, followed by drinking water vaccination route with HI titres ranging between 2.3 and 4.0. The lowest titres were from the feed vaccination route where HI titres ranged between 1.6 and 3.0. Following the trial challenge experiment, the entire control group died on the 3rd and 4th day after infection. However 70 % of the chickens immunized with the Nobilis® ND Inkukhu vaccine by using either the eye-drop or drinking water route survived the trial challenge. Only 20 % of the chickens from the group immunized through the feed route survived the trial challenge. Evidently both the eye-drop and drinking water

vaccination routes were efficient in preventing infection of Newcastle disease virus. The efficiency of the vaccine using either of the routes can be enhanced by having boosts at 3-month intervals on the first year of a vaccination campaign and then at 6-month intervals on the second year onwards.

From the questionnaire survey, 96 % of the owners mentioned that the indigenous name in Sesotho for Newcastle disease is **Mokoiyo**. The questionnaire also revealed that outbreaks occur mainly in spring months between August and October. The majority of the respondents indicated that they would prefer to vaccinate their flocks using the drinking water route. They also proposed that extension officers should be involved in the vaccination exercises. It was evident that in the majority of households in Qwa-Qwa women were the ones who were primarily responsible for taking care of the chickens.

It will be valuable in the future if village communities in Qwa-Qwa were educated in the control of poultry diseases, in particular on Newcastle disease. Through workshops and seminars, and also by assisting in vaccination programs, extension officers in rural areas can play a critical role in the growth of free ranging poultry farming in poor rural communities in South Africa.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 BACKGROUND

Free ranging chickens, also referred to, as backyard chickens are mostly owned by village communities in rural areas of South Africa. They are bred and raised for their meat and eggs as they play a major role in family consumption. These chickens are also important in performing traditional rituals, are used in bartering and also given as gifts for visiting relatives. However there are different constraints in free ranging village chicken production such as, mortalities due to disease, poor technical support, predation, theft and poverty.

Many researchers in different countries of Africa, Asia and Australia have mentioned Newcastle disease as the major cause of mortalities in village chicken production. In South Africa statistics from the National Department of Agriculture have shown that there has been 231 reported outbreaks of Newcastle disease between 1995 and 1999 from both the commercial sector and the village chicken sector.

In the commercial sector Newcastle disease is kept under control mainly by vaccinations, whilst there are no vaccination campaigns aimed at controlling Newcastle disease in free ranging village chickens. In a recent study conducted by Thekisoe *et al.*, in year 2000, Newcastle disease was found to be existing in the rural Qwa-Qwa villages. This led to the current study which is primarily aimed at developing a vaccination strategy against

Newcastle disease in free ranging village chickens in Qwa-Qwa. This will hopefully assist in controlling Newcastle disease and thereby improve free ranging village chicken production in Qwa-Qwa, South Africa.

1.2 VILLAGE POULTRY PRODUCTION PROBLEMS AND POTENTIAL

Poultry is by far the largest livestock group in the world and is estimated to be about 14 000 million, consisting mainly of chickens, ducks and turkeys (FAO, 1999). In total, poultry products (eggs and meat) constitute 30 % of all animal protein consumed worldwide. Within the last 10 years this proportion has increased from 20 % and is predicted to increase to 40 % before the year 2015 (IFPRI, 2000). Poultry production in most developing countries is based mainly on scavenging backyard systems. It has been estimated that 80 % of the poultry population in Africa is found in traditional production systems. Little attention is given to this means of production even though 30 – 100 % of the animal protein consumed in some villages comes from this source (Gueye, 1998).

In the wake of animal protein deficit due to poverty, some African countries supported poultry production in the hope of providing the human population with nutritious and affordable animal products. This support usually occurred in the form of improved day-old chicks and feeds, promoting the establishment of local hatcheries, supply of veterinary drugs and the provision of technical support and controls or bans over imports of cheap poultry products. As a result of that support, the poultry sector has grown

rapidly throughout the African continent. Over the last decade, growth in the African poultry population was estimated at 29 % (Gueye, 2000).

There is an enormous deficit between the animal protein produced in Africa and that required to feed the continent's 550 million people. The deficit is attributed to low productivity of livestock rather than to low numbers. The poultry industry in Africa involves both scavenger and exotic (European) breeds. Most of the people in Africa live in rural areas and have a very low income. They cannot afford to buy poultry products from commercial farms. Scavenger chickens are their main source of animal protein. In the first instance they are cheap to produce. Secondly people find it much easier to kill a chicken for family consumption than, say, a goat, sheep or cow. The larger livestock are kept mainly for prestige or as a sign of wealth. The higher the number of animals one keeps the higher one's status in the community and also the wealthier one is regarded (Musiime, 1991).

With the exception of urban areas in northern and southern Africa, poultry production in Africa is undertaken through an extensive system at village or family level. Almost every village household keeps domestic chickens (on average between 5 and 20 birds) and it is estimated that village fowl make up more than 80 % of the total fowl population in Africa, estimated at 1 068 million in 1995 (Gueye, 1998). Under these circumstances, poultry provides a good source of protein and ready cash for villagers, which in turn helps to sustain the village economy and contributes to the prevention of urban migration. The benefits of family poultry production go directly to the rural poor, in most cases to the women who are the principal caretakers (Dwinger *et al.*, 2000).

Poultry diseases are a result of various disease causing agents. There are fungal diseases caused by fungi and yeasts, bacterial diseases such as salmonellosis, viral diseases which include paramyxoviruses which cause Newcastle disease and parasitic diseases such as coccidiosis caused by protozoa called coccidia of the genus *Eimeria*. There are also ectoparasites found on poultry such as lice, mites and ticks which cause skin irritations, blood and production losses and are vectors for disease causing agents. It is very difficult to quantify losses due to diseases in village poultry, as there is little published information (Jordan and Pattison, 1996).

Diseases contribute a lot to mortalities of chickens especially to the village chickens as they receive little or no veterinary attention due to a lack of knowledge of the owners about poultry diseases. In a study which investigated backyard chickens in Zimbabwe which were not vaccinated, it was found that the mean mortality rate due to disease was estimated at between 3 and 100 % (mean 25 %) mostly in young birds under 6 weeks old (Kelly *et al*, 1994).

From a survey on village chicken losses conducted in Zimbabwe by Kusina *et al.*, (2000), they reported that, basically losses were due to diseases, parasites and predators. The extent and severity of diseases were reported to be seasonal with greatest magnitude of losses occurring during the hot dry season. They made a Venn diagram showing causes of poultry losses (Figure 1). The central circle represents all poultry losses in the area. The size of the marker representing a problem or cause of loss and the distance from the centre indicates the ease with which poultry can be lost through that particular cause. The smaller the area and the further the marker is away

from the centre, the less important the problem is as a cause of village poultry loss. It is evident that Newcastle disease (ND) is depicted as the biggest cause of poultry loss.

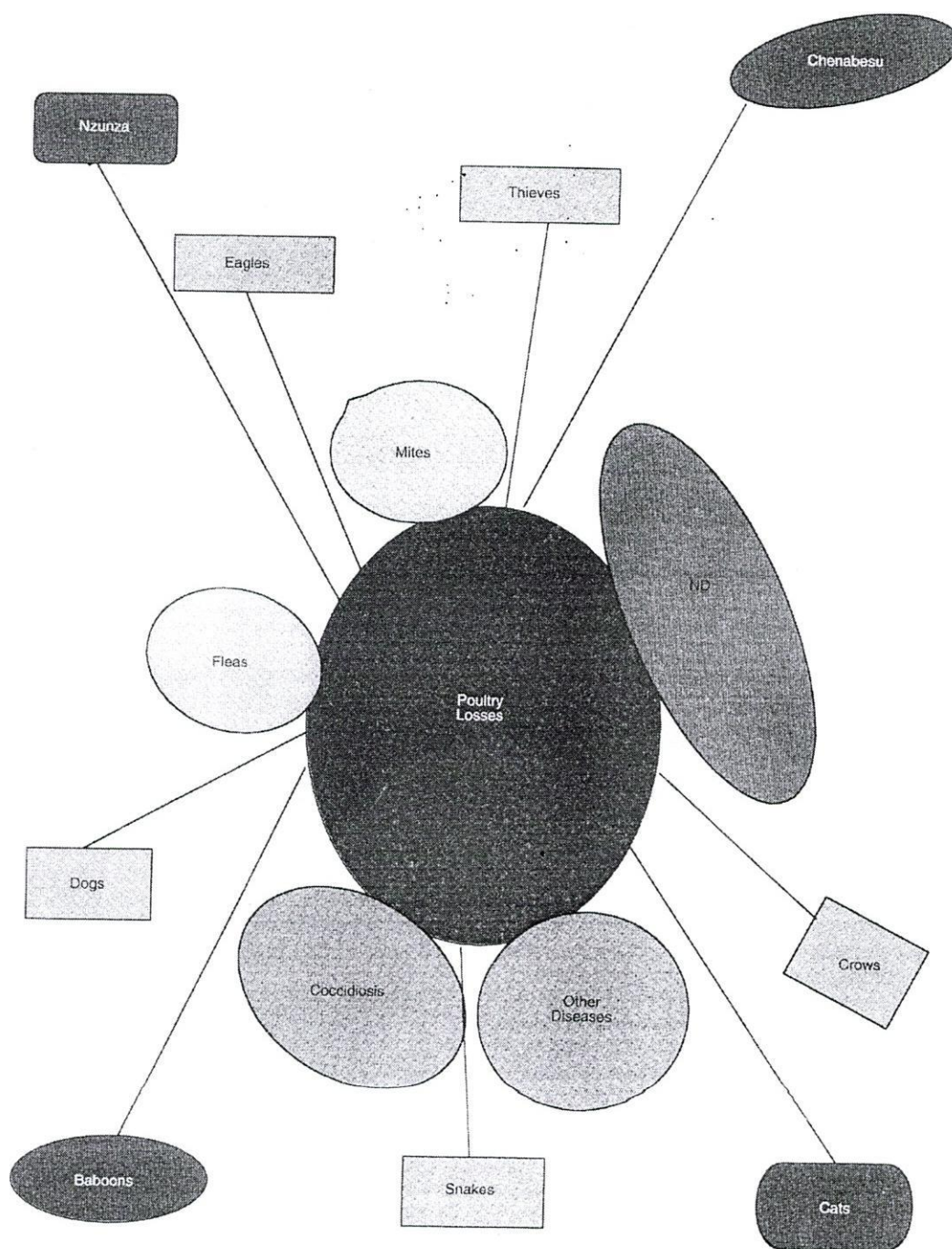


FIGURE 1: Venn diagram showing causes of poultry losses (Kusina *et al*, 2000)

This situation prevails in many other African countries (Kitalyi, 1998). Data from Yongolo (1996) on village poultry studies conducted in Tanzania support the argument that Newcastle disease is the most devastating disease of village chickens.

The importance of village chickens in the economy of developing countries has been coming to the fore far more noticeably over the past few years. Not only are they the most numerous poultry type in the world, but when they do not die from Newcastle disease they play a very important role in the cash flow of the poor rural population (Cumming, 1991). The village poultry system has been described on many occasions by a number of authors (Huchzermeyer, 1993; Aini, 1990) as small flocks, nil or minimal inputs, with low output and periodic decimation of the flocks by Newcastle disease.

Chickens are usually maintained as a source of meat, with few eggs being consumed or sold, as hens incubate practically every egg. When young birds reach a weight of about 1 kg, which takes up to 20 weeks, the birds are either sold or consumed by the household. In most countries the village chicken is highly prized as a gourmet meat and thus the price paid is frequently well above that paid for a similar sized commercial broiler chicken. The production from these flocks is low, seldom exceeding much more than 2.5 birds per month (Cumming, 1991).

Newcastle disease is the most important worldwide virus infection of poultry (Alexander, 1991; McFarren and McNulty, 1993). Although it is endemic in most countries of Africa (Bell, 1991), the clinical disease usually follows an epidemic pattern, with outbreaks occurring at times of climatic stress

(Awan *et al.*, 1994). In South Africa Newcastle disease was first diagnosed in 1944. A slaughter out policy was followed until 1971, after which a vaccination policy was introduced. A few outbreaks occurred in 1991 and in 1993 severe outbreaks occurred with high mortalities in poultry. Transmission of the disease was mainly due to movement of live chickens from the initial outbreak in the Northwest province (Verwoerd, 1998 unpublished). The statistics from the National Department of Agriculture shows reported outbreaks from 1995 – 1999. In 1995 alone, 61 outbreaks occurred throughout the country, of which most reports were from commercial poultry. These figures are however questionable as most of the outbreaks especially in rural areas have gone unreported (Tsibane, 2000).

Outbreaks of Newcastle disease in rural areas are mostly unreported due to lack of veterinary extension services in those areas, or to the fact that people do not know that it is a controlled disease that should be reported. To some people the loss of 5 chickens may not seem so important that they have to travel long distances from their villages to report to the nearest State Veterinarian's office. According to the affected owners, it is not of any great significance, as they will not be reimbursed for the dead chickens or benefit in any way from reporting the dead chickens. These combined factors contribute to the possible false picture of a low incidence of the disease that is reported in rural areas of South Africa (Tsibane, 2000).

1.3 SOCIO-ECONOMIC PROBLEMS IN VILLAGE CHICKEN PRODUCTION

Village poultry in Africa has evolved to be robust and sustainable, infectious diseases, particularly Newcastle disease, are important and sometimes a catastrophic constraint (Alders and Spradbrow, 1999). Vaccination against Newcastle disease is technically feasible (Biswas *et al.*, 1996; Wambura *et al.*, 2000) but an economically non-sustainable control strategy for village chickens in Africa (Sonaiya *et al.*, 1999). A variety of options to reduce control costs are being explored, including the production of vaccines by developing country institutes (Spradbrow and Copland 1996; Wambura *et al.*, 2000).

In Lesotho (a country with similar village conditions as in Qwa-Qwa, South Africa), the majority of poor live in rural and peri-urban areas. It is worthy to note that chickens are a resource available to the poorest families. Women have always kept chickens for various reasons, particularly as petty cash to cater for minor but essential household needs such as medical care, food, *etc.* Chickens are also used in several cultural rituals and traditional doctors use them for healing purposes. It is also not uncommon to offer a chicken as a gift (Lebajoa, 2000).

Among other African countries Lesotho is rated as having a high literacy level. Village chickens have played a significant part in the education of the rural people as live chickens and/or eggs are sold to raise money for school fees. They provide a cheap resource of protein in comparison with mutton or beef, which are even less healthy in terms of having high levels of

undesirable fat. As such it is more common to find poultry meat in the diet of children and they always carry eggs to school as a snack. Thus, chickens play a significant role in the rural economy and household food security (Lebajoa, 2000).

Commercial poultry production is mostly practiced on commercial farms and it requires large amounts of capital. There are different research institutes established to improve commercial poultry. The objective of these institutes is to emphasize poultry research and education to improve egg and meat production. This has resulted in the growth of different disciplines e.g. poultry breeding, poultry nutrition, poultry physiology, product technology and housing which today assume vital positions in animal production and health sciences (Reddy, 1991). This has no value for the majority of poultry farmers in the rural areas of the developing countries that cannot afford to use research results, as they are not applicable due to peculiar socio-economic conditions prevailing in such countries and regions.

The Qwa-Qwa district in the north-eastern Free State of South Africa has many villages where the Basotho people still live in traditional lifestyles. They own and breed various domestic animals such as cattle, sheep, goats, pigs and poultry. They own poultry for domestic consumption of eggs and meat. The average flock number of chickens in Qwa-Qwa is 18 with two or three males and the rest being females and chicks. Other domestic birds owned in Qwa-Qwa are geese, turkeys, ducks and pigeons. The chicken houses are made of inexpensive materials such as old corrugated iron and wire mesh. Poultry farmers in Qwa-Qwa use aloe plants, potassium permanganate and Epsom salt in order to prevent diseases. The insecticidal

powders (carbamate) called blue death powder (Robertson's (Pty) Ltd., Alrode) and carbadust (Effecto, Silverton) are used to control parasites and a disinfectant called Jeyes fluid (carbolic acid) (Adcock Ingram, Industria) is used when cleaning the poultry houses (Thekisoe *et al.*, 2003). Special emphasis should be laid on research and development best suited for maximizing poultry production in developing countries especially in rural areas.

1.4 NEWCASTLE DISEASE

1.4.1 Epidemiology of Newcastle disease

Newcastle disease is a devastating viral disease that occurs in most countries of the world (Aini and Ibrahim, 1990). This disease is a major problem in the rearing of village chickens (Spradbrow, 1990). The disease may manifest itself in different ways depending on the strain of the virus involved and the presence of immunity in the affected flocks. Newcastle disease is caused by a group of closely related viruses that form the avian Paramyxovirus type 1 (PMV-1) (Jordan and Pattison, 1996), and was the first member of the genus paramyxovirus to be isolated from birds in 1926 (Alexander, 1995). The incubation period varies with the strain, and is generally 4 to 5 days (range 2 to 15 days). The virus is readily inactivated by formalin, alcohol Merthiolate, lipid solvents, lysol and ultraviolet light (Bratt and Clavell, 1972).

It has been reported that in intensively managed poultry systems the main route of infection is respiratory/aerosol, and can spread rapidly. Feed, trucks, personnel, *etc.*, can also act as potential mechanical carriers. The mode of

transmission from bird to bird is dependent on the organs in which the virus multiplies. Birds showing this respiratory disease presumably shed virus in aerosols of mucus and it is known that birds in an atmosphere containing such aerosols become infected (Alexander, 1991).

Viruses that are mainly restricted to intestinal replication may be transferred by ingestion of contaminated faeces, either directly or in contaminated food or water, or by inhalation of small infective particles produced from dried faeces (Jordan and Pattison, 1995). In rural poultry the spread of the Newcastle disease virus is not as rapid as in commercial poultry, it can take weeks to pass through a typical village chicken flock. Faecal-oral transmission seems to be more common in free range scavenging poultry than the respiratory route. Newcastle disease can also infect humans and cause inflammation of the mucus membrane of the eyes (Botha *et al.*, 1997).

1.4.2 Pathogenesis of Newcastle disease virus

A striking feature of Newcastle disease virus strains and isolates is their ability to cause quite distinct signs and severity of the disease, even in the same host species. Based on the disease produced in chickens under laboratory conditions Newcastle disease viruses have been placed in five pathotypes (Jordan and Pattison, 1996):

- **Viscerotropic velogenic** Newcastle disease viruses cause a highly virulent form of disease in which haemorrhagic lesions are characteristically present in the intestinal tract.
- **Neurotropic velogenic** Newcastle disease viruses cause high mortality following respiratory and nervous signs.

- **Mesogenic** Newcastle disease viruses cause respiratory and sometimes nervous signs with low mortality.
- **Lentogenic respiratory** Newcastle disease viruses cause mild or inapparent respiratory infection.
- **Asymptomatic enteric** Newcastle disease viruses cause inapparent enteric infection.

However such groups should be regarded only as guide as there is always some degree of overlap and some viruses are not easily placed in a specific pathotype.

Over 200 different species of birds have been reported to be susceptible to natural or experimental infections of Newcastle disease virus and these include ducks, geese, turkeys, chickens and pigeons. The clinical signs vary considerably according to the virulence and tropism of the Newcastle disease virus involved, the species of bird, the age of host, the immune status of the host and environmental conditions. Chickens infected with the virulent Newcastle disease virus may die without showing any signs of illness. Common symptoms include lethargy and inappetance. Severe respiratory distress and gasping, greenish diarrhoea and marked decrease in egg production. Sometimes deformed eggs may be produced. Respiratory signs such as mild rales and sneezing can be detected by careful observation. Nervous signs of tremor, torticollis, convulsions and paralysis of wings and legs cannot be seen until the disease is advanced. An infected chicken fluffs its feathers. Other domestic birds such as turkeys and pigeons may also be affected. Normally ducks are resistant to the disease but on occasions, ducklings may be affected (Alders and Spradbrow, 2000).

1.4.3 Diagnosis of Newcastle disease

Newcastle disease can be diagnosed by various methods; it can be by observation of symptoms, serological assays, virus characterization and isolations and by postmortem examination of carcasses. Viruses causing respiratory disease may induce inflammation of the trachea, often with haemorrhages. The air sacs may also be inflamed and appear cloudy and congested. Viscerotropic viruses cause haemorrhagic lesions in the intestinal tract, particularly the proventriculus. Where neurotropic viruses have been dominant, the central nervous system has neuronal degeneration, perivascular degeneration, cuffing of lymphocyte cells and proliferation of the endothelial cells (Jordan and Pattison, 1996).

Isolation of viruses is from cloacal swabs (faeces) and tracheal swabs. From dead bird intestines are sampled together with organs and tissues associated with the clinical signs, for example, the brain if nervous signs were present. The viruses can be grown in various culture media. Modern methods of virus identification include polymerase chain reaction (PCR) and by direct sequencing of amplified cDNA (Stauber *et al.*, 1995). Other important identification tests for Newcastle disease virus include haemagglutination (HA) as well as electronmicroscopy.

The most commonly used method to indicate exposure of birds to Newcastle disease is by detection of antibodies from serum samples. Various tests may be used to detect antibodies to Newcastle disease virus in poultry sera and tests based on virus neutralization (VN) or enzyme linked immunosorbent assay (ELISA) reactions have been used in support of the diagnosis of Newcastle disease. Currently the haemagglutination inhibition (HI) test is

widely used. The value of any serological method in the diagnosis of disease is clearly dependent on the expected immune status of the birds involved (Della-Porta and Spencer, 1989).

1.4.4 Control of Newcastle disease

Regardless of whether control is applied at international, national or farm levels, objectives are either to prevent susceptible birds from becoming infected, or to reduce the number of susceptible birds by vaccination. Internationally, countries have policies that regulate the imports and exports of chickens with regard to the history of the diseases in countries concerned. Some countries do not vaccinate and would not want any form of Newcastle disease virus introduced to their domestic poultry. Others allow only specific live vaccines and consider some vaccines to be unacceptably virulent (Alexander, 1991).

At national level control policies are directed at prevention of the introduction of the virus and prevention of its spreading within the country. Most countries have restrictions on trade on poultry products such as eggs and live poultry (Alexander, 1991). Some countries have adopted eradication policies with compulsory slaughter of infected birds. National policies usually include restrictions of movement or marketing of birds within a quarantine area around the outbreak. Others require prophylactic vaccination of birds even in the absence of outbreak while some have a policy of ring vaccination around outbreaks to establish a buffer zone (Jordan and Pattison, 1996).

At farm level and village conditions the most effective way of disease control and prevention is by practice of good hygiene and vaccination. In

poorly managed, overcrowded, badly ventilated conditions with inevitable underlying bacterial infections, even the mildest live vaccine strains may produce disease sufficiently severe to mimic Newcastle disease virus of high pathogenicity. Good hygiene complemented by good management is therefore of critical importance at all times and not merely during an epizootic of disease. Hygiene measures should involve both maintaining the birds in a healthy environment and imposing some degree of biosecurity which entails the control of movement by people, vehicles and wild birds or other poultry and footpaths (Jordan and Pattison, 1996).

1.5 SIGNIFICANCE OF SERODIAGNOSIS FOR DISEASE INFECTION IN POULTRY

The history of laboratory testing closely follows developments in the field of immunology in general. Serological investigations were a natural outgrowth of the study of immunity, and the time period from 1900 to 1950 was called the era of international serology. Serology is the study of non-cellular components in the blood called serum. Because viral infections manifest a number of clinical symptoms that may be similar to those of other types of infections, laboratory testing offers the primary technique for differentiating viral infections from other conditions. Detection of viral antibody is usually the easiest and the fastest method of determining if clinical symptoms are due to the presence of a viral infection (Reeves and Todd, 1996).

1.5.1 Serological epidemiology

Serological epidemiology is the investigation of disease and infection in populations, by the measurement of variables present in serum. A range of constituents of serum can be measured, including minerals, trace elements, enzymes and hormones. One of the constituents of serum that is frequently measured, is the specific activity of globulins. Alternative terms for antibody measurements are titration and assay. Antibodies provide evidence of current and previous exposure to infectious agents. Their assay is commonly employed in veterinary medicine as a relatively efficient and cheap means of detecting this exposure. In practice, other body fluids, such as saliva, milk, vaginal and other mucous secretions may also be used for serological epidemiological investigations. Animals with detectable antibody titres are seropositive; animals with no detectable antibodies are seronegative and those previously seronegative and now seropositive are said to have seroconverted (Gummow, 2000).

1.5.2 Phases of antigen-antibody binding

The combination of antigen with specific antibody can be thought of as occurring in three distinct phases: primary, secondary and tertiary. The primary phenomenon involves the combination of an individual binding site on an antibody molecule with a single epitope or determinant site on an antigen. These reactions are reversible and occur in milliseconds and can be measured indirectly by techniques such as immunofluorescence, radioimmunoassay and enzyme immunoassay. Secondary phenomena, however, can be measured more readily and these include: precipitation,

agglutination, and complement fixation. Inflammation, phagocytosis, deposition of immune complexes, immune adherence, and chemotaxis are all *in vivo* reactions that are classified as tertiary phenomena (Weir and Stewart, 1997).

1.5.3 Haemagglutination Inhibition (HI) test in chickens

Avian viruses that agglutinate erythrocytes include Newcastle disease, avian influenza, infectious bronchitis and adenovirus (Egg Drop Syndrome). The inhibition of haemagglutination by specific antibodies is the basis of the haemagglutination inhibition test. It is a convenient and economical serologic tool that has been applied extensively to the control of several avian diseases such as Newcastle disease, by measuring vaccine response and evidence of past infection. The basic components of the haemagglutination inhibition test are the haemagglutinating antigen, the serum in serial dilutions and thus decreasing concentrations, and the erythrocyte suspension. Erythrocytes can be taken from a single chicken if experience has shown that the donor is satisfactory; otherwise, a pool from a minimum of three chickens is recommended. The tests may be done in tubes or microtest plates (Beard and Wilkes, 1985). For all paramyxovirus serotypes, birds that have not been immunized or infected usually have HI titres of less than 1:8 (2^3). In this research project haemagglutination inhibition tests for Newcastle disease have been used to measure the antibody status and response of experimental chickens in Qwa-Qwa before and after immunizations.

1.6 BASIC IMMUNOLOGY OF POULTRY

Animals live in a potentially hostile world filled with a bewildering array of infectious agents of diverse shape, size, composition and subversive character which would very happily use their hosts as rich sanctuaries for propagating their selfish genes, hence animals developed a series of defence mechanisms at least their equal in effectiveness and ingenuity. It is these defence mechanisms which can establish a state of immunity (Latin *immunitas*, freedom from) against infection and whose operation provides the basis for a subject called immunology (Roitt, 1997). It is essential that a chicken has a functional immune system to best protect it against infectious diseases and that the poultry owners have an understanding of the basic functioning of the immune system in order of fully understanding the concept of vaccination and the relevance of serology. The immune system consists of a number of components that together form an effective barrier against most pathogens. These components can be broadly divided into two categories, innate immunity and specific acquired or adaptive immunity (Breytenbach, 2000).

1.6.1 Innate immunity

Innate immunity involves immunity that is not intrinsically affected by prior contact with antigen, *i.e.*, all aspects of immunity not directly mediated by lymphocytes. In other words no matter how many times this system is exposed to a specific antigen, the response will remain constant provided the system is functional. The simplest way to avoid infection is to prevent microorganisms from gaining access to the body. The first line of defence is

by the skin, the secretion of mucous, ciliary action, the lavaging action of bactericidal fluids (e.g. tears), gastric acid and microbial antagonism (Figure 2). If penetration of microorganisms occurs, they are destroyed by soluble factors such as lysozyme and by phagocytosis with intracellular digestion (Roitt, 1997).

Microbial and other foreign particles trapped within the adhesive mucous are removed mechanically by ciliary movement, coughing and sneezing. The microbial antagonism is associated to the commensal bacteria found in the gastrointestinal tract and urogenital system which suppresses the growth of many pathogenic bacteria by competition for essential nutrients or by production of inhibitory substances such as lactic acid creating an unfavourable environment for bacterial growth. There is also a process called phagocytosis (Figure 3). Two types of cells are responsible for phagocytosis, namely: the polymorphonuclear neutrophils and the macrophages (Breytenbach, 2000).

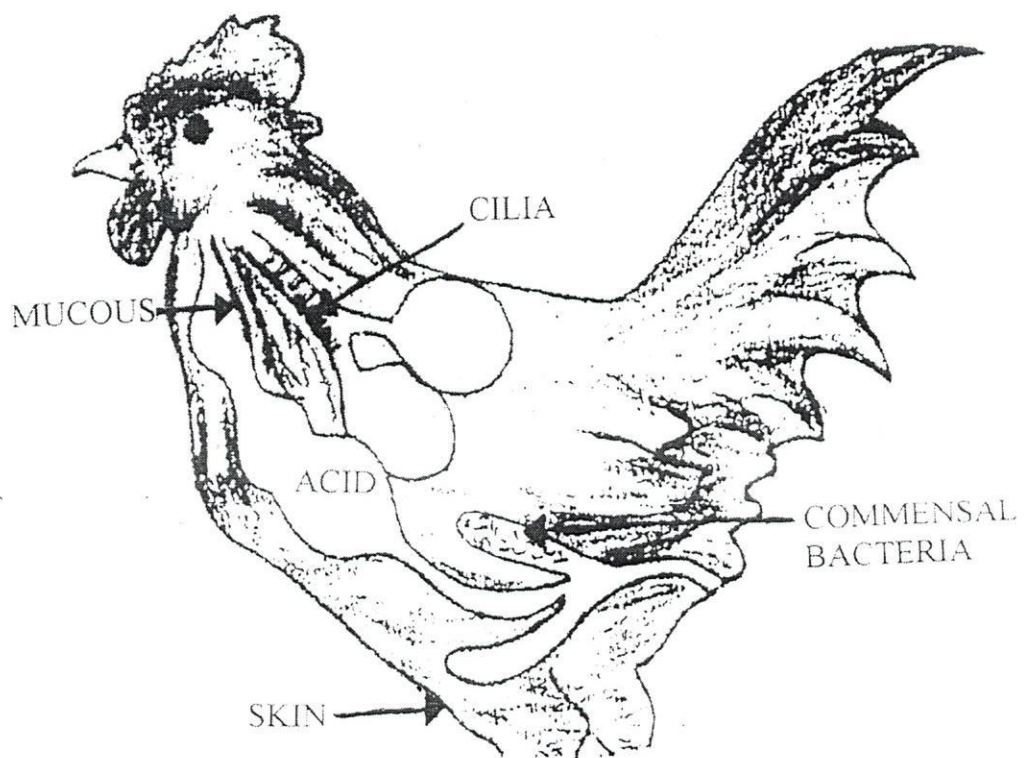


FIGURE 2: Innate immune system of a chicken (Breytenbach, 2000).

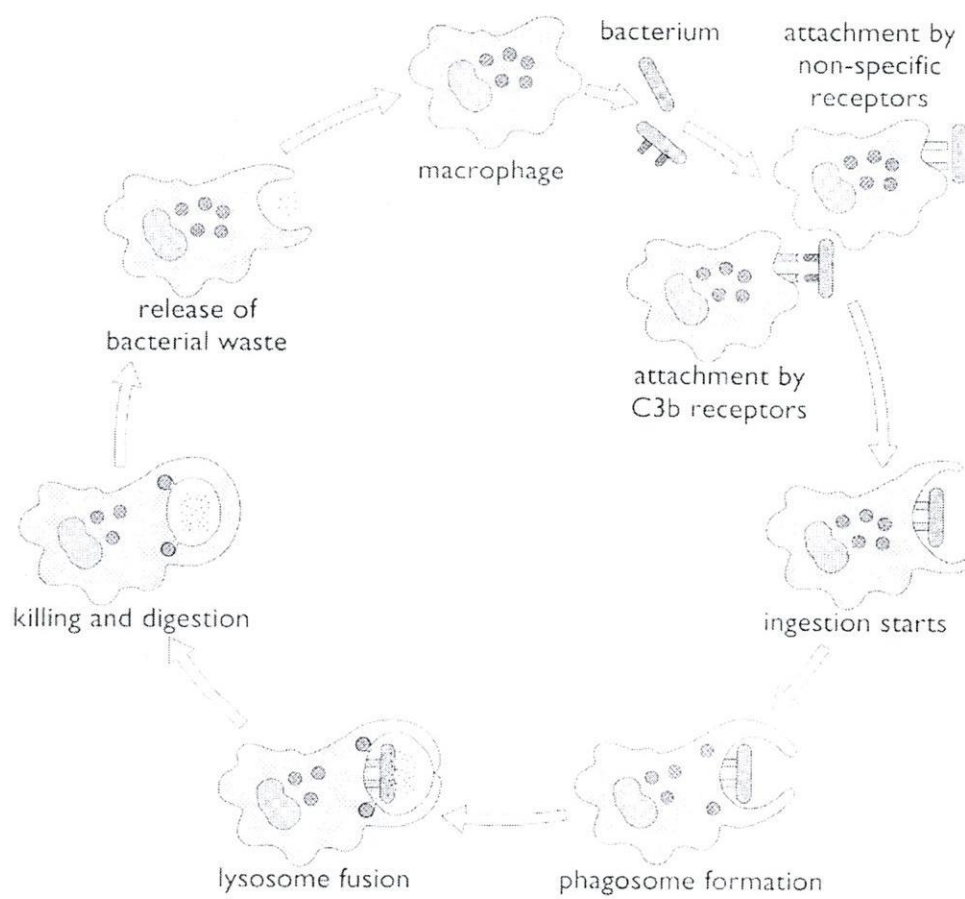


FIGURE 3: The process of phagocytosis (Staines *et al*, 1994).

In the second line of defence there is a complex series of some 20 enzymatic proteins, found in plasma and referred to as the complement system. When triggered the complement system results in a cascade of reactions where the product of one reaction is the enzymatic catalyst of the next. Certain micro-organisms such as viruses infect cells and actually replicate in the host cell. It is however clear that the body needs to destroy these viruses before they start to replicate. Natural killer cells specialize in inflicting damage on target cells that represent a threat to the body, *e.g.*, tissue cells which undergo malignant change or become infected. Natural killer cells differ in their mechanisms of target cell recognition but employ identical lytic mechanisms (Figure 4) (Reeves and Todd, 1996).

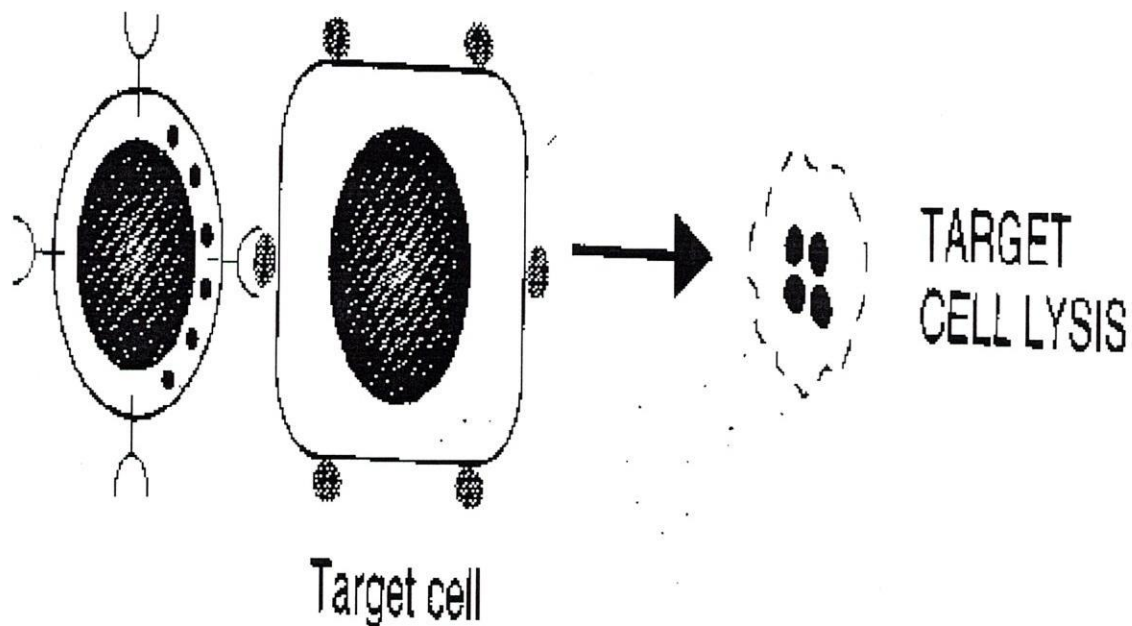


FIGURE 4: Lytic mechanism of natural killer cells (Abbas *et al*, 1997)

1.6.2 Specific acquired immunity

Antibodies have been brought about by the evolutionary processes aimed at bridging the gap between the innate system and a highly specific defence mechanism. Antibodies are collectively called immunoglobulins (Ig). Immunoglobulins consist of five different classes (isotopes) that differ in the structure of their H chains. These H chains differ in antigenic reactivity (serologically), in carbohydrate content, and size. Most importantly they confer different biological functions on each isotope (Mbat, 2000a).

Many biological properties are attributed to antibodies, these include neutralisation of toxins, immobilisation of micro-organisms; neutralisation of viral activity; agglutination (clumping together) of micro-organisms or of antigenic particles; binding with soluble antigen leading to formation of precipitates (which are readily phagocytized and destroyed by phagocytic cells); activating serum complement to facilitate the lysis of micro-organisms or their phagocytosis and destruction either by phagocytic cells or by killer lymphocytes. Still another important biological property of antibodies is their ability to cross the placenta from the mother to the fetus. Those antibodies that have the capacity to agglutinate particulate antigens or micro-organisms are called agglutinating antibodies or agglutinins; those that activate or fix complement in the presence of antigen are termed complement fixing antibodies (Mbat, 2000b).

This function of producing hundreds of different antibodies with specific recognition sites for specific micro-organisms is fulfilled by B-lymphocytes (Figure 5), so named as they differentiate in the Bursa of Fabricius. Each B-

lymphocyte is programmed to produce only one antibody that it places on its outer surface as a receptor. The molecules in the micro-organism which react with antibodies are called antigens, and when a specific antigen reacts with a matching surface antibody the B-lymphocyte is activated. The activated B-lymphocyte divides into antibody producing plasma cells, each manufacturing hundreds of antibodies identical to the original antibody. These antibodies attach themselves to the antigens of the pathogenic micro-organism, effectively presenting it to the macrophages and complement system. The plasma cells then develop into memory cells, so that when next there is exposure to the same specific antigen memory cells will be capable of rapidly responding, producing even higher levels of antibody (Breytenbach, 2000).

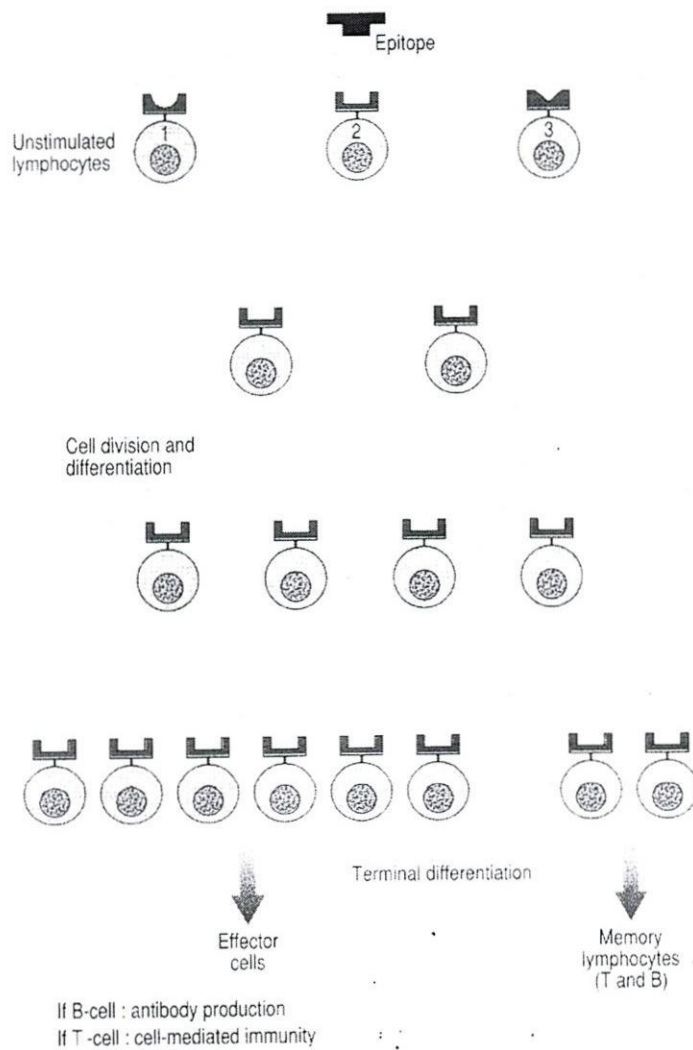


FIGURE 5: Production of antibodies (Weir and Stewart, 1997)

1.7 IMMUNISATION

During most of their lives, animals require to survive numerous and varied infectious onslaughts, particularly in southern Africa where the variety of infectious agents which cause disease in livestock is legion. To face these onslaughts animals require an efficient and versatile defence mechanism. Immunisation entails the protection of susceptible animals against infectious diseases by the administration of vaccines (van Dijk *et al.*, 1994).

1.7.1 Vaccines

A vaccine is the preparation of micro-organisms or their antigenic components which can induce protective immunity against the appropriate pathogenic bacterium, protozoan or virus but which does not itself cause disease. Vaccines may be composed of killed pathogenic micro-organisms (killed vaccines), or live non-pathogenic strains of virus, bacterium or protozoan (live vaccines), or may be composed of isolated protein antigens (subunit vaccines) (Mbati, 2000c).

There are basically two types of vaccination, namely, passive immunisation and active immunisation. Passive immunisation involves the transfer to one individual, antibodies formed from another. For example, the transfer of maternal antibodies to progeny through the egg. Passive immunisation with antibody gives immediate protection, it does not last very long because antibodies survive in the circulation for only a few weeks before they break down and are removed (van Dijk *et al.*, 1994).

By active immunisation an individual is intentionally immunised with killed or live micro-organisms to induce a state of specific immunity. The protection given by this sort of immunisation can in some cases be life long and is very easy to boost by a repeat immunisation (secondary immune response) (Figure 6). Because immunisation creates a lot of memory lymphocytes, these can make a rapid response to infection so that the infection does not cause disease. (van Dijk *et al.*, 1994).

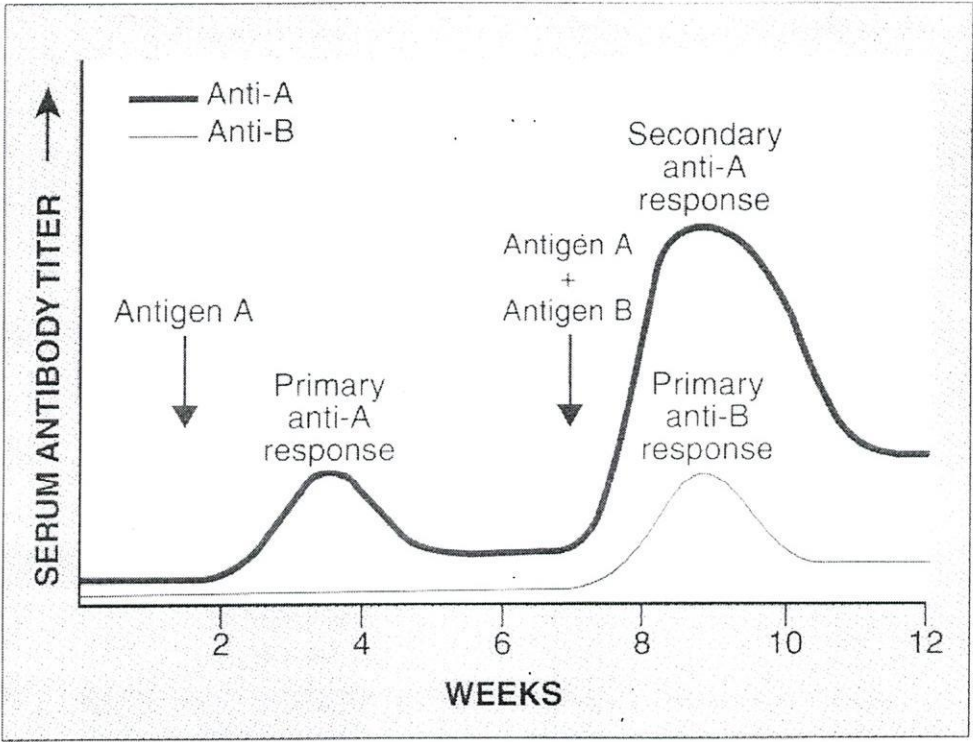


FIGURE 6: Secondary immune response (Abbas *et al.*, 1997).

1.7.2 Vaccines for Newcastle disease

Basically there are three types of commercially available vaccines for Newcastle disease: live lentogenic, live mesogenic and inactivated vaccines. Live lentogenic vaccines are usually derived from field viruses which have been shown to have low pathogenicity for poultry but produce an adequate immune response. Typical vaccine strains are Hitchner B1 and La Sota-possibly the two most widely used vaccines and F strain and V4 (Jordan and Pattison, 1996). Table 1 shows comparison of Newcastle disease vaccines.

Table 1: Comparison of Newcastle disease vaccines (Alders and Spradbrow, 2000)

	Live	Inactivated
1.	Contains a small amount of living virus which replicates; cheaper	Must contain a large amount of inactivated virus; more expensive
2.	Can be administered by many routes: eye drop, intranasal, spray, drinking water, oral, injection	Must be injected
3.	Stimulates all forms of immunity	Stimulates only antibody-based immunity
4.	Duration of immunity varies according to route of administration, usually not more than 4 months	Duration of immunity approximately 6 months
5.	Difficult to store (except thermostable live vaccines, e.g. I-2)	Less difficult to store
6.	Not dangerous to vaccinator	Dangerous to vaccinator through accidental injection

1.7.3 Live, thermostable Newcastle disease vaccines

A thermostable vaccine enables distributors and users to reduce the problems associated with inadequate cold chains in the field. The heat resistant V4 vaccine against Newcastle disease has yielded encouraging results in Cameroon (Bell *et al.*, 1995), Ghana (Amakye-Amin *et al.*, 1998) and many countries in Southeast Asia (Spradbrow, 1993-94). Ideris *et al.*, 1987) reported that the V4 vaccine is thermostable, retaining its activity for 12 weeks at a temperature of 28° C in freeze dried form.

The Australian Centre for International and Agricultural Research (ACIAR) commissioned workers at the Virus Laboratory in the University of Queensland to produce a seed virus that could be made available without cost to laboratories in developing countries (Bensink and Spradbrow, 1999). The result was a strain I-2, which was amplified in eggs from a disease free flock to form a master seed. The strain I-2 which underwent laboratory tests in several countries has proved to be protective against local virulent strains of the Newcastle disease virus. In Vietnam it has been officially recognized as the Newcastle disease vaccine for village chickens, after extensive laboratory and village trials (Tu *et al.*, 1998). Field records in Mozambique indicate that I-2 Newcastle disease vaccine provides approximately 80 % protection in the field in the face of an outbreak (Pagani, 1999).

Thermostable live Newcastle disease vaccines spread from vaccinated to unvaccinated birds when housed together (Alders *et al.*, 1994). The degree of spread under village conditions is less when birds roost in trees and

horizontal transmission should not be seen as a reliable substitute for vaccinating village chickens.

Nobilis[®] ND Inkukhu (Intervet S.A.) is a freeze dried vaccine that in the freeze dried form is stable for up to 7 days in temperatures not exceeding 30° C. At such temperatures the infectivity titre remains stable for seven days. Once reconstituted with a diluent it should be treated as any standard freeze dried Newcastle disease vaccine. Furthermore, once removed from refrigeration for an extended period, it must be used within the seven-day period and not returned to refrigeration for further storage. The expiry date printed on each vial is valid only when the vaccine is held constantly under refrigeration.

This vaccine is aimed primarily at the small-scale commercial farmer in outlying areas. It offers this type of farmer the ease of transporting the vaccine from the supplier (with refrigeration) to his/her farm (often without refrigeration) without the cold chain required for conventional freeze dried vaccines. The vaccine strain ND Clone LZ.58 was originally marketed by Mycopharm in the Delvax range. The vaccine Delvax ND Clone LZ.58 has proven its efficacy in numerous poultry producing countries as a primer and booster vaccine against Newcastle disease. It is a clone of the La Sota strain and will induce some post-vaccinal reactions in chickens. Post-vaccinal reactions occur during the first week following vaccination and include a mild snick and a very slight rise in mortality (less than 0.2%) (Alders and Spradbrow, 2001).

1.7.4 Testing Newcastle disease virus vaccine for efficacy and safety

The standard tests for Newcastle disease virus vaccines includes tests for extraneous micro-organisms that indicate the microbiological purity of the vaccine, tests for safety that show the vaccine is innocuous, potency tests that measure the antigen content of the vaccine and efficacy tests that demonstrate the immunisation potential of the vaccine. Efficacy tests should be conducted under conditions similar to those that will prevail when the vaccine is used in the field. Organisations that are contemplating pilot trials on village chickens may first wish to conduct their own efficacy tests, as an indication that the pilot trial should precede. The efficacy test should indicate that, at least under optimal conditions, the test vaccine would protect against local strains of Newcastle disease virus. A basic efficacy test compares the response of vaccinated and control chickens to challenge with a virulent strain of the micro-organism. The Newcastle disease efficacy test should use local pathogenic field isolates of Newcastle disease virus in that particular region or country (Spradbrow, 1999).

This study was aimed at investigating the most efficient route for vaccinating village chickens against Newcastle disease in Qwa-Qwa. The three different routes which were compared included: administration via drinking water, administration via feed and eye drop administration. Blood samples were also collected frequently to determine the effectiveness of the vaccine by checking the haemagglutinin unit through haemagglutination inhibition test and also to determine the intervals for revaccination. The trial challenges were conducted to determine the effectiveness of the vaccine when chickens were challenged with the virulent Newcastle disease virus. The questionnaire survey aimed at evaluating the perceptions of farmers

about vaccines and vaccination against Newcastle disease. The study is expected to serve as part of Newcastle disease control and prevention program in the Qwa-Qwa district of the north eastern Free state.

CHAPTER 2

OBJECTIVES

2.1 Statement of the problem

Chicken raising is the most widespread stock breeding activity both in general and within households in village communities. Newcastle disease introduces a high risk factor into chicken-raising, therefore various strategies need to be established to ensure production (Bagnol, 2000). Newcastle disease appears to be a major problem, although it can be controlled with standard vaccines. Vaccination is seldom practiced in the villages. Conventional vaccination regimes are difficult to apply to free ranging chickens that are kept in small numbers. The control of Newcastle disease in village chickens would allow greater production of chicken meat and eggs and would provide an incentive for controlling other diseases and for improving husbandry (Ideris *et al.*, 1990).

A recent pilot study on diseases of free ranging chickens that was conducted in the Qwa-Qwa district of the north eastern Free State province revealed that in 1998 entire flocks died due to Newcastle disease virus infections, and that currently only 5 % of chickens were exposed to Newcastle disease infection, which means that the rest are at risk of infection should outbreaks occur. It also emerged that free ranging chickens in Qwa-Qwa villages are never vaccinated against Newcastle disease or any other poultry disease (Thekisoe *et al.*, 2003).

This study is therefore formulated on the basis of developing a strategy for vaccinating village chickens against Newcastle disease in South Africa. Proper control of Newcastle disease in village chickens would benefit both the village communities and the commercial poultry sector, as it has been stated in the past that village chickens are apparently a source of Newcastle disease virus in commercial poultry (Spradbrow, 1999). A suitable route of vaccine administration that is both simple and effective will be determined and recommended by this study. This study will play a major role in assisting to control Newcastle disease in the rural Qwa-Qwa communities and hopefully this will dramatically improve production and farming capabilities in the village poultry sector of South Africa.

2.2 General objective

To develop a strategy for vaccinating free ranging village chickens against Newcastle disease using Nobilis® ND Inkukhu vaccine (Intervet South Africa).

2.3 Specific objectives

1. To compare the effectiveness of different routes (eye-drop, feed and drinking water) of vaccinating free ranging village chickens against Newcastle disease. The most efficient route will be the one that stimulates the highest immune response as measured by haemagglutination inhibition test.

2. To determine ideal intervals of revaccinating free ranging village chickens against Newcastle disease. Serological tests for Newcastle disease will be conducted at regular intervals after vaccination to monitor persistence of specific anti-Newcastle disease antibodies.
3. To conduct trial challenges on experimentally vaccinated free ranging Qwa-Qwa chickens in isolation using a South African isolate of velogenic Newcastle virus, in order to determine the level of protection offered by the Nobilis® ND Inkhuku vaccine through the three routes of treatment administered.
4. To address the issue of ease and convenience of vaccine application.
5. To conduct a questionnaire survey in order to evaluate the perceptions of village chicken owners on poultry vaccines, the most acceptable vaccination route and gender issues pertaining to village chicken husbandry.

CHAPTER 3

MATERIALS AND METHODS

3.1 AN OVERVIEW OF THE STUDY AND FIELD WORK

This research project was conducted in Thibella village (Figure 7) of the Tsheseng area in Qwa-Qwa district, for a period of 16 months from March 2001 to August 2002. A sample of approximately 700 chickens from 80 households was vaccinated 4 times at 8-week intervals, approximately 210 chickens were bled 8 times at 4-week intervals during the trial. There was a preparatory phase from March 2001 to August 2001 during which a research proposal was prepared, followed by a series of meetings with stakeholders. The study was started with a meeting with Dr. Sello Maboe the State Veterinarian, with whom aims and objectives of the experiment were discussed. Secondly there was a meeting with the chief extension officer, Mr. Tsholo Keele, at Tsheseng Agricultural offices. He then recommended the extension officer, Mrs Matshidiso Mota, to assist in the entire research project. There was also a meeting in May 2001 with the tribal council officer Mr. Makhilithi Mgomezulu who then organised a meeting with Thibella community that was held in June 2001 at the tribal council hall. In this meeting the attendants were informed of the vaccination study and objectives of the research were also explained. All the poultry owners who attended the meeting registered their names and addresses.

Between July 2001 and August 2001, there was a house-to-house search and location of households that had chickens (Figure 8). Between September 2001 and October 2001, chickens were bled from the identified households,

and seroassays for Newcastle disease were conducted. All the tested samples were negative for Newcastle disease and these results confirmed that vaccinations could be conducted. Vaccination trials were started in December 2001, and the chickens were revaccinated 3 times during the study whilst blood samples were collected monthly until August 2002. A trial challenges with a virulent Newcastle disease virus was conducted in July 2002 on 40 experimental chickens in the Poultry Reference Laboratory isolators at the University of Pretoria. Post-mortems were thereafter conducted on all experimental chickens for detection of Newcastle disease signs.

3.2 DESCRIPTION OF THE STUDY AREA

Qwa-Qwa lies in the Eastern Free State (28° 50' E, 28° 35' S) province of South Africa. It is at 1600m above sea level, and the mean annual rainfall of the north eastern Free State is given as 800mm, and the mean maximum monthly temperatures range between 21°C and 30°C. The mean minimum temperatures range between 6°C and 19°C (Vrey and Smith, 1980).

3.2.1 Geology

The eastern Free State Province with its beautiful landscape consists of the rocks of the Karoo super-group, of the Beaufort and Stormberg groups, with the soil types belonging to the Clovelly-Avalon, Katspruit, Kroonstad-Katspruit and Sterkspruit. Numerous dolerite dykes and occasional sills have intruded the Karoo formations and a few small dolerite diatremas have

also been recorded in Qwa-Qwa, which contain sedimentary breccias (Kritzinger and Pieterse, 1987).

3.2.2 Rainfall

The eastern Free State lies within the summer rainfall region of South Africa with more than 85 % of the precipitation occurring during the period of September to March, mainly in the form of thunderstorms. The annual precipitation on the lower lying plateau, occurring in the Harrismith and Kestell localities is approximately 700 mm per annum, increasing gradually to approximately 1 340 mm per annum in Qwa-Qwa (Drakensberg mountains) as one progresses in a southerly direction. Hailstorms are common, occurring three to five times per annum. Snowfall occurs in the high-lying south with the heaviest fall occurring towards the end of June (Kritzinger and Pieterse, 1987).

3.2.3 Atmospheric temperature

The temperature in the eastern Free State Province may be described as being cool to moderately warm. The average temperature during mid-winter is 7°C and during mid-summer is 17.9°C. The average daily minimum temperature during winter is 4.6°C and the average daily maximum temperature during summer is 29.9°C. Cold spell temperature decreases in excess of 5°C can occur many times a year, lasting two to three days per year. Hot spells with temperatures in excess of 35°C also occurs for four to five days at a time. During September and October temperatures are

regarded to be unstable, but during April-May are quite stable (Kritzinger and Pieterse, 1987).

3.2.4 Vegetation

The eastern Free State Province of South Africa belongs to the grassland biome, with five vegetation types namely: moist cool highveld, moist cold highveld, wet cold highveld, afro-mountain and alti-mountain grassvelds (Moffet, 1997). The vegetation can also be described as highland sourveld, which changes into Themeda Festuca veld at heights in excess of approximately 2 200 m. In the almost pure sourveld, red grass (*Heteropogon contortus*) predominates and these are easily reduced, through over-grazing, to tough grass (*Eragrotis plana*) and wiregrass (*Elionums argenteus*) (Kritzinger and Pieterse, 1987).

Trees and grasses are fairly rare while *Leucosidea sevicea*, *Trimeria grandifolia* and *Hereomorpha arborescens* are most common. When overgrazing occurs, the first mentioned type tends to become hardy. A few examples of upright yellow wood (*Podocarpus latifolius*) occur in sheltered ravines. In the Themeda-Festuca alpine veld, mostly red and wiregrass and *Festuca costata* is to be found. The last two grass types are unpalatable grasses. The shrubs are limited to mainly *Leucosidea sericea* and *Erica-Helichrysum* (Kritzinger and Pieterse, 1987).

Thibella village is situated in the southern part of Qwa-Qwa (Figure 8) in an area called Tsheseng. It is approximately 20 km from the Qwa-Qwa campus of the University of the North.



FIGURE 8: Map showing households vaccinated at Thibella village
(Schlumberger Ltd., 2000).

3.3 EXPERIMENTAL PROCEDURES

All the experimental procedures were conducted in the Parasitology Research Program laboratory of the School of Life Sciences of the University of the North Qwa-Qwa Campus; Thibella village in Qwa-Qwa as shown in Figure 8 and the Poultry Reference Laboratory at the Faculty of Veterinary Science of the University of Pretoria.

3.3.1 Blood sample collection

Blood samples were collected from 3 chickens in each household from a total of 60 households during the first bleed between September and October 2001 before vaccinations. Thereafter blood samples were collected every month from January until September 2002 from the same chickens as they were marked with cable tiers on their legs (Table 2). Blood samples were collected from the wing veins of chicken with a sterile 18-gauge needle (Kenxin®) into 7-ml silicon coated BD-vacutainers® (Becton, Dickinson and Co.) (Plate 1).

Table 2: Dates on which chickens were bled before and after vaccinations

1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th
Sept-Oct 2001	14 Jan 2002	11 Feb 2002	11 March 2002	15 April 2002	13 May 2002	10 June 2002	23 Sept 2002

3.3.2 Serum preparation and storage

In the laboratory blood samples were placed under sunlight for 1 hour, thereafter samples were placed into a medifriger centrifuge (P-Selecta®, Spain) and were spun at 2000 rpm for 1 hour at 20°C, then serum was harvested. For those samples which did not form enough serum, they were stored overnight at 25°C in a dry incubator (Labcon® R.S.A.) for serum to separate from plasma (Plate 2). The following day the samples were centrifuged and serum harvested. Serum was harvested into 3.8-ml cryogenic vials (Cellstar® and Nalgene®) using 500µl micropipettes (Elkay®) (Plate 3). It was then stored at -35°C and later transported to the serology laboratory of the Poultry Reference Laboratory, University of Pretoria, where haemagglutination inhibition tests for Newcastle disease were conducted (Plate 4). The haemagglutination inhibition titre responses were measured at 4-week monthly intervals for a period of seven months after the first and the second vaccinations in December 2001.

3.3.3 Haemagglutination inhibition test for Newcastle disease

Test procedure (Standard Operational Procedure-Poultry Reference Laboratory, 2000)

Using a 96 well round bottom plate (Sterilab-Sterilin®), each well of all the plates to be tested was filled with 50µl Veronol buffer (using either the Titertek® multi drop for larger numbers of plates or 8 channel micropipette-Tranferpette®(Germany) for small number of plates). To the 1st row (well

A1 to H1) 50µl of each serum which had to be tested was added and diluted till the end of the 12th row. There was 50µl of diluted serum left in the tips after the last dilution on the 12th row. The same procedure was applied with all the microtiter plates. To each well 50µl of antigen (diluted) using either the titertek multidrop or an 8 channel pipette- Tranferpette®(Germany) was added. It was left to stand for 30 minutes at room temperature. Thereafter to each well 50µl of red blood cells (RBC) added. It was left to stand for 60 minutes at room temperature. The results were thereafter read.

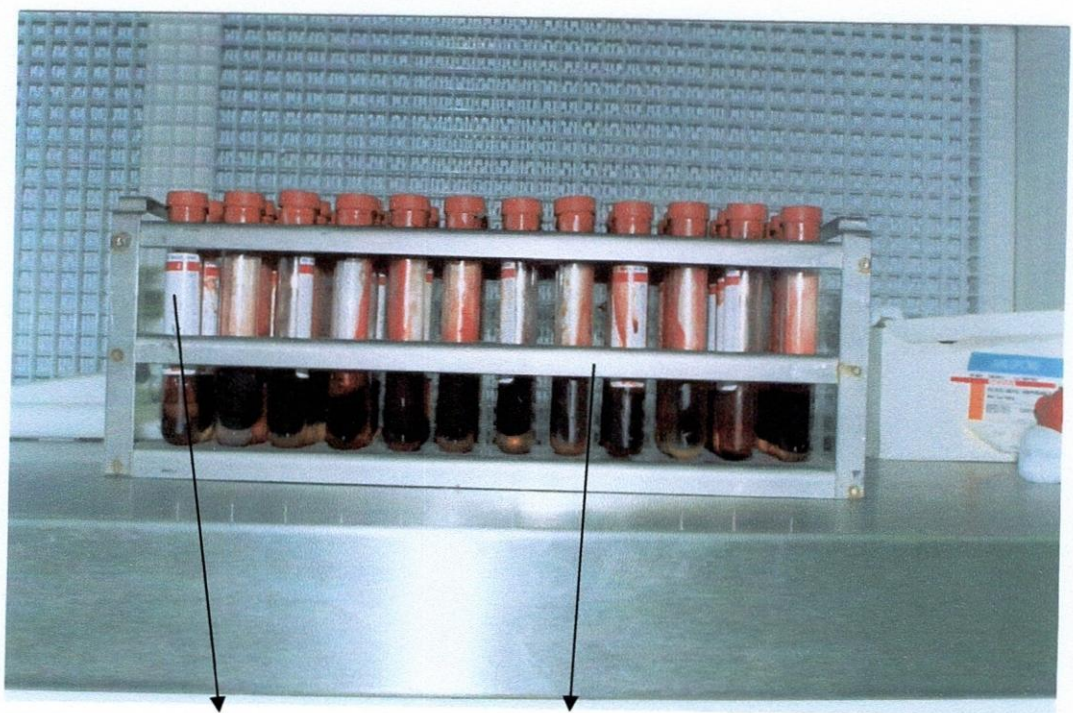
Control plate

Fifty µl Veronol buffer was added to each well in a microtiter plate using the titertek multi drop. 50µl of the positive control serum was added to wells A1 and B1. Fifty µl of the negative control serum was added to wells C1 and D1. Fifty µl of the diluted antigen was added to wells E1 and F1. Two fold serial dilutions of the contents of wells A1 to F1 were made by removing 50µl of the content of the first well and adding it to the next well. This was repeated until reaching end of the plate, then the last 50µl was discarded. 50µl of the diluted antigen was added to each well from A1 to D12 (positive and negative control rows). This was left to stand for 30 minutes. Then 50µl of the 0.3% red blood cells suspension was added to all of the wells on the plate. This was left to stand for 60 minutes and then results were read.

Interpretation of results

The antigen that was added to the wells has haemagglutinin on its surface that can bind to the red blood cells and form haemagglutination. If the serum tested has antibodies for that antigen, they will bind and there will not be any

haemagglutination (that is haemagglutination inhibition). Due to the inhibition red blood cells will sediment out and will form a button at the bottom of the well. But if there are no antibodies in the tested serum the antigen and the red blood cells will bind and form a haemagglutination (that is no pellet formation of red blood cells) (Poultry Reference Laboratory, 2000).



(a)

(b)

PLATE 1: Blood samples in vacutainers after bleeding

(a) Vacutainer (b) Test tube rack



PLATE 2: Blood samples placed in dry incubator overnight.



PLATE 3: Serum stored in cryogenic vials

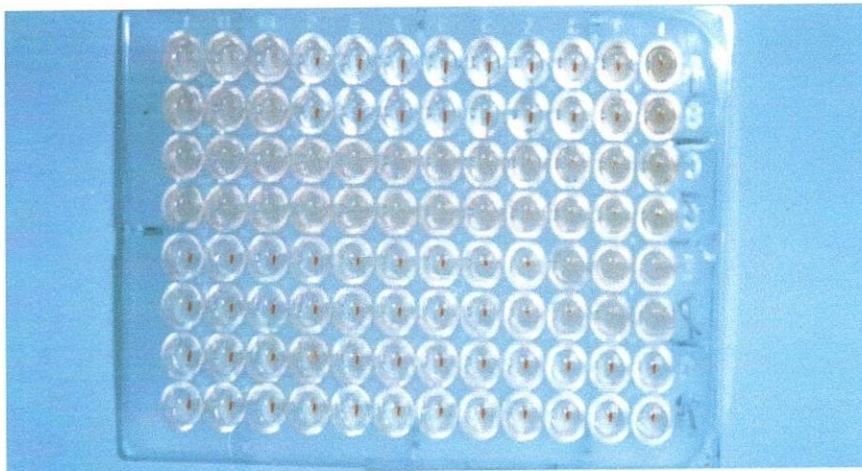


PLATE 4: Plate showing haemagglutination inhibition test for Newcastle disease.

3.3.4 DETERMINATION OF REVACINATION INTERVALS

The revaccination intervals were determined using the HI titre responses that were measured by conducting haemagglutination inhibition tests for Newcastle disease. The HI tests were conducted from blood samples collected from the vaccinated flocks and the control every month after the first vaccination was conducted.

3.3.5 IMMUNISATION

The type of vaccine used in this study is called **Nobilis® ND Inkukhu** vaccine (Intervet South Africa). The Nobilis® ND Inkukhu is a live freeze-dried vaccine containing Newcastle disease virus. The vaccine is produced in eggs derived from specific pathogen free (SPF) flocks tested free from *Salmonella*, mycoplasmas and avian viruses. Each dose of Nobilis® ND Inkukhu contains at least 10^6 EID₅₀ clone LZ.58. The freeze-dried pellet contains stabilizers. The vaccine was prepared in accordance to Intervet procedures from the brochure supplied together with the vaccine vials, which includes reconstitution in the morning 1 hour before administration. Three different vaccination routes, eye-drop, drinking water and feed were used to administer the vaccine. Plates 5, 6 and 7 shows materials used in each vaccination route. Immunisations were conducted on four occasions on dates reflected by Table 3.

Table 3: Dates on which immunisations were conducted

1 st	2 nd	3 rd	4 th
4 Dec 2001	19 Dec 2001	15 March 2002	23 July

3.3.5.1 Eye-drop vaccination route

Correct dilution of the vaccine is critical. The eyedroppers were already calibrated (30 ml per 500 doses). The ideal volume for the small eye of a chicken was a drop that is approximately 15 µl. All chickens in each poultry house were vaccinated using the eye-drop route. The owners were notified a day before vaccinations not to release their chickens until they were vaccinated the following morning. Chickens in the control group were given eye-drops of pure water.

Vaccine mixture was prepared as follows:

A 500 dose vaccine vial was mixed with 1000 drops from the diluent bottle supplied by Intervet South Africa. Two drops of vaccine were applied per bird into an eye and a nostril. Vaccine was used for a period of 1 hour after preparation and was stored in a cool box for the entire vaccination period.

3.3.5.2 Drinking water vaccination route

The vaccine was prepared at the village and then distributed to each household identified as part of the study. Owners were notified a day before to remove drinking water for chickens overnight in order to ensure that they were thirsty in the morning when the vaccine was supplied. Chickens in the control group were given pure water.

Vaccine mixture was prepared as follows:

Five litres of distilled water was mixed with 10g of Elite® (Clover, S.A.) skim milk powder (low fat) in the morning at the laboratory. The solution was allowed to stand for 30 minutes whilst travelling from the University to Thibella village. The 500-dose vaccine was mixed and given to the fowls with each household receiving 50 to 70 ml (depending on the flock size) that was approximately 3 doses per chicken.

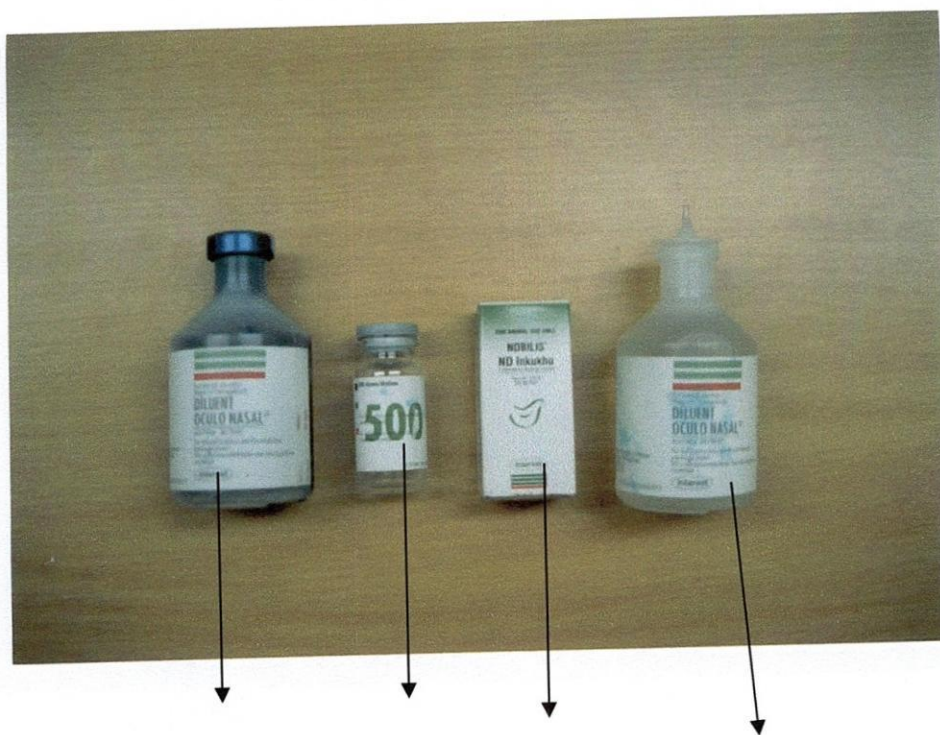
3.3.5.3 Feed vaccination route

One kilogram of Iwisa maize meal (Premier Milling Co.) was cooked at the Parasitology laboratory of the University of the North Qwa-Qwa campus and was left overnight to cool down. In the morning it was taken to the village to be mixed with the vaccine. The vaccine was prepared at the village and then distributed to each household identified as part of the study. The porridge vaccine mixture was administered to the birds in their houses and to those roaming around within 1 hour after preparation. Again the owners were notified a day before that they should not give their chickens feed in the morning until they were given feed vaccine in order to ensure that chickens would eat the vaccine-porridge mixture. Chickens in the control group were given pure hard porridge.

Vaccine mixture was prepared as follows:

One kilogram of maize meal was cooked in 2 litres of water for 15 minutes. The cooked maize meal porridge was allowed to cool overnight and then taken to the village in the morning. A 500 dose vaccine was mixed in 500ml distilled water. This vaccine mixture was then mixed with 2.5kg cooked

porridge. A 10g vaccine – porridge mixture (depending on the flock size) was given to each household identified as part of the study and was calculated as 2 doses per bird.



(a)

(b)

(c)

(d)

PLATE 5: Materials used in eye-drop vaccination route

(a) Diluent (b) Vaccine vial (c) Vaccine vial container
(d) eye-dropper



(a) (b) (c)
PLATE 6: Materials used in drinking water vaccination

Route

(a) Skim milk powder (b) Water container (c) Vaccine vial



(a) (b) (c)
PLATE 7: Materials used in feed vaccination route

(a) Maize meal (b) Vaccine vial (c) Porridge (pap)

3.3.6 DETERMINATION OF VACCINATION EFFICACY

(Standard Operational Procedure - Poultry Reference Laboratory, 2002)

A challenge trial was conducted to determine the efficacy of the vaccination. All chickens that were used as experimental animals in this research project were certified free of Newcastle disease virus infection by haemagglutination inhibition test at the beginning of the study. A total of 40 experimental chickens were used in trial challenges to test the efficacy of Nobilis ND Inkhuku vaccine. Each vaccination route (eye-drop, drinking water and feed) was represented by 10 experimental free ranging chickens collected from the village. The control group was also represented by a sample size of 10 chickens. There were 5 free ranging chickens from the control group in Thibella village and a further 5 were the specific pathogen free (SPF) chickens obtained from the Poultry Reference Laboratory of the University of Pretoria. A total of 8 isolators were used containing 5 chickens each. The trial challenge was conducted as follows (Poultry Reference Laboratory, 2002):

- (i) Day 1: Experimental chickens were placed in the isolators (Plates 8, 9 and 10) and were left overnight to acclimatise to the new environment.
- (ii) Day 2: All chickens were tagged on legs with rings of different colours so that they could be identified individually. All the chickens were weighed using Kubota Ltd, Richter Scale, model KA-10 (Plate 11). They were then bled and blood samples were taken for haemagglutination inhibition test for Newcastle disease.

- (iii) The chickens were then challenged with a local velogenic Newcastle disease strain that had been isolated from the tracheae of infected chickens.
- (iv) The virus challenge dose was 0.2 ml (approximately 1×10^6 viruses) allowing for a total dose of 10^4 EID₅₀ (EID = egg infection dose) and the chickens were challenged intramuscularly.
- (v) The chickens were then placed in the isolators set at a temperature of 25°C, and pressure at 50 Pascal.
- (vi) From day three until the last day of the experiment, chickens were observed twice a day (in the morning and afternoons) and the observations were recorded.
- (vii) Chickens that were found dead were taken for post-mortems while those showing nervous signs or found in lateral recumbence for more than 12 hours were euthanased.
- (viii) The entire experiment lasted for 10 days.
- (ix) The SPF chickens and the control chickens were expected to start dying within 4 days and the vaccinated chickens with weak immune protection were expected to die after 6 days.

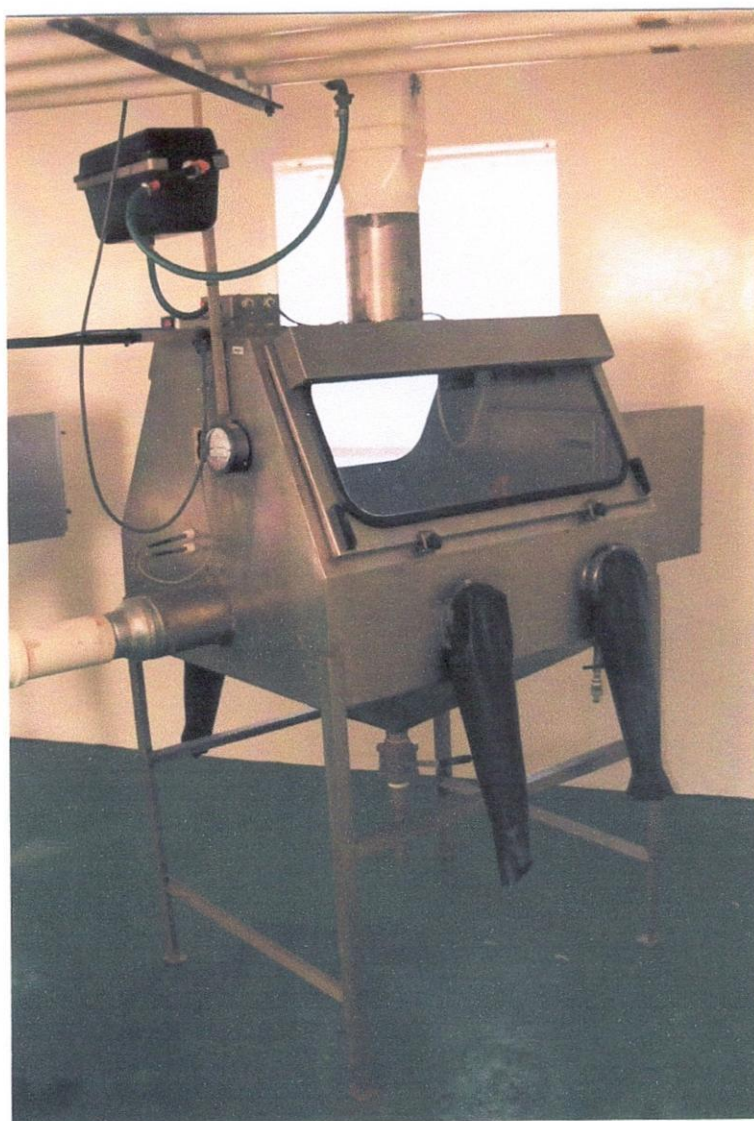


PLATE 8: An isolator used during a trial challenge experiment at the Poultry Reference Laboratory, University of Pretoria.



PLATE 9: Free ranging chickens in the isolator during trial challenge experiment.



PLATE 10: Specific pathogen free chickens in an isolator during trial challenge experiment.

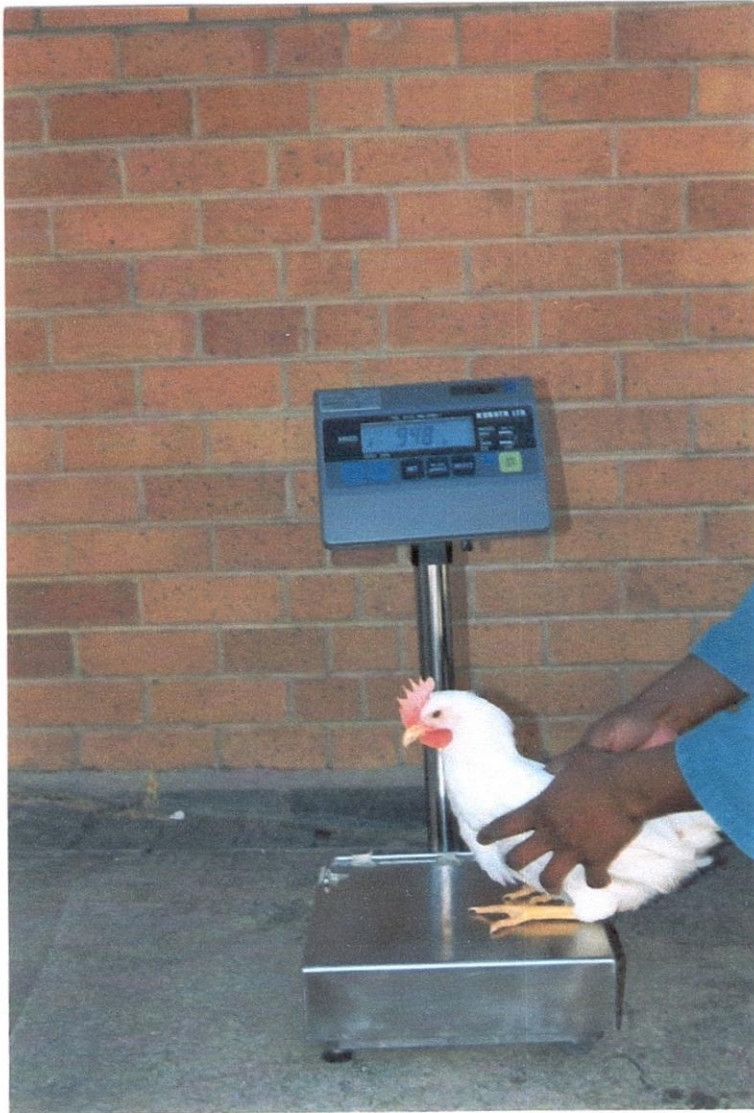


PLATE 11: Weighing chickens on a Richter scale during a trial challenge experiment.

3.3.7 POST-MORTEM

Post-mortems were conducted on all the experimental chickens at the Poultry Reference Laboratory post-mortem room. The chickens were dissected on ventral area so that the internal organs are exposed (Figure 10). The visceral organs of the respiratory system and the digestive system were examined for lesions that are known as symptomatic for Newcastle disease virus infection such as proventricular, ceecal tonsil haemorrhages, sinusitis and tracheitis.

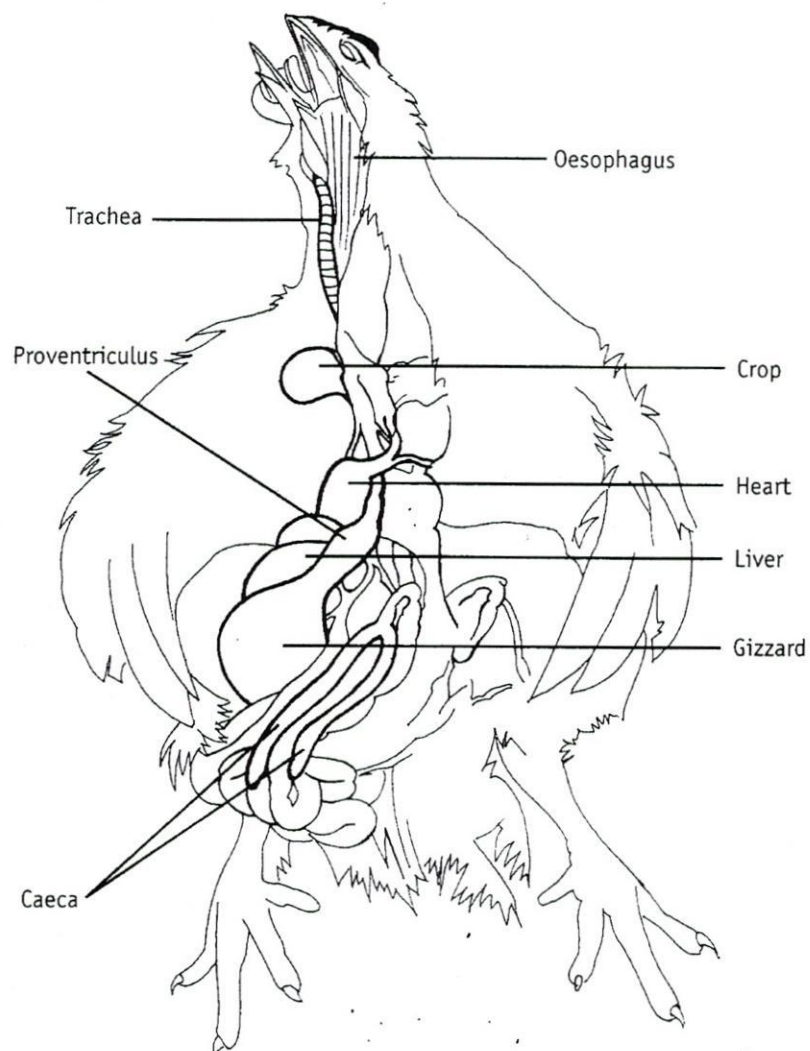


Figure 9: Diagram showing how internal organs of a chicken were exposed during postmortems (Alders and Spradbrow, 2001).

3.3.8 Questionnaire survey

A total of 55 questionnaires were administered to owners of free ranging chickens used in the vaccination experiment in Thibella village. The owners were asked questions verbally and at the same time appropriate answers were recorded by the interviewer in the questionnaire forms. The main aim of the survey was to evaluate the most acceptable vaccination technique by the owners. The types of questions included in the survey were also seeking information on the demographics of poultry owners, their perceptions on vaccines, interest to buy the vaccines, their experiences with extension officers and poultry husbandry practiced. A sample of a questionnaire used is illustrated in Appendix 1.

CHAPTER 4

RESULTS

4.1 ANTIBODY RESPONSE FROM VACCINATED CHICKENS AS MEASURED BY HAEMAGGLUTINATION INHIBITION TEST

The HI titres were analysed using Mixed Procedure under Statistical Analysis System (SAS). This is a statistical procedure recommended for unbalanced data or data characterised by missing values and it provides efficient estimates (SAS/STAT Software, 1997). The eye-drop vaccination route had the highest HI titres, followed by drinking water vaccination route. The feed vaccination route had the lowest titres compared to the other two routes (Table 4).

Table 4: Monthly haemagglutination inhibition titre averages per vaccination route

Year 2002	Eye-drop	Drinking water	Feed	Controls
January	4.38	3.46	2.36	0.4
February	4.22	2.58	1.63	0.4
March	3.38	2.9	2.8	0.2
April	2.7	2.3	2.7	0.1
May	4.1	4	5	0.8
June	3.9	3.6	2.8	0.8
September	4.4	3.2	3	0.05
MEAN	4.0	3.1	2.8	0.4

There was no significant difference ($p = 0.0829$) of mean HI titres per route of vaccination between the months. There was no evidence of interaction between the vaccination routes and the months ($p = 0.8108$) *i.e.* there were no occasions where the HI titre responses of certain routes were found to be similar in any particular month (Table 5).

Table 5: p-values of treatments v/s months

Source of variation	p-value
Treatments	< 0.0001
Months	0.0829
Method v/s. months	0.8108
Error mean square (MSE)	1.34143

4.1.1 COMPARISON OF TREATMENTS

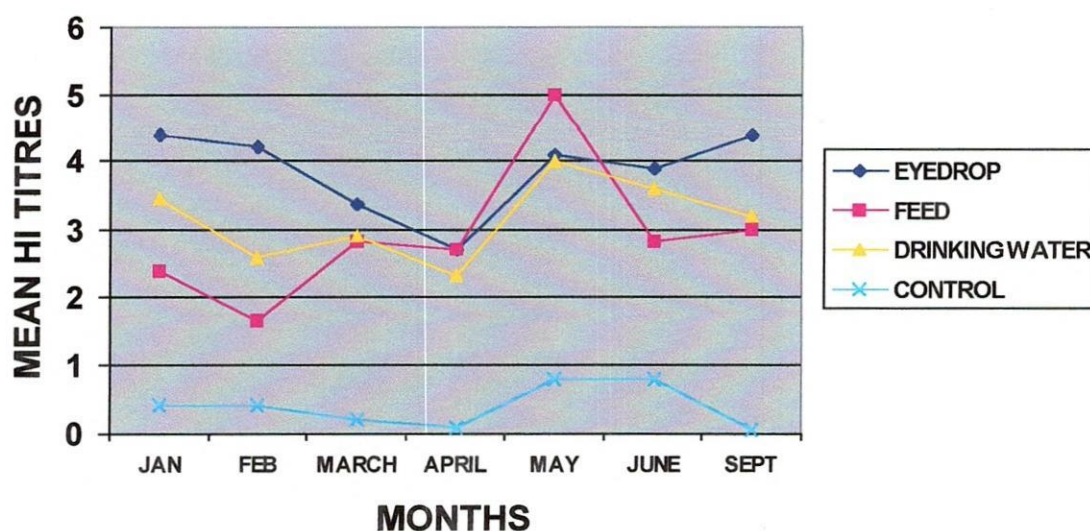
The 3 routes of vaccination (eye-drop, drinking water and feed) differed significantly ($p < 0.0001$) in HI titre response when compared to the negative control, with vaccination routes having better HI titres than the control. Eye-drop and drinking water vaccination routes did not differ significantly ($p = 0.2135$) when their HI titres were compared. However there were significant differences ($p = 0.0020$) of HI titres between eye-drop and feed routes and also between feed and drinking water (Table 6).

Table 6: Comparison of HI titre response in eye-drop, feed, drinking water and control treatments (p-values)

Contrasts	p-values
Eye-drop v/s Feed	0.0020
Eye-drop v/s Drinking water	0.2135
Feed v/s Drinking water	0.0449
Control v/s Eye-drop	< 0.0001
Control v/s Feed	< 0.0001
Control v/s Drinking water	< 0.0001

Chickens from the negative control group had uniform mean HI titre responses which were all below 1 HI titre for every month bled. However the 3 vaccination routes (eye-drop, feed and drinking water) had variable HI titre responses for each month bled as shown by Figure 10.

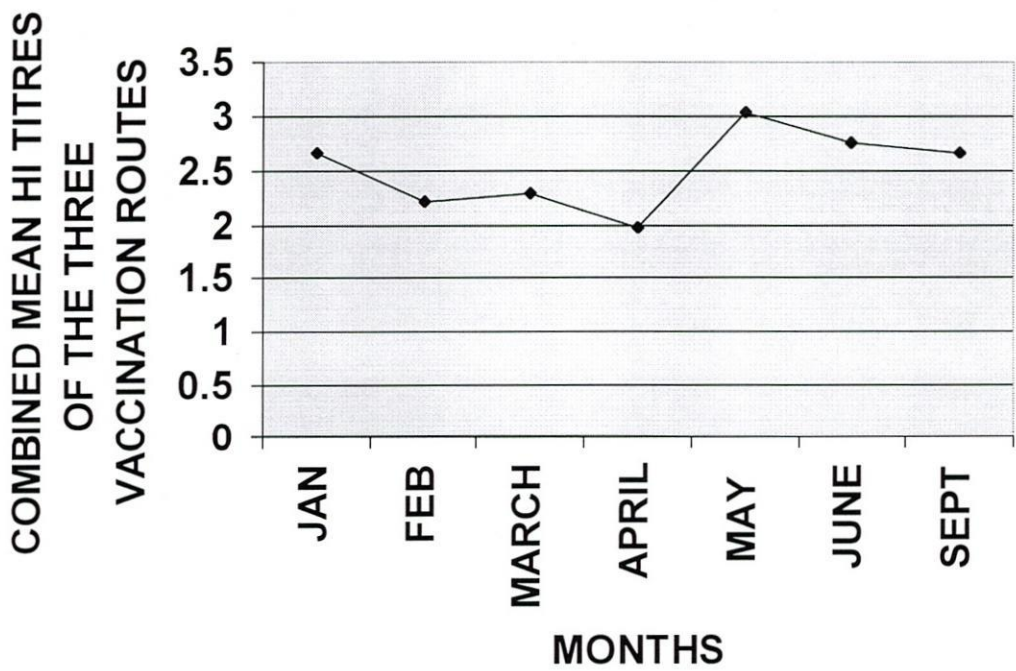
FIGURE 11: Monthly mean HI titres per route (eye-drop, drinking water and feed) and negative control



4.2 DETERMINATION OF REVACCINATION INTERVALS

The mean monthly HI titres of the 3 vaccination routes (eye-drop and drinking water) decreased in February, March and April 2002) after the first vaccination which was conducted in December 2001. In the feed group there was however a slight increase of HI titres in March and April. Chickens were revaccinated in April. In May after revaccination in April, there was an increase in HI titre response from the 3 vaccination routes, but once again in the following months there was a decline of HI titre responses (Figure 11).

FIGURE 11: Combined mean HI titre responses of the 3 vaccination routes (eye-drop, drinking water, feed) over a period of seven months of vaccination



4.3 DETERMINATION OF VACCINATION EFFICACY

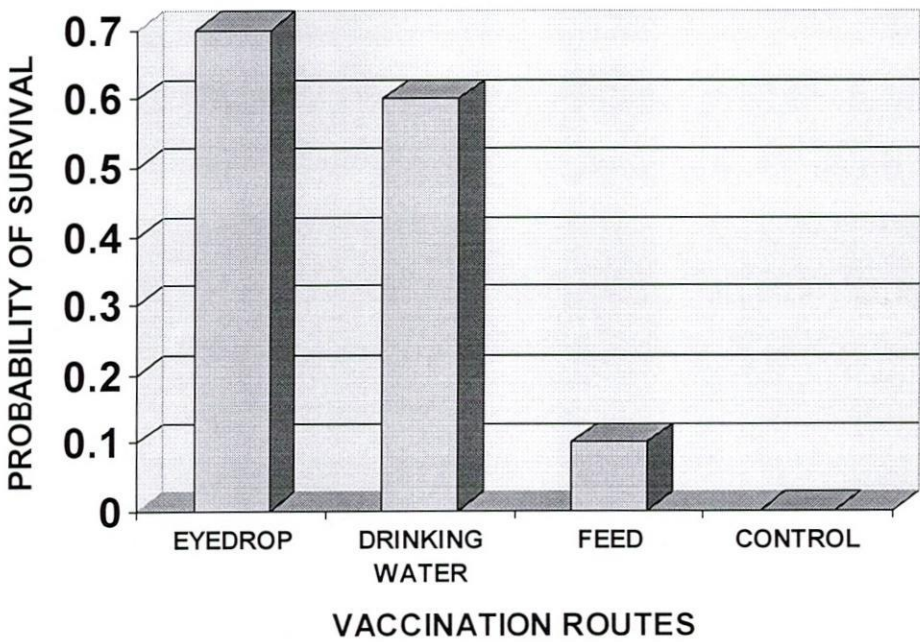
Each chicken was weighed and bled for haemagglutination inhibition response before being challenged. The mass of chickens was measured to distinguish between young and old chickens since their ages were not known (Table 7). The trial challenge data was analysed using PROG Logistic Statistical Analysis procedure which analysis the probabilities (SAS/STAT Software, 1997).

Table 7: Weight and HI titres of experimental chickens in isolators

Isolator 1 Eye	Weight (g)	HI Titres	Isolator 5 SPF-Control	Weight (g)	HI Titres
1	1286	4	21	921	0
2	1026	4	22	774	0
3	1136	0	23	663	0
4	1162	7	24	689	1
Isolator 2 Feed			25	789	1
5	1141		Isolator 6 Feed		
6	1365	2	26	1269	0
7	1071	1	27	1380	0
8	682	0	28	1456	1
9	981	0	29	889	1
Isolator 3 Water			30	1090	1
10	502	1	Isolator 7 Water		
11	530	0	31	1608	6
12	417	0	32	1461	2
13	451	1	33	1255	0
14	513	2	34	429	0
Isolator 4 Control			35	1505	0
15	1403	2	Isolator 8 Eye		
16	1701	1	36	995	4
17	1758	1	37	1566	6
18	1590	1	38	1229	7
19	1748	1	39	1177	4
20	1712	1	40	1721	4

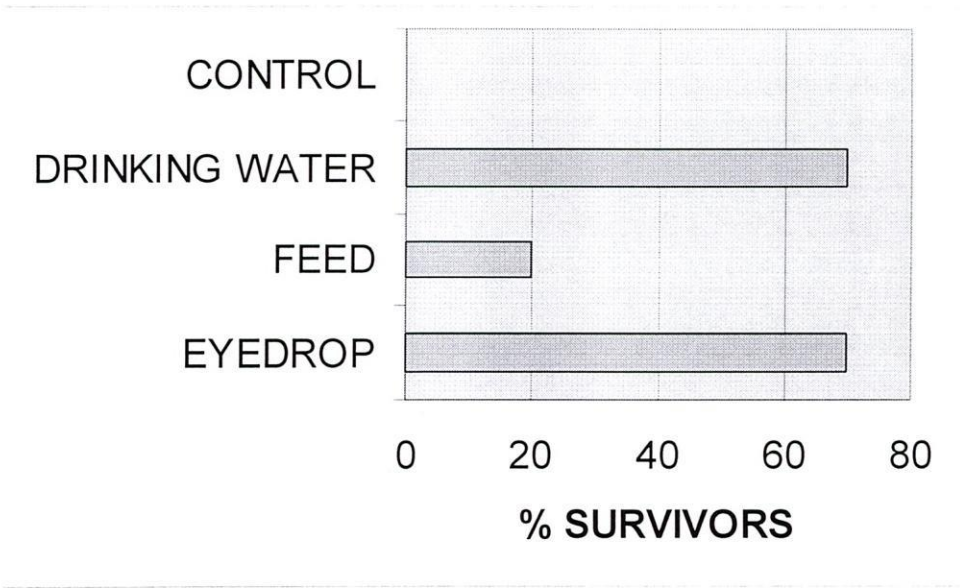
There was a substantial influence of haemagglutination inhibition titres on survival of chickens challenged with Newcastle disease virus, *i.e.*, chickens with higher HI titres had more chances of survival against challenge than those with lower titres. The eye-drop and the drinking water group had more chickens with high HI titres (3 HI or higher) hence they had higher number of survivors. The feed and control groups had low HI titres (0 and 1 HI's) hence the low numbers of survivors in the feed group and no survivors in the control (Figure 12).

FIGURE 12: **Comparison of probabilities of survival from HI titre responses of eye-drop, drinking water, feed and control vaccination routes in trial challenge experiment.**



In the trial challenge experiment 70 % of the chickens immunized through the eye-drop or drinking water route survived, whilst only 20 % of those immunized through the feed vaccination route survived (Figure 13).

FIGURE 13: Percentage survival of chickens immunized through different routes when challenged with Newcastle disease virus



4.4 POSTMORTEMS

Chickens that died during the trial challenge were stored in a freezer and the survivors were euthanized, thereafter post-mortems were conducted on all experimental chickens. Out of 40 experimental chickens 88 % had different Newcastle disease lesions and 12 % had no lesions (Table 8). The unvaccinated control chickens had severe lesions, whilst the vaccinated chickens from 3 treatments (eye-drop, feed and drinking water) had mild lesions.

Table 8: Lesions on visceral organs after Newcastle disease trial challenge experiment

Eyedrop		Water		Feed		Controls	
#	Lesion type	#	Lesion type	#	Lesion type	#	Lesion type
1	CTH	1	GOOD	1	S, T, PVH	1	PVH, S, T, P
2	CTH	2	GOOD	2	CTH, KC, S, T, N, HM	2	CTH, PVH, S, T, AS
3	CTH, KC	3	GOOD	3	PVH, KC, N, HM, LO	3	PVH, S, T, AS, P, OO
4	CTH, S	4	GOOD	4	CTH, S, T, P, HM	4	PVH, S, T, AS, P, OO
5	CTH, KC, S	5	GOOD	5	CTH, PVH, S, T, GN	5	CTH, PVH, S, T, OO, P
6	CTH, OO	6	OO	6	CTH, KC, S, LO, N	6	CTH, S, T
7	S, HM, SM, OO	7	CTH, S	7	CTH, KC, S, LO, N	7	CTH, T, SM
8	CTH, PVH, KC	8	CTH, KC, S, T, H, N	8	N	8	PVH, KC
9	CTH, OO	9	S, N, PVH	9	CTH, KC, S, N	9	S, T
10	OO	10	CTH, S, KC, T	10	CTH, SM	10	PVH, S, N

KEY: (Lesion abbreviations)

S – Sinusitis; **T** – Tracheitis; **P** – Pneumonia; **PVH**- Proventricular haemorrhage; **CTH** - Cecal tonsil haemorrhage; **GH** - Gastro intestinal haemorrhage; **OO** – Oophoritis; **SM** – Splenomegaly; **HM**– Hepatomegaly; **KC** - Kidney congestion; **LO**- Lung oedema; **N** – Nephrosis; **AS**-Airsaculitis.

4.5 QUESTIONNAIRE SURVEY

Questionnaires were administered to 80 households that participated in the vaccination research project in Thibella village, and only 55 (70 %) free ranging chicken owners provided responses.

4.5.1 Demography

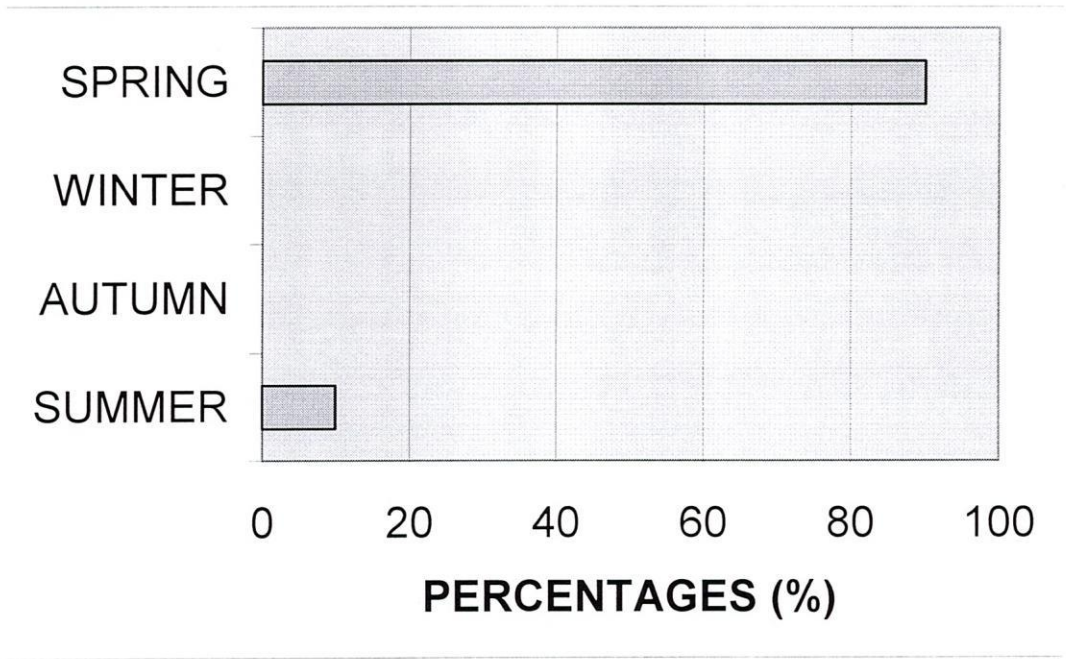
A total of 50 (90 %) out of 55 free ranging chicken owners interviewed were not employed (unemployed or pensioners).

4.5.2 Newcastle disease vaccination

A total of 96 % of the interviewees mentioned that the indigenous name in Sesotho for Newcastle disease is **Mokoioy**, and they considered Newcastle disease as a priority disease in Qwa-Qwa, whereas 4 % were not sure of the name. All chicken owners (100 %) interviewed had not observed Newcastle disease symptoms on other birds such as turkeys, geese and ducks. Only 2 % had knowledge of the existence of Newcastle disease vaccines. A total of 95 % of the interviewees were positive that vaccines are effective in disease prevention whereas 5 % were not sure. Two percent of the owners would

prefer to vaccinate their chickens by themselves, whereas 98 % would like to see vaccinations been done by the extension officers. The interviewees mentioned that they have observed Newcastle disease outbreaks in spring (90 %) and summer (10 %) months (Figure 14).

Figure 14: Seasonal outbreaks of Newcastle disease in Qwa-Qwa according to owners observations



Majority of the free ranging chicken owners in Thibella village chose drinking water as the preferred route for vaccination (Table 9).

TABLE 9: Preferred route of vaccination by free ranging chicken owners in Thibella village

Vaccination route	Percentage (%)
Eye-drop	2
Feed	8
Drinking water	90

The free ranging chicken owners in Thibella were willing to pay for the vaccine at different costs as reflected by Table 10.

TABLE 10: Amount that can be afforded by owners for purchasing Newcastle disease vaccine

Amount	Percentage (%)
R2 per flock	40
R5 per flock	50
R10 or more per flock	10

4.5.3 Interaction with extension officers

All the free ranging chicken owners in Thibella mentioned that they had had no interaction with extension officers on matters concerning poultry diseases. They also believed that extension officers were not well qualified in handling poultry diseases. Interviewees also stated that there had never been agricultural campaigns or educational workshops organised for poultry rearing or poultry diseases in their region.

4.5.4 Birds in a household

All the free ranging chicken owners interviewed in Thibella reported that their chickens range freely during the day and made contact with wild birds such as hawks and swallows. Chickens were the preferred domesticated birds owned by villagers in Thibella when compared to turkeys, ducks, geese and pigeons (Table 11).

TABLE 11: Percentages of birds owned by households

Type of birds	Percentage (%)
Chickens	90
Turkeys	2
Ducks	2
Geese	4
Pigeons	2

4.5.5 Gender aspects of village chicken husbandry

In 93 % of the households interviewed the woman is the one who decides when to eat chickens or eggs, whereas in 3 % of the household it's the husband who makes such a decision. In 4 % of the households both make the decision. It is the primary responsibility of the women and children to take care of the chickens in Thibella village (Table 12).

TABLE 12: Family members who take care of the birds

Family member	Percentage (%)
Women	50
Husbands	3
Children	20
All	27

Most of the chicken houses at Thibella households are constructed by husbands, 25 % by women and only in 10 % of the cases are they constructed by all members of the households.

4.5.6 General comments

During the interviews 5 % of the owners mentioned that they would like to be trained in conducting the vaccinations against poultry diseases and would be happy to be educated on poultry diseases generally. Some owners (10 %) interviewed stated that it would be convenient if vaccines and other treatments for poultry diseases were sold at shopping centres that are closer to their village. The questionnaire has also revealed that majority (75 %) of the free ranging chicken owners mentioned that they loose most of their chickens from theft and predation of chicks by hawks.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

In most developing countries of Africa, Asia Central and South America, keeping of village poultry is a constant backdrop to village life. The flocks contain mostly scavenging chickens that subsist mainly on household scraps. Almost without costs the flocks provide eggs and live birds for sale or barter and eggs and poultry meat for human consumption. Improvements in village poultry production could improve food security and increase rural incomes, thereby greatly benefiting poor rural communities. In many countries aid agencies and non-governmental organizations are approaching poultry rearing as an activity that will specifically benefit the women of the village communities (SADC Planning Workshop, 2000).

It is estimated that annual outbreaks of Newcastle disease kill 70 – 80 % of unvaccinated village chickens (Spradbrow, 1993/94). Outbreaks of Newcastle disease are unpredictable and discourage villagers from paying proper attention to the husbandry and welfare of their chickens. Newcastle disease virus infections substantially reduce the benefits from village chicken production, and are a constraint to even modest intensification of the production system (Ronohardjo, 1984; Anon, 1986).

The only effective and viable means of control for Newcastle disease in village chickens is by vaccination. However the main problem of vaccination against Newcastle disease remains to be post vaccination reactions, which cause performance losses and strongly increase the risk of respiratory infection (Comte, 1999). Effective vaccine application is a

crucial part of modern poultry production. Common reasons for vaccine failure includes: vaccine incorrectly stored, cold chain broken, chickens vaccinated at the wrong time, disinfectant in water, water temperature too high, too much or too little water, vaccine expired, vaccine faulty or containing wrong strains, lighting or ventilation not reduced and syringes poorly calibrated (Hewson, 2000).

It has not been an easy exercise conducting this research project. There were various constraints, which partly interfered with the smooth progress of the study. It should be noted that free ranging chicken owners are sensitive when their chickens are bled, so few chickens had to be used during bleeding sessions. Although most owners were cooperative some would not honour appointments as agreed upon, especially when asked not to release chickens before the start of bleeding or vaccination procedures. This study is a fieldwork study conducted mainly in the village, and poor weather was also a disturbing factor, especially during rainy days. Reported cases of chicken theft in the village also reduced the number of sampled chickens in some households. When conducting studies of this sort it is important to discuss and explain to all stakeholders (State veterinarian, extension officers, traditional leaders and the community at large) in villages the purposes of the study.

This study aimed at developing a strategy for controlling Newcastle disease in free ranging village chickens in Qwa-Qwa by vaccination with Nobilis ND Inkukhu vaccine. Three different routes of vaccination were used to apply the vaccine namely: eye-drop, drinking water and feed vaccination routes. A trial challenge experiment was also conducted to test the efficacy

of the vaccine. The results as discussed in this chapter will hopefully be part of a solution for combating Newcastle disease in free ranging chickens in rural areas of developing countries.

5.1 EYE-DROP VACCINATION ROUTE

In this study the eye-drop vaccination route had mean HI titres ranging between **2.7** and **4.4** and were the highest as compared to the other vaccination routes used. In a similar study conducted in Mozambique by Dias *et al*, (2000) where they compared different routes of vaccination, the highest titres were achieved in groups where the vaccine was administered via the eye-drop route. At the beginning of this study, experimental chickens were certified to be seronegative with all having 0 HI titres, which meant that they were neither infected nor vaccinated against Newcastle disease. However after immunizations, chickens vaccinated via this route developed serum antibodies for Newcastle disease virus as measured by haemagglutination inhibition test for Newcastle disease. The stimulation of a high humoral immune response in chickens immunized by the eye-drop route was mainly responsible for the protection observed when 70 % of the birds survived following experimental challenge with Newcastle disease virus.

The main advantage of the eye-drop vaccination route in the current study was that precise and accurate doses were given to each and every immunized chicken. In similar experiments from the Australian Centre for International Agricultural Research (ACIAR), conducted by Young (1991) on alternatives to oral administration of Newcastle disease vaccines to village chickens. The

findings of those studies revealed that the eye-drop method results in high levels of protection. Young (1991) also reported that the advantage of using eye-drop route is that ten times less vaccine per dose is required; the dose per chicken is known; the identity of the vaccinated birds is also known so that each owner can make sure that all his birds are vaccinated. Further, it is important to mention that in the current study the apparatus for eye-drop application as provided from the manufacturers were user friendly (a vaccine vial is mixed with the solvent/ diluent which then makes 30ml per 500 dose bottle) and could be easily applied by the chicken owners and extension officers. Table 13 summarizes the advantages and disadvantages of eye-drop vaccination route.

5.2 DRINKING WATER VACCINATION ROUTE

The drinking water administration route produced mean HI titres ranging between **2.3** and **4.0** HI titres. This route also effectively stimulated the immune response of vaccinated chickens resulting in high antibody production. In the trial challenge experiment 70 % of the chickens previously immunized using the drinking water route were protected from the Newcastle disease virus. In a similar study on thermostable Newcastle disease vaccines in Tanzania conducted by Foster *et al*, (1999), the eyedrop and drinking water method offered 70 % protection when challenged with their local velogenic Newcastle disease virus.

Similar results to this study have been reported in a research project on experimental trials with a thermostable Newcastle disease virus (strain I₂) in commercial and village chickens in Tanzania. In that study Wambura *et al*,

(2000) reported that when they compared the 3 routes of vaccination, drinking water route was second to eye-drop route in offering protection against challenge. The drinking water vaccination route is easy to apply as the vaccine mixture is simply poured in the chicken drinking containers.

It is however very important to have the cooperation of the chicken owners as water has to be removed from the chicken houses the previous evening before the chickens are vaccinated, so as to make sure that they are thirsty. Again the chickens have to be within their houses or be in a closed confinement where drinking water is given, and this also requires the cooperation of the chicken owners. Table 13 summarizes the advantages and disadvantages of drinking water vaccination route.

5.3 FEED VACCINATION ROUTE

The present study experimentally introduced this feed route for comparison with other vaccination routes and does not form part of the recommended routes from the manufacturers of the Nobilis® ND Inkukhu vaccine (Intervet South Africa).

Chickens vaccinated through feed route produced mean HI titres ranging between **1.6** and **3.0**. These titres were significantly lower when compared to the other two vaccination routes used in this study. In a similar study to evaluate an oral Newcastle disease vaccine in Thailand, Tantaswasdi *et al*, (1992) reported that a V4 Strain vaccine was effective when given intranasally or in drinking water but the vaccine was not effective when supplied with uncooked rice.

It is important to mention that not all chickens feed on exact doses as estimated when the vaccine is applied. Some chickens eat more, probably older chickens consume faster and more than younger ones and also the dominant and intimidating chickens in the flocks fed more than the others. Ideris *et al*, (1990) in their study to vaccinate chickens against Newcastle disease with a food pellet vaccine concluded that their chickens developed HI antibodies when immunised with V4-UPM food pellet vaccine and that they were protected (65-75 %) from parenteral and contact challenge with virulent Newcastle disease vaccine. However the present study had 20 % survivors and this might be due to differences in types of vaccines used.

In the feed route, 37 % of the serum samples collected after vaccinations tested by haemagglutination inhibition test for Newcastle disease were positive. This meant that there was presence of antibodies for Newcastle disease. However these positive HI titre responses of chickens in feed route were lower when compared to other two routes used in this study and this corresponded with low percentage (20 %) of chickens (in feed route) which survived during an experimental challenge with virulent Newcastle disease virus. This low percentage of positive HI titres in this route might be due to the fact that vaccine did not trigger enough stimulation of the humoral immune response which resulted in low production of antibodies. Another reason for poor production of antibodies by this route could be that the vaccine mixture might not be attaching very well to the porridge. In a study conducted by Spradbrow and Samuel (1991) on oral Newcastle disease vaccination with V4 virus in chickens whereby they compared the oral route with drinking water, intraocular and intramuscular routes. They reported that, feed vaccination had to be applied twice before it raised substantial

antibody response whereas in other routes there was antibody response from single application. Therefore to produce adequate antibody response to feed vaccination route, multiple vaccinations are recommended and this might not be economic for vaccination in village environments. Table 13 summarizes the advantages and disadvantages of feed vaccination route.

TABLE 13: Summary of advantages and disadvantages of eye-drop, drinking water and feed vaccination routes using Nobilis® ND Inkukhu vaccine in free ranging village chickens

Vaccination route	Advantages	Disadvantages
Eye-drop	• Accurate and precise doses per chicken known. • Individual chicken vaccinated can be easily identified	• Requires a lot of manpower for catching each chicken. • Few chickens (approx. n=140) can be vaccinated within 1 hour
Drinking water	• Larger number (approx. n=250) of chickens can be vaccinated within 1 hour. • No manpower needed	• Exact doses of vaccine per chicken not known. • Not easy to identify vaccinated chickens in large flocks

Feed	• Larger number (approx. n=210) of chickens can be vaccinated within 1 hour. • No manpower needed. • Can be given to chickens even when not inside houses.	• Exact doses of vaccine per chicken not known. • Not easy to identify vaccinated chickens in large flocks.
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5.4 CONTROLS

A group of experimental chickens selected as the control group were given doses of pure water as eye-drops or pure drinking water or pure hard porridge (pap) all of which were not mixed with the vaccine. The control also had 0 HI titres before vaccination and during the entire experiment had negative titres (there was no presence of antibodies for Newcastle disease). The HI titres in this group ranged between **0.05** and **0.8**, titres that were far too low to be protective against Newcastle disease virus infection. This was also evident during the trial challenges whereby the experimental control group had 100 % mortalities within the first 4 days of challenge trial. In a similar study conducted by Wambura *et al*, (2000) similar results were obtained whereby 100 % mortalities were observed in the control group within 3 – 5 days after inoculation with the virus.

The postmortems revealed that chickens from the control group (unvaccinated chickens) had severe lesions observed from internal organs such as sinuses; trachea; proventriculus, ceecal tonsils, kidneys, intestines, spleen and lungs. Vaccinated chickens from different routes (eye-drop, drinking water and feed) had mild lesions. A similar vaccination experiment conducted in Malaysia by Ideris *et al*, (1990) showed that all chickens in their control group died irrespective of the route of challenge. Their postmortem examinations of chickens died revealed haemorrhages in the proventriculus, intestines and ceecal tonsils. The trachea and lungs were very congested.

The control group has been instrumental in proving that there is antibody response stimulated by the Nobilis® ND Inkukhu vaccine when administered to free ranging village chickens which had never been vaccinated against Newcastle disease virus before. The control group has also been of importance as proof that the trial challenges were conducted successfully and in accordance with application procedures. It is also important to mention that a few individual chickens (3 %) in the control group had detectable but insignificant Newcastle disease antibodies. This could have been due to handling the control mixtures with equipment that had been used in the vaccine mixtures by mistake. In one case the owner of one flock mentioned that he had been giving his chickens a certain treatment for Newcastle disease although he was not sure whether it was a vaccine or not. Another possibility is that the vaccinated chickens might have infected the control chickens through aerosols whilst scavenging around as some flock/households are close to each other.

5.5 DETERMINATION OF REVACCINATION INTERVALS

The findings of this study have shown a decreasing trend of HI titres on the second, third and fourth months after vaccinations until the chickens were revaccinated. Revaccination induces a higher production of antibodies and this results in higher immune response than in the initial vaccination (Abbas *et al.*, 1997). This observation is an indication that the chickens should be revaccinated after every 3 months in the first year of vaccination campaign in order to boost the initial vaccination. For example, if chickens were vaccinated in January they would then need to be revaccinated in May the same year and so on. The same program of vaccination had also been suggested by Alders *et al.*, (2002) in the training manual for controlling Newcastle disease in village chickens. In free ranging village chickens there are always new clutches of chickens hatched all through there year, this therefore means that chickens will have to be vaccinated every year. However, one can suggest that on the second year vaccinations be conducted every six months (2 times in a year) considering financial strains that might be problematic for annual vaccinations in poor rural environments.

5.6 DETERMINATION OF VACCINATION EFFICACY

To determine the efficacy of the vaccine, experimental chickens were challenged with a South African velogenic Newcastle disease virus and it was observed that 70 % of the birds immunized through either drinking water or eye-drop route survived. This shows the extent to which these vaccination routes can raise antibody response that can offer protection for the chickens against challenge. Dias *et al.*, (2000) also observed that after

natural outbreaks of Newcastle disease in villages of Mozambique where vaccination trials with NDV4-HR vaccine were conducted, the eye-drop vaccination route was the one that offered adequate protection to chickens. They stated that approximately 80 % of the chickens vaccinated via eye-drop route survived natural challenge with virulent Newcastle disease virus. When the vaccine was administered twice via drinking water, a survival rate of 65 % was achieved.

Only 20 % of the chickens from the birds immunized via the feed vaccination route survived the trial challenge. This result corresponds with low HI titres that were produced by this group during the entire vaccination study. Oral vaccination of chickens with thermostable vaccines (*e.g.* NDV4 HR and I2) has been successful in some developing countries. One problem with food based Newcastle disease vaccination is low recovery of the virus from some grains (especially maize), a consequence of either binding or inactivation (Ideris *et al.*, 1990). However it is important to highlight that all the experimental chickens were challenged with 1 million (1×10^6) viruses which is a load far more than what free ranging chickens will get during infection or outbreaks in the field or natural environment.

The trial challenge experiment in this study has proved that Nobilis® ND Inkukhu vaccine is efficient in offering protection to free ranging chickens. The study has demonstrated that the eyedrop and drinking water vaccination routes are more efficient than the feed vaccination route because the experimental chickens in eye-drop and drinking water routes produced higher HI titres with correspondingly significant protection against trial challenge with Newcastle disease virus. This is supported by Figure 12 in

Chapter 4, which demonstrates the probabilities of survival of chickens immunized from HI titres produced by 3 different vaccination routes and the control.

5.7 QUESTIONNAIRE SURVEY

A questionnaire survey was conducted mainly to evaluate the perceptions and knowledge of free ranging chicken owners on Newcastle disease vaccines, gender issues, and interaction with extension officers. This survey has served to demonstrate similarities and differences in free ranging chicken rearing in the Qwa-Qwa area and other developing countries with similar village environments.

Ninety percent of the free ranging chicken owners from Thibella village in Qwa-Qwa were unemployed. Chickens in the traditional poultry systems provide scarce animal protein in the form of meat and eggs, and are available for sale or barter in societies where cash is not abundant (Gueye, 2000). Therefore village chickens can play a vital role of improving diet and poverty alleviation in Thibella community if production is improved. Although the majority of the owners are unemployed they showed interest in paying for the vaccine.

The majority (98 %) of the free ranging chicken owners interviewed in Thibella preferred the vaccination to be conducted by extension officers. It will therefore be important to train and educate extension officers on vaccinations for poultry. There is also a need to train members of the

community who will be the community vaccinators assisting the extension officers.

Many interviewees (90 %) mentioned that they have experienced Newcastle disease outbreaks in spring months of August to October. This might be due to climatic stresses of winter, which weakens the immune system of the chickens. One can suggest that one of the winter months be included in the vaccination program with the intension that the immune system is well primed by spring, with the expectation that at least the beginning of spring the humoral immune system of the chickens will still be producing antibodies for Newcastle disease due to vaccination.

Of the 3 vaccination routes, 90 % of the free ranging chicken owners interviewed preferred the one employing drinking water. This is due to the ease of administering the vaccine, as water is simply mixed with the vaccine and given to chickens. The other vaccination routes were not popular because with the eye-drop route a lot of manpower is required for chasing and catching each and every chicken in the flock, while with feed route, porridge has to be cooked specifically for the purpose of using this immunization route.

All interviewees mentioned that there is no interaction between themselves and the extension officers. The private sector including the commercial poultry sector can play a vital role, such as donating funds for training village extension officers and for vaccination campaigns. Not only does the community stand to benefit from this, but the risk of disease spread from rural to commercial poultry will also be reduced (Tsibane, 2000). This is a

very crucial point in livestock production, especially in villages. There will be a need from the side of the government and academic institutions to improve the relations between the community and extension officers.

All the interviewees stated that their chickens have contact with wild birds such as hawks and swallows. It has been proven that more than 200 species of birds are susceptible to natural or experimental Newcastle disease virus infections and it seems probable that many more are fully susceptible (Jordan and Pattison, 1996). Therefore these hawks and swallows might act as reservoirs of Newcastle disease virus or might increase the transmission of the virus from one flock to another during outbreaks. In addition to the risk of transmission that might be caused by the hawks, the owners mentioned that chicks are lost due to predation by hawks.

It is crucial to remember that the care of poultry is just one of the tasks that rural women undertake (Alders and Spradbrow, 2001). The questionnaire survey in this study has also shown that in majority of the households in Thibella women are the one's who take care of the chickens. Projects pertaining to vaccination of chickens in this area should be structured in such a way that it accommodates women as main role players. One can further suggest that there should be more female extension officers working on village poultry campaigns. In many rural areas, women have not received formal education and so speak only local languages. Consequently, it is important that project meetings, training sessions, *etc*, be conducted in the local language and that informal methods of training be employed, especially where illiteracy is common (Alders, 1996).

5.8 RECOMMENDATIONS

The findings of this study have led to the following recommendations:

- (i) The Nobilis® ND Inkukhu vaccine be used in vaccination campaigns against Newcastle disease in free ranging chickens in rural village communities. It is thermostable and easy to transport to rural village areas. In addition it produces a sufficient antibody response in free ranging chickens and has proved to be efficient in protecting them against laboratory challenge with local virulent Newcastle disease virus.
- (ii) The recommended route of administration of the vaccine be through drinking water. It should be used in vaccination campaigns since it has proved to be the easiest to apply and is the most favoured route by the free ranging chicken owners.
- (iii) The other vaccination routes such as the eye-drop and spray (although not used in this study) can be applied in village conditions in campaigns where there is enough manpower and finance to purchase the equipment required for the spray method.
- (iv) Revaccination should be conducted at 3-month intervals on the first year of a vaccination campaign and from the second year onwards revaccinations can be conducted on 6-month intervals considering the financial difficulties in rural village areas. Vaccinations must be conducted annually as young susceptible chicks are hatched throughout the year.

(v) There should be training courses organized to develop the skills of the extension and animal health officers with regard to their knowledge on poultry diseases and how to conduct vaccination campaigns in village communities. This requires involvement and cooperation of government departments, academic institutions such as Universities, Technikons, agricultural colleges and agricultural research institutes.

(vi) Selected community members who are willing to be trained in conducting vaccinations be identified, and whose role would be to assist and complement the role of extension officers.

(vii) Most importantly there is a need for financial support from the government, business communities and the producers of these vaccines for the vaccination campaigns to take place in rural village areas. The people initiating such vaccination campaigns will have to canvas for sponsorships for the projects.

In conclusion, the sustainable control of Newcastle disease in village chickens will not be achieved easily. However, the findings of this study do provide a basis in formulating programs for vaccination campaigns for free ranging village chickens in South Africa. The successful control of Newcastle disease in village chickens will result in empowerment and poverty relief to impoverished village chicken farmers. In the long-term success in vaccination against Newcastle disease may change both the conceptions which exist at present, that village chicken production system is a low input/low output system.

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APPENDIX I

QUESTIONNAIRE

Date:.....

Questionnaire no:

DEMOGRAPHY

Owner name: -----

House no: -----

Employment: -----

Village: -----

NEWCASTLE DISEASE VACCINATION

What is the Local name for Newcastle disease?

Do you consider it a priority disease in Qwa-Qwa?

YES	NO

In which seasons of the year are ND outbreaks more likely to occur?

SUMMER	AUTUMN	WINTER	SPRING

Have you observed symptoms of ND in other birds than chicken?

GEESE	DUCKS	PIGEONS	TURKEYS

Were you aware of the existence of ND vaccine before this research?

YES	NO

Are vaccines effective in disease prevention?

YES	NO

How much can you afford to pay for the vaccine?

R2	R5	R10 OR MORE

Which route are you willing to use?

FEED	WATER	EYE DROP

Do you prefer vaccination by:

SELF	EXTENSION OFFICER

INTERACTION WITH EXTENSION OFFICERS

How often do you meet with extension officers?

EVERY 3 MONTHS	EVERY 6 MONTHS	ONCE A YEAR

Do you think they are well qualified for poultry disease?

YES	NO

Which agricultural campaigns do they usually organise?-----

Are there any educational workshops organized on poultry diseases?

YES	NO

BIRDS IN A HOUSEHOLD

What type of birds do you have in a household?

CHICKENS	GEESE	DUCKS	PIGEONS	TURKEYS

Do your birds have contact with wild birds when outside their houses?

YES	NO

If yes what are those birds?

EAGLE	HAWK	SWALLOWS	OTHER

GENDER ISSUES OF VILLAGE CHICKEN MANAGEMENT

Who takes care of the chickens?

FATHER	MOTHER	CHILDREN	ALL

Who decides when to eat chickens or eggs?

FATHER	MOTHER

Who does the construction of the chicken house?

FATHER	MOTHER	CHILDREN

GENERAL COMMENTS:

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