The effect of Water quality on the efficacy of Newcastle Disease Vaccine

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DEDICATION

To my Mother
To my Father
To my sister and brothers
To all who concern with improving of
poultry industry

ACKNOWLEDGMENTS

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ABSTRACT

In this study, samples of water sources commonly used to prepare Newcastle disease vaccine in poultry were collected for analysis and experimental vaccination trials. Water samples were collected from different areas; artesian well from Omdurman, shallow well from Elsagai, tap water was collected from Shambat, surface water from Elgiraif and commercially bottled water.

Water samples were tested to determine the quality of water using the main parameters; pH, conductivity, TDS, TSS, turbidity and salinity.

Turbidity results showed great variation between the water sources examined (artesian well, shallow well, tap water, surface water and bottled water) 4, 5, 0, 48 and zero N.T.U respectively for group A, B, C, D and E. TSS analysis resulted in 0.8, 1.4, 0, 32 and zero mg\L respectively. P^H levels were between 6.6, 7.4, 7.21, 7.18 and 7.32 respectively. Conductivity results were 309, 928, 253, 253 and 207 respectively. TDS results were 148, 442, 120, 127, and 96 mg\L respectively. Salinity results 0.1 were equal for all groups.

A number of 40 chicks in each group were vaccinated on the 14th day of age with LaSota vaccine of Newcastle Disease prepared in artesian well (group A), shallow well (group B), tap water (group C), surface water (group D) and bottled water (group E). Blood was collected every week till the 5th week and serum was tested for antibodies (Ab) against ND using hemagglutination inhibition (HI) test to determine the best immune response.

Based on the experiment results of the geo mean HI titers for group E (bottled water) was the best group with regards to immune response. From the 1st to the 5th week, Ab titers were 5.05, 6.47, 4.39, 3.95, and

3.52 $\log_2$ respectively. The second best immune response was that of group C (tap water) with 4.54, 6.38, 4.52, 3.95 and 3.63 respectively. Group A gave 2.78, 4.41, 4.39, 4.38 and 3.52 $\log_2$ respectively. Group B results were 3.52, 4.89, 4.28, 3.32 and 3.26 $\log_2$ respectively. On the other hand, group D results showed great difference from other groups with 2.49, 3.95, 4.94, 3.14 and 2.88 $\log_2$ respectively.

The present study revealed great variations in water quality as measured by pH, conductivity, TDS, TSS and turbidity and in the immune response when these five water sources were used to prepare LaSota vaccine in chickens. It is recommended after this study to use bottled water or tap water to prepare drinking water vaccines in poultry farms in Khartoum State.

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease that shows a range of clinical signs, from sub-clinical to sudden very high mortality, depending on virus strain and host. ND has a devastating effect on commercial poultry production. No accurate estimation is available for the cost of ND to the commercial industry but the cost would include death and loss of production due to clinical disease. ND is an Office International des Epizooties (OIE) list A disease and subject to international regulations (Alexander, 1990).

Newcastle disease virus (NDV) has a worldwide distribution, and has a large host range. It is caused by a virus (Avian paramyxovirus-1) that belongs to the genus Rubula virus of the family Paramyxovridae (Murphy *et al*., 1999). NDV strains and isolates have been grouped into five pathotypes that relate to the disease signs: viscerotropic velogenic (VV), neurotropic velogenic (NV), mesogenic, lentogenic and asymptomatic enteric (Alexander, 1990).

Newcastle disease was first reported in Sudan in 1951 (Anon, 1950-1951). The disease took a virulent form and caused high mortality (Khogali, 1971). The virus was isolated and identified for the first time in Sudan in 1962 in a natural outbreak of the disease (Karrar and Mustafa, 1964).

The main types of vaccines are live lentogenic vaccines, live mesogenic vaccines and inactivated vaccines (Payla and Rweyemamu, 1991). Many vaccination programms were practiced in commercial farms in Sudan using different routes of administration that include intranasal, spray, drinking water and intraocular. However, the commonly used route particularly for large flocks is the drinking water route.

The aim of ND vaccination via drinking water route is to vaccinate a high proportion of the birds in the flock to prevent or minimize the effect of the disease. The presence of a choanal cleft in poultry also allows this route to be employed for most respiratory diseases (Cargill, 2006).

Vaccination via drinking water is adequate under conditions of low risk of challenge with mesogenic and velogenic strains of field ND (Sluis, 2002) the efficacy of this route varies as a result of water quality. Evaluation of water quality could be done by many ways. It can be tested for the presence of bacteria and other microbes, for the levels of minerals that occur naturally in the water, and for other chemical and physical factors. It is essential to appreciate the significance of limits set on chemical parameters defining water quality.

Poultry farms may use water from net work or municipal sources from wells, streams, ponds, lakes, rainfall catchments and springs to prepare poultry vaccines. Because of its very nature of potential hydrogen bonding, water is an excellent solvent for both inorganic and organic substances.

Water quality is characterized by its taste, acidity, alkalinity, odour, colour, turbidity, salinity, electrical conductivity, PH, biochemical oxygen demand and hardness.

Drinking water is of concern to poultry producers due to its great variability in quality and its potential for contamination. Naturally occurring surface and ground water always contain inclusions ranging from low to very high concentrations that can affect negatively on the diluted vaccines and its efficacy. In spite of vaccination, the virus remains a potential threat to commercial poultry producers, due to many factors. Vaccine mishandling or improperly used is one of those factors.

Many poultry farms used different watering systems to vaccinate their flocks against ND and other diseases, although of vaccination some

flocks show signs of the disease. Water quality has affect on vaccine efficacy, the good water quality; the more efficient vaccine. In this study we examined the differences between water sources commonly used in Khartoum State to dilute poultry vaccines, and their effects on immune response.

Experimental Design

14 days old- layer chicks were grouped into five groups; each group received the recommended dose of LaSota vaccine, diluted in different sources of water (artesian wells, shallow wells, tap water, surface water and bottled water) via drinking water administration. Collection of serum and estimation of levels antibodies against ND in order to determine the best source of water that gives the highest immune response.

Objectives:

1. To determine the physical parameters of five water sources commonly used to prepare poultry vaccines in Khartoum State; namely artesian wells, shallow wells, tap water, surface water and bottled water.

2. To assess immune response in chickens when ND vaccine is prepared in these five water sources.

CHAPTER ONE
LITERATURE REVIEW

1.1. Newcastle Disease

Newcastle disease is the most important viral disease of poultry; chickens are the most susceptible host. The incubation period varies with strain of virus, and is generally 4 to 5 days (range 2 to15 days). ND has been reported to infect more than 240 species of bird.

1.1.1. Definition

Newcastle disease is caused by *avian paramyxo virus type 1* (APMV-1) strains of the genus *Rubula virus* that belongs to the subfamily *Paramyxovirinae*, which belongs to the family *Paramyxoviridae.*The genus Rubula virus includes mumps virus, human Para influenza viruses 2 and 4, Newcastle disease (ND) virus (APMV-1), and the other avian paramyxo viruses (APMV-2 to APMV-9) (Alexander, 1991).

1.1.2. History

Newcastle disease was not recognized until 1926, the first report coming from the city now known as Jakarta in Indonesia. In that year and the following year it was recognized in other parts of Asia and in England. The outbreak in England centered in Newcastle upon Tyne and it is to this town that the disease owes its common name. The disease has restricted local names in many countries and the Indian name for the disease, Ranikhet, persists in several parts of Asia. Fowl Pest is a legal term that encompasses both Newcastle Disease and Fowl plague; caused by influenza type A (Copland, 1987).

1.1.3. The disease in Sudan

In Sudan Newcastle disease was first reported in Khartoum in 1951(Anon, 1950-1951).Since then the disease has been regularly mentioned in all reports of Sudan Veterinary Services. Diagnosis was based on the picture of the disease, but the virus was isolated and identified for the first time in 1962 (Karrar and Mustafa, 1964 and Eisa, 1974). According to Ballouh *et al.,* (1983), twelve of the Newcastle disease virus strains were isolated from the country during 1963-1979. Eight of them were velogenic and four were mesogenic. During the year 1984-1985, four virus strains were isolated from outbreaks around Shambat village (Haroun *et al.*, 1992). In another study, six strains were isolated from outbreaks in the country between 1988-1991. All isolates were similar in that they killed chicken embryos quickly in MDT values, embryo lethal dose 50(ELD50), had higher ICPI and characteristics of the viscerotropic velogenic strains of Newcastle disease virus (Khalafalla *et al.*, 1992).

1.2. Newcastle Disease virus

1.2.1. Classification

The Newcastle disease virus can be classified into five pathotypes based on the clinical signs induced in infected chickens (Beard and Hanson 1981):

i. Viscerotropic Velogenic (VV) characterized by high mortality with intestinal lesions.

ii. Neurotropic Velogenic (NV) high mortality following nervous signs.

iii. Mesogenic pathotypes with low mortality, respiratory and nervous signs, reduced egg production.

iv. Lentogenic pathotypes has mild or in apparent respiratory infection, deaths confined to young chickens.

v. A symptomatic enteric (a pathogenic) known by in apparent intestinal infection.

1.2.2. Virion Properties

Newcastle disease virus is RNA virus with helical capsid symmetry and has a non segmented, single stranded genome of negative sense. The RNA has a molecular weight of about 5x10. Nucleotide sequencing of the NDV genome has shown it to consist of 15.156 nucleotides. The avian paramyxoviruses consist of pleomorphic particles that are usually rounded and 100-500 nm in diameter or are represent as filamentous forms about 100 nm across. Surface projections on the envelope approximately 8 nm long represent the Haemagglutinin-Neuraminidase molecule, with the Fusion molecules forming smaller projections. The capsid of avian paramyxoviruses is assembled in the cytoplasm and becomes enveloped by modified cell lipoprotein membrane as the virus is budded from the cell surface (Alexander, 1990).

1.3. Biological activity

Newcastle disease virus has two main functional glycoproteins which are inserted in the envelope; one (HN) possesses haemagglutination and neuraminidase activities, the other (F) fusion protein. During the infection process, the HN protein is responsible for attaching the virus to the cell and the F protein brings about fusion between the cell and virus membranes to allow the genetic material to enter the cell (Alexander, 1991).

1.3.1. Haemagglutinin activity

The Haemagglutinin glycoprotein of the virus finds receptors on the red blood cells of many species and agglutinates these cells. Red blood cells are not a target for the virus in the chickens, but many laboratory procedures with ND virus employ the haemagglutination inhibition reaction as serological test for detection of the virus (Copland, 1987).

1.3.2. Neuraminidase activity

The enzyme neuraminidase (mucopolysacharide N-acetyl neuralminyle hydrolase) is a part of HN molecule. The activity of this enzyme is to allow the eventual release from the cell surface and the separation of the agglutinated cells (Ackerman, 1964).

1.3.3. Cell fusion and haemolysis

Newcastle disease virus and other paramyxoviruses may cause haemolysis of RBCs or fusion of cells by essentially the same mechanism. Attachment at the receptor site during replication is followed by fusion of the virus membrane with the cell membrane. This may result in fusion of two or more cells together, a similar process is involved in the syncytia formation that may occur when virus particles are budded from cells in which they have been produced (Alexander, 1991).

1.4. Route of infection

Newcastle disease virus can infect through the respiratory tract, the ocular mucous membranes and the digestive tract; although this usually requires very high doses of virus depending on the virulence of the strain. The virus shed from the respiratory tract in aerosol form, or in the faeces causing food and water contamination and used as a source of infection of

other birds especially in large commercial poultry units. In multi-age farms the infection spread mainly by aerosol. Vaccines contaminated with virulent ND virus have also initial outbreaks within flocks. It is generally accepted that the virus is not transmitted through eggs (vertical transmission) the exception may be the non-pathogenic strains, as they do not cause the death of embryos (Alders and Spradbrow, 2001).

1.5. Pathogenicity of NDV

Variation in pathogenicity can be explained by the biochemistry of the two surface proteins. The precursor proteins of Velogenic viruses are susceptible to proteases in many types of cells, so that many types of cells are able to release active viruses. With less virulent viruses, only one of the surface glycoproteins is readily cleaved to active form. Both glycoproteins of a virulent viruses apparently have very few cell types are able to produce active virus. Variations in pathogenicity, target organ specificity and age susceptibility could all be a function of interactions between viral glycoproteins and cellular proteases. It is well recognized that strains of NDV differ in organ specificity, that vary with age of chicken and this may reflect variation in protease production (Alders and Spradbrow, 2001).

1.6. Diagnosis
1.6.1. Clinical signs

The clinical signs of ND vary considerably according to the virulence and tropism of the ND virus involved, the species of bird, the age of host, the immune status of the host and environmental conditions. As a result, none may be regarded as a specific sign of ND.

Chickens infected with virulent ND virus strains may die without showing any signs of illness. Respiratory signs such as mild rales and

snick can be detected by careful observation. Other signs include severe respiratory distress and gasping, swelling of the head and neck, greenish diarrhea, marked decrease in egg production and some times deformed eggs may be produced. Nervous signs characterized the neurogenic strains included tremor, torticollis, convulsions and paralysis of wings and legs will be seen in advanced phase of the disease. Mortality may be very high, often reaching 50% to 100%. Other domestic poultry such as turkey and pigeons may also be affected (Alders and Spradbrow, 2001).

1.6.2. Post mortem findings

Post mortem findings are characteristic but not definitive. ND can be suspected if the following lesions are encountered, particularly in combination (and when the flock history is consistent with an ND outbreak). Congestion and mucous exudates in the trachea; congestion of lung (heavier than normal) lungs sink in water; haemorrhages of the proventriculus mucosa; haemorrhagic and necrotic ulceration of lymphoid patches of the intestine, caeccal tonsils and bursa of fabricius Congested ovarian follicles in chicken in lay (Alders and Spradbrow, 2001).

1.6.3. Agent Identification

Diagnosis of all avian paramyxoviruses is by isolation and identification of the virus. Virus isolation is usually done by the inoculation of embryonating chicken egg. For NDVs it is necessary to test for pathogenicity to confirm diagnosis as disease for which statutory control measures should be enforced.

The growth of viruses in the embryonating chicken can be detected by embryo death; ability of the amniotic allantoic fluid (AAF) to cause

haemagglutination (HA) of washed chicken erythrocytes (Chairman *et al.,* 1998).

Alternatively, the presence of ND virus can be confirmed by the use of reverse-transcription polymerase chain reaction (RT-PCR) using nucleoprotein-specific. Also, the presence of virus serotypes can be confirmed by using this technique.

1.7. Serological tests

Avian serological tests consists of a combination of classical test methods, such as the agar-gel precipitation (AGP) test, the agglutination test, the virus –neutralization (VN) test, enzyme-linked immunosorbent assay (ELISA) and the haemagglutination-inhibition (HI) test. The HI test is the most widely used because of its convenient and economical property in measuring vaccine response and evidence of the disease (Alexander, 1991).

1.7.1. Agar-gel immunodiffusion test (AGID)

Known as Agar gel precipitation (AGP) or double immunodiffusion test (DID), is the simplest to set up, requiring only positive and negative control sera, concentrated antigen, and appropriate agar antibodies to disease agents (Beard, 1970).

1.7.2. Agglutination test

Plate agglutination tests are used as screening tests for many diseases. These tests use stained or unstained antigen mixed with either whole blood or serum. It is important to run confirmatory tests because plate agglutination tests can give false positive and false negative reaction (Alexander, 1991).

1.7.3. Haemagglutination inhibition test

The HI test is used to titrate antibodies to avian paramyxoviruses. HI is a naturally occurring activity with avian influenza viruses, ND and adenoviruses (Alexander, 1991).

1.8. Immunity to NDV

Chicken infected with NDV produce antibodies (Abs) six to ten days after viral exposure, reaching a peak in two to four weeks (Yuan, 1999). Antibody production is rapid; Haemagglutination inhibiting Abs can be detected within four to six days of infection and persists for at least two years. Maternal Abs protects for three to four weeks after hatching (Murphy *et al*., 1999).

Immunoglobulin (Ig) IgG and IgM are confined to the circulation and does not prevent respiratory infection. Locally produced IgA Abs play an important role in protection in both respiratory tract and intestine (Murphy *et al*., 1999).

1.9. Prevention and control

Velogenic Newcastle disease outbreaks, even large ones, have been contained and eradicated under favorable circumstances. The techniques used are usually; the identification of infected properties, with the destruction of the entire chicken population and disinfection before restocking; enforcement of strict quarantine to limit spread of the disease and use of vaccines to produce buffer areas with protected birds. There are enormous difficulties involved in slaughter and burying large numbers of birds, in disinfecting large poultry farms and in vaccinating large numbers of chickens rapidly (Alders and Spradbrow, 2001).

Veterinary considerations are not the only ones in eradication campaign. The destruction of birds that are still healthy can be difficult to

justify. Surviving birds protected by vaccination are some times processing plants in action. Carcasses from these salvage operation could be a source of further outbreaks. Vaccination alone does not achieve eradication (Alders and Spradbrow, 2001). National control policies are directed at prevention of introduction of the virus and prevention of its spread within the country. To prevent the introduction of NDV, most countries have restrictions on trade in poultry products, eggs and live poultry (Alexander, 1991).

1.10. Vaccination of NDV

Vaccination is the only effective tool in controlling ND. The control can be achieved by good hygiene combined with immunization, both live-virus vaccines containing naturally occurring lentogenic virus strains and inactivated virus (injectable oil emulsions) being commonly used. These vaccines are effective and safe and may be administered via drinking water or by aerosol or nostril droplets, or beak dipping. Laying hens are revaccinated every 4 month; protection against disease can be expected about a week after vaccination (Ward *et al*., 2000).

Study was done to develop a strategy to control Newcastle disease ND in free ranging village chickens using the Nobilis ND Inkukhu vaccine (Intervet South Africa). The study was conducted at Thibella village in Qwa-Qwa, South Africa from April 2001 to October 2002. Three different routes of vaccination (administration via eye-drop, drinking water and feed) were investigated. The haemagglutination inhibition (HI) test was conducted monthly in order to measure the antibody response of village chickens after immunization against Newcastle disease using a South African isolate of velogenic ND virus; challenge trials were conducted to

determine the efficacy of the vaccine. A questionnaire was provided to evaluate perceptions of farmers on vaccinations.

The eye-drop vaccination route produced the highest HI titres ranging between 2.7 and 4.4, followed by the drinking water vaccination route with titres ranging between 2.3 and 4.0. The lowest titres were from the feed vaccination route which ranged between 1.6 and 3.0. Following the challenge, the entire control group died on the third and fourth day after infection. However, 70% of the chickens immunized by using either the eye-drop or drinking water route survived the challenge. Only 20% of the chickens from the group immunized through the feed route survived. Evidently both the eye-drop and drinking water routes were efficient in preventing disease. Necropsies showed that vaccinated chickens had mild lesions whilst control chickens had severe lesions compatible with Newcastle disease. The efficacy of the vaccine using either of the routes can be enhanced by administration of booster vaccinations at 3-month intervals during the first year of a vaccination campaign and then at 6-month intervals from the second year onwards. The majority of the owners indicated that they would prefer to vaccinate their flocks using the drinking water route (Thekisoe *et al.*, 2004).

1.11. Vaccination against Newcastle disease (ND) in Sudan

The first vaccination against ND in Sudan was performed in 1951 with a vaccine imported from South Africa (Anon, 1951-1952). In 1958, a wet live vaccine containing the Muketeswar strain (mesogenic) was used after severe outbreak of ND in Khartoum (Ali, 1978). Komarov (K) strain (mesogenic) was obtained from Lebanon in 1962 and propagated in chicken embryos to produce a freeze-dried vaccine at the Central Veterinary Research Laboratory (CVRL) (Ali,

1978). This vaccine was issued for use in 1963 (Fadol, 1991). The HI test and challenge were used to assess the immune response of vaccinated chickens. The vaccine was recommended to be given by the I\N route to four –week old chicken and repeated at the point of lay (Ali, 1978). Further investigation showed that the time for first vaccination could be at three weeks of age when the maternally derived antibody titre decreases to below 3 Iog_2 (Haroun and Hajer, 1989). In another investigation to modify the NDV vaccination programme in Sudan, the F strain was suggested for early vaccination of 1-7 day old chicks (Mahasin *et al*., 1980). Tabidi *et al*., (1998) using K vaccine found that the aerosol route gave higher antibody titers compared with other routes.

1.12. Newcastle disease virus vaccines

Most of commonly used vaccines are cultures of NDV containing either lentogenic or mesogenic strains of the virus. These are introduced into chicken by some suitable route and they multiply, provoking the production of antibody and probably also of cell mediated immunity. Even Lentogenic vaccines can cause some clinical reaction in vaccinated chicken, especially if other disease agents are present. Mesogenic viruses produce better immune responses, but because of their higher pathogenicity; their use is restricted to mature birds that have already received a course of lentogenic vaccine. The more invasive mesogenic viruses produce a better primary response, and they are wildly used as booster vaccines. The practice of using killed vaccines to give long-term protection in laying birds is increasing; these vaccines are chemically inactivated and are often included with an oil adjuvant or some other adjuvant to increase the immune response. They appear to be most effective in birds that have already some degree of immunity because of

vaccination with a living vaccine. Killed vaccines are also subject to interference by maternally derived antibodies. Controls for the production of killed vaccines are often less strict than those for living vaccines (Alders and Spradbrow, 2001).

1.12.1. Live vaccines

Newcastle disease live vaccines are divided into two groups: lentogenic and mesogenic vaccines (Palya and Rweyemamu, 1991).

1.12.1.1. Lentogenic strain vaccines

Within this group, there is considerable range in virulence. Examples of these vaccines are LaSota (Asplin), Hitchner B1, V4 and I_2 a virulent thermo stable strains (Alexander, 1991) and (Tu *et al*., 1998).

The most common method of application for live Lentogenic vaccines used worldwide is via the drinking water (DrW), I\N, eye dropping (I/O) and beak dipping (BD) routes. The application of live vaccine by sprays and aerosols is also very popular due to the ease with which large numbers of birds can be vaccinated in a short time (Alexander, 1991).

1.12.1.2. LaSota vaccine trials

Comparative studies in Bulgaria were carried out on the effect of the vaccination of broilers against Newcastle disease with LaSota strain through the single application in the drinking water on the 21st day and the newly introduced vaccination at the age of four days, using the spray method, as well as the combined method--spray vaccination on the fourth day and giving the vaccine twice on the 21st day. It was found that the last method confers better immunity and higher

immunity against the disease as compared to offering the vaccine only once on the 21st day (Semov *et al.,* 1976). In Bulgaria, chickens were immunized against Newcastle disease with live vaccines Hitchner B1 and LaSota by the spray method with particles of 50-100 micron and by aerosols with particles of 17-20 microns. Vaccinations were made under experiment and production conditions with chickens aged 5, 10 and 15 days. Vaccination of 5 and 10-day-old chickens was not always successful which necessitated their revaccinations. It was found that spray reimmunization with flocks or aerosol one with Atomist enhance antibody titer and resistance degree sufficiently for prophylactic purposes. The aerosol method is more efficacious than the spray method; under production conditions, it is more effective and readily applicable. Single immunization of 15-day-old chickens after both methods is effective and increases chicken immunity to the end of the fattening period (Runtev and Sizov, 1980). Two schemes of vaccination against Newcastle disease (A and B) were tested on broiler-chickens and two schemes (C and D), on parent form birds. Vaccine of the LaSota strain was used for the broilers and was administered twice at differing age after the spray method. For the parent birds LaSota strain vaccine was used also twice after the spray method, plus a vaccine of the strain Komarov twice intramuscularly and as aerosol (scheme C) and the vaccine strain LaSota spray and aerosol alone (scheme D). Immunity induced in birds after immunization was followed serologically by the titer of serum antihaemagglutinins and by provocation with a highly virulent Newcastle disease virus strain. The 4 schemes of immunization tested comparatively to ensure solid immunity of birds to Newcastle disease. Better production index results were obtained from broiler chickens

vaccinated after scheme B and parental form birds vaccinated after scheme C (Khadzvier *et al*., 1979).

Two types of locally produced live vaccines (HB1 and LaSota – lentogenic strains) and inactivated oil adjuvant (IOAV) vaccine were used to compare the efficiency of three vaccination techniques, namely drinking water, ocular and spray on broiler chicks. The ocular route of vaccination on one-day-old chicks followed by a booster dose on the third week through the same route induced a significantly higher level of haemagglutination inhibition antibody titre. The highest mean antibody titre was $\log_2 6.6$ and 93.3% of the chicks were protected from the challenge. The spray technique induced a lower antibody titre (peak of $\log_2 5.9$) and only 53% of the chicks in this treatment survived against the challenge. The results of this study showed that the ocular route is superior to the spray technique and the drinking water route. The economic analysis result showed that the ocular HB1 and LaSota vaccine administration method to 1- and 21-day-old chicks gave the highest revenue followed by the drinking water method. In terms of total cost, the injection method required the highest cost (0.21 birr/chick) followed by the ocular method (0.18 birr/chick). The marginal cost of vaccine administration is too small compared with marginal revenues from relative effectiveness of the methods. The internal rate of return for the ocular method was very high. The results of sensitive analysis on revenues from different vaccination methods indicate that a 25% reduction in broiler price reduces the marginal revenue from the ocular method by 12 487 birr but this still does not prove that the ocular method is economically viable for small- and medium-scale poultry farms (Degefa *et al*., 2004).

1.12.1.3. The Mesogenic strain vaccines

The Mesogenic strain vaccines are suitable only for secondary vaccination of birds due to their greater virulence. Examples of these are Muketeswar, K and Roakin strains. They usually require inoculation by wing –web (WW) stabbing or I\M injection (Alexander, 1991).

1.12.2. Inactivated vaccines

Inactivated vaccines are usually produced from infective AF treated with betapropiolactone or formalin to kill the virus and mixed with a carrier adjuvant such as aluminum hydroxide or mineral oil (Cross, 1988).

1.12.3. Thermostable vaccines

Thermostable vaccines are those which are resistant to high temperatures. Examples of them are the non-pathogenic Australian viruses V_4, I_2 and northern Ireland Ulstar 2C (Hanson and Spalatin, 1978). The main advantages of thermostable vaccines are:

1-Thermostability; they have the possibility of reaching sites beyond the cold chain in a viable state.

2-Ease of administration; they can be applied by the farmers at the village level.

3- They can spread from vaccinated to non-vaccinated chickens in close contact (Alders and Spradbrow, 2000).

1.13. Water effect on vaccine efficacy

Chicken producers rely on administration of vaccines to protect flocks against Newcastle disease, infectious bronchitis, laryngotracheitis and infectious bursal disease. The drinking water route

is often used as it requires minimal labour and is associated with mild or unapparent vaccine reaction. Unfortunately, the efficacy of vaccine administered in drinking water varies according to water quality and technique. Attaining adequate antibody levels can influence protection in the event of exposure to field challenge with consequential effects on performance including liveability and financial return (Sluis, 2002).

1.14. Water Quality considrations

High quality drinking water may be defined as water which contains inclusions which promote vitality and lack inclusions causing morbidity and mortality. As the volume of non drinkable water increases and the technology for measurment of inclusions improves, there is increasing a ware of water inclusions and their effects on health and nutrition (Sluis, 2002).

The ability to achieve a guidline value within a driking-water supply deppends on a number of factors, including the concentration of the chemical in the raw water; control measures employed through out the drinking-water system, nature of the raw water (ground water or surface water, presence of natural background and other componants); and treatment processes already installed (WHO guidline, 2006).

1.14.1. Microbial aspects

The greatest microbial risks are associated with ingestion of water that contaminated with human or animal (including bird) faeces, faeces can be a source of pathogenic bacteria, viruses, protozoa and helminthes.

Faecaly derived pathogenes are the principal concerns in setting health, based targets for microbial safety. Microbial water quality often varies rapidly and over a wide range. Short-termpeeks in pathogen

concentration may increase disease risks considrably and may trigger outbreaks of water borne diseases, further more, by the time microbial contamination is detected, many lifes will be exposed. Securing the microbial safety of drinking-water supplies is based on the use of multiple barriers, from catchment to consumer to prevent the contamination of drinking water or to reduce contamination to levels not injurious to health.

While water can be a very significant source of infectious organisms, many of the diseases that may be water borne may also be transmitted by other routes, including person to person contact, droplets and food intake. Depending on circumstance and in the absence of water borne outbreaks, these routes may be more important than water borne transmission (WHO guideline, 2006).

1.14.2. Chemical aspects

There are many chemicals that may occur in drinking water; however, only a few are of immediate health concern in any circumstance. There are few chemical constituents of water that can lead to health problems resulting from a single exposure, except through massive accidental contamination of driking-water supply (WHO guideline, 2006).

1.15. Analytical Methods

Various collections of standard or recommended methods for water analysis are published by a number of national and international agencies. It is often thought that adequate analytical accuracy can be achieved provided that all laboratories use the same standard method (WHO guideline, 2006).

1.15.1. Volumetric titration

Chemicals are analyzed by titration with standardized titrate. The titration end-point is identified by the development of colour resulting from the reaction with an indicator, by the change of electrical potential or by the change of pH value (WHO guideline, 2006).

1.15.2. Colorimetric Methods

Colorimetric methods are based on measuring the intensity of colour of a colored target chemical or reaction product. The optical absorbance is measured using light of a suitable wavelength. The concentration is determined by means of a calibration curve obtained using known concentration of the determinant.

Other methods used as the UV method, which similar to the colorimetric method. For ionic materials, the ion concentration can be measured using an ion-selective electrode (WHO guideline, 2006).

1.16. Critical parameters of drinking-water quality

The principal risks to community health associated with water supplies. There is traditional tests use to establish the safety of supplies. Some agencies refer this strategy as minimum monitoring, while others use the term critical parameter testing. The parameters recommended for the minimum monitoring of community supplies are those that establish the hygienic of the water (WHO guideline, 1997).

1.16.1. Turbidity

Turbidity is important because it affects both the acceptability of water to consumers, and the selection and efficiency of treatment processes, particularly the efficiency of disinfection with chlorine since it

exerts a chlorine demand and protects microorganisms and may also stimulate the growth of bacteria.

In all processes in which disinfection is used, the turbidity must always be, preferably below 1 NTU (Nephelometric Turbidity Unit).

Turbidity in drinking-water is caused by particulate matter that may be present from source of water as a consequence of in adequate filtration or from resuspension of sediment in the distribution system. It may also be due to the presence of inorganic particulate matter in some ground water. Turbidity is an important operational parameter in process and can indicate problems with treatment processes. No based guideline value for turbidity has been proposed; ideally, however median turbidity should be below 0.1 NTU (WHO guideline, 1997).

1.16.2. Total dissolved solids TDS

Total Dissolved Solids is a measure of the total ions in solutions. The platability of water with a TDS level of less than 600 mg/L is generally considered to be good; drinking water becomes significantly and increasingly unplatable at TDS levels greater than about 1000 mg/L. The presnce of high levels of TDS lead to excessive scaling formation (WHO guideline, 2006).

1.16.3. Hardness

Hardness is atendence to precipitate soap and form scales on heated surface. Temporary hardness is due to presence of calcium and magnesium bicarbonates. Perminant hardness is caused by calcium and magnesium sulfate. Hardness is a problem in plugging up watering devices. Sodium and potassium produce no hardness. Water softeners remove hardness but not TDS (Jensen *et al*., 1977).

The taste threshold for the calcium ion is in the range of 100-300 mg\L, depending on the associated anion, and the taste threshold for magnesium is probably lower than that for calcium. In some instances, consumers tolrate water hardness in excess of 500 mg\L. Depending on the interaction of other factors, such as pH and alkalinity, water with hardness approximately 200mg/L may cause scum formation. Soft water, with a hardness of less than 100mg/L, may on the other hand, have a low buffering capacity (WHO guideline, 1997).

1.16.4. pH

Although pH usually has no direct impact on consumers, it is one of the most important operational water quality parameters. Careful attention to pH control is necessary at all stages of water treatment to insure satisfactory water clarification and disinfection. The pH should preferably be less than 8; however, low pH water is likely to be corrosive. Alkalinity and calcium management also contribute to the stability of water and control its aggressiveness.

The optimum pH required will vary in different supplies according to the composition of the water and the nature of the concentration of the materials in the range 6.5-8.0 (WHO guideline, 1997).

1.16.5. Conductivity

Conductivity is the measurment of a solutions ability to conduct an electrical current. The unit of measure is often expressed as milliohms. Absolutely pure water is actually a poor electrical conductor. It is the substances (or salts) dissolved in the water which determine how conductive the solution will be. There for, conductivity can be an excellent indicator of water quality.

The higher the conductivity, the more salts are dissolved in water. By comparing conductivity readings on a regular bases, there will be differences occurred in different sources of water. Nutrient defficiencies are possible when water is too pure (low conductivity) or if the relative concentrations of some nutrients are unbalanced (i.e calcium-magnesium) (Jensen *et al.*, 1977).

1.16.6. Water salinity

Salinity is expressed by the a mount of dissolved salt content of water, and it has been traditional to express salinity not as percent, but as part per million, which is approximately grams of salt per liter of solution. In other disciplines chemical analyses of solutions, and thus salinity is frequently reported in mg/L or ppm (Lewis, 1980).

1.16.7. Alkalinity

It is important to note that testing the alkalinity is much more important than generally recognized. Alkalinity dictates how much influence the waters pH will have on the soil and nutrient availability. In addition, alkalinity has a very great effect on the ease and difficulty of reducing the pH of water (Lewis, 1980).

1.16.8. Chlorine

Chlorination using an in-line proportioner (a device for accurately injecting the correct proportion of chlorine into the water line) has been successful in poultry operations if the residual chlorine level in the waterers is at least 1 milligram per liter (mg/L) (Roland, 1987). Most individuals are able to taste or smell chlorine in drinking-water at concentrations well below 5 mg/L, and some at levels as low as 0.3mg/L. At a residual free chlorine concentration of between 0.6 and

1.0 mg/L, there is an increasing likelihood that some consumers may object to the test. The taste thershold for chlorine is below the health-based guideline value (WHO guideline, 1997).

1.16.9. Dissolved Oxygen

The disolved oxygen content of water is influenced by the source, raw water temperature, treatment and chemical or biological processes taking place in the distribution system. Depletion of dissolved oxygen in water supplies can encourage the microbial reduction of nitrate to nitrite and sulfate to sulfide. It can also cause an increase in the concentration of ferrus iron in solution (WHO guideline, 1997).

CHAPTER TWO
MATERIALS and METHODS

2.1. Water sampling

A volume of 200 ml water was collected in sterile bottle to avoid contamination and samples were closed by a rubber or stopper until it was filled. Samples were collected from different water supplies to represent the different water systems in poultry farms. The volume of sample was sufficient to carry out all tests required. Preferably not less than 100 ml. Each sample collected separately and transported within 2 hours to Institute of Environmental Studies- University of Khartoum for analysis.

2.1.1. Artesian well

Collection of Artesian well sample was from Elsarha-farm in Omdurman locality. The distribution system used was pipes connected with the main pipe; and a pump used to push water from the well. This well measured more than 40 meters in depth. The sample was collected from the main pipe or tube connected with the well, continuously operates the pump for 5 min and then we collected our sample from the mid stream. This sample represents the ground water characteristics.

2.1.2. Shallow well

Collection of Shallow well sample was from Elsagai-farm in Khartoum North locality. This well was less in depth than Artesian well from several meters to around 30 meter under ground. One main pipe connected with a pump to push water from the well and to keep it in storing tank; this tank feeds the farm. The sample was collected from the tap of storing tank in sterile bottle.

2.1.3. Potable water (Tap water)

Tap water sample had taken from a poultry farm in Shambat; in this farm watering system used was a service pipe directly connected with the net work; which supplies Bahry locality. The tap opened fully and water kept run for 2-3 min, and then we collected our sample from the mid stream.

2.1.4. Surface water

In monitoring stream and surface water, establish sampling locations at critical sites. The sample was collected from a canal connected directly with the main stream of River Nile in Elgiraif- poultry farm. The time of collection was in afternoon from the mid stream.

2.1.5. Bottled water

The sample was bought from commercial bottled water named by Soba water; the sample used was 500 ml in sterile bottle.

2.2. Water analysis

After collection; samples were analyzed and evaluated at the Institute of Environmental Studies- University of Khartoum. By using the Standard Method for Examination of water and waste water Manual (APHA, 1998), the samples were tested for the following parameters:

2.2.1. Turbidity

After receiving to the laboratory, we analyzed water samples firstly for turbidity, using Spectrophotometer to estimate the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity. This

method named by Nephelometric Method and the evaluated unit is Nephelometric Turbidity Unit (NTU).

2.2.2. pH

pH value was calculated by using electrometric pH meter (HANA Instruments HI 8314). The main idea of this apparatus is to determine the activity of hydrogen electrode.

2.2.3. Total Dissolved Solids (TDS)

A well-mixed sample is filtered through a standard glass fiber filter, and the filtrate is evaporated to dryness in a weighed dish using Analytical Balance Meter A 80 and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids.

2.2.4. Total Suspended Solids (TSS)

A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained to the filter is dried to a constant weight at 103° to 105°C. The increase in weight of the filter represents the total Suspended Solids.

2.2.5. Chlorine

The method used in determining free chlorine and chloramines called (DPD) method and it is extension of the N, N-diethyl-p-phenylenediamines. For evaluation mixtures of various chloro-compounds presenting in water we added glycine before reacting the samples with DPD reagent. Differentiation is based on the fact that glycine converts free chlorine instantaneously into chloroaminoacetic acid but has no effect on other chlorites.

2.2.6. Conductivity and Salinity

The two tests have same apparatus named by Conductivity meter HACH Co 150.

2.3. Virus strains

2.3.1. LaSota strain

LaSota vaccine strain was a vaccinal strain (INTERVET, Holland) produced as freeze-dried ampoules of 1000 dose. It was used in the present study for vaccination of chicks.

2.3.2. I_2 Strain

Live NDV vaccine containing the Lentogenic thermostable I_2; isolated in Australian Center for International Agriculture Research (ACIAR). It was used in the present study as an antigen in HI test. One freeze-dried ampoule was reconstituted in D.D.W and propagated in E.E as below.

2.4. Chicken Embryonated eggs

Chick embryos were obtained from the poultry farm of the Department of Microbiology, Faculty of Veterinary Medicine University of Khartoum and were incubated in the incubator at $37\,^{o}$C.

2.5. Inoculation of Embryonated egg

The working seed of I_2 strain was prepared by inoculation of 9-11 day-old chicken embryonated eggs. Embryonated eggs were candled before inoculation in a dark room to check for embryo viability. A line was marked around the air sac and across was made 2-4 mm over the air sac with a pencil. Eggs were then swabbed with 70% alcohol and a pore was made on the cross. Eggs were then inoculated

by allantoic route with sterile disposable 1 ml syringes. Each egg received 0.1 ml of the inoculums and the pores were sealed with melted paraffin wax. Eggs were then incubated at 37 ^{o}C and candled daily to check for embryo death for five days. Embryo death during 24 hours of inoculation was considered as non-specific and discarded. At day five post inoculation (pi), all eggs were chilled at 4 ^{o}C for at least two hours and the allantoic fluid (AF) aseptically collected into sterile vials.

2.6. Harvest of I$_2$ strain

Eggs were removed from the refrigerator, disinfected using 70% alcohol and then the shell over the air sac was removed using sterile forceps and the chorioallantoic membranes rupture. Aminoallantoic fluid (AAF) was then aspirated with sterile syringe into sterile bijou bottles.

2.7. Experimental chicks

A total of 200 male chicks of Longman breed were used in this study. They were obtained as one-day-old chicks from Ayad Farm, Khartoum. Chicks were divided into five groups and reared in special metal cages till the required age. Experimental chicks were used in the present study to determine the effect of water sources used to prepare LaSota vaccine by drinking water administration.

2.8. Collection of blood

Chicks used in vaccination trials were sampled for blood by heart puncture or from the wing vein using disposable syringes and then the blood was left overnight at room temperature to clot and then

centrifuged at 1000 rpm for 10 minutes. Separated serum samples were stored at -20 $^{\circ}$C till used.

2.9. Dilution of the vaccine

The vial containing 1000 dose per bird was dissolved into 5 ml of water for injection (double distilled water), the 4 ml was discarded and the volume was retained to 5 ml; each group of chicken received 1 ml of dissolved vaccine diluted in 1 liter of different waters.

2.10. Preparation and sterilization of glassware

Glassware like beakers, flasks, pipettes, centrifuge tubes, bijou bottles, measuring cylinders were boiled in water with a detergent for 20 minutes and rinsed in running water five times to remove detergent completely. Dissecting equipments like forceps, scissors and scalpel handlers were sterilized after washing by dry heat at 180° C for 30 minutes. Microtitre plates were sterilized by putting portion of detergent as potassium dichromate on the wells, then in the following day, they were rinsed in tap water and soaked and then rinsed in three changes of distilled water (DW) and left to dry at room temperature.

2.11. Haemagglutination (HA) and Haemagglutination inhibition (HI) test

2.11.1. Collection and preparation of RBCs

Blood was collected from the wing vein of healthy 8-10 weeks-old chicken into 5 ml sterile syringe with EDTA powder added the blood then clarified by centrifugation at 1000rmp for 10 minutes. The supernatant was discarded and equal volume of sterile phosphate buffer saline (PBS) was added to pack RBCs and then centrifuged at 1000rpm

for 10 minutes. This procedure was repeated three times and pack cells were then diluted to 1% for use in HA and HI tests.

2.11.2. HA test procedure

A volume of 0.025 ml of phosphate buffer saline (PBS) was dispensed into each well of U-bottomed microtitre plate.

Two-fold serial dilutions of 0.025 ml volumes of the virus suspension were made across the plate. 0.025 ml of 1% (v/v) chickens RBCs is dispensed to each well.

The solution was mixed by tapping the plate gently. The RBCs were then allowed to settle for 30 minutes at room temperature. The HA titer was the reciprocal of the last dilution showing Haemagglutination.

2.11.3. Determination of Haemagglutination Inhibition (HI) Test
2.11.3.1. Preparation of 4HA unit (4HAU) of the virus

HA test was performed on undiluted I_2 virus suspension. The last well showing HA was considered 1HAU and accordingly, the 4HAU was calculated accurately from the initial range of dilutions. The virus suspension was then diluted to contain 4HAU per 0.025.

2.11.3.2. Procedure of HI

A volume of 0.025 ml of PBS was dispensed into each well of the U-bottom microtiter plate. Two fold serial dilutions of 0.025 ml volumes of the tested sera were made across the plate. 4HAU virus\antigen in 0.025 ml was added to each well and the plate was left for 30 minutes in room temperature for antigen/antibody reaction. 0.025 ml of 1% (v\v) chicken RBCs was added to each well and, after gentle mixing, the RBCs were allowed to settle for 40 minutes at room

temperature. The HI titer was the reciprocal of the last dilution showing complete inhibition haemagglutination.

The HI titer was the highest dilution of serum causing complete inhibition of 4HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.025 ml PBS only) were considered.

CHAPTER THREE
RESULTS

3.1. Analysis of water sources
3.1.1. Turbidity

The highest turbidity result in water samples was found to be 48 N.T.U in surface water and the lowest turbidity result in water samples were found to be zero in tap water and bottled water (Figure 1).

3.1.2. pH

The highest result of pH in water samples was found to be 7.4 in Shallow well and the lowest pH result was found to be 6.6 in Artesian well (Figure 2).

3.1.3. Total Dissolved Solids (TDS)

The highest result TDS was found to be 442 mg/L in Shallow well and the lowest result was found to be 96 mg/L in Bottled water (Figure 3).

3.1.4. Conductivity

The highest Conductivity result was found to be 928 in Shallow well and the lowest result was found to be 207 in Bottled water (Figure 3).

3.1.5. Total Suspended Solids (TSS)

The highest TSS result was found to be 32 in surface water and the lowest result was found to be zero in Bottled water and Tap water (Figure 4).

3.1.6. Chlorine

Results of free chlorine were found to be 0.2 mg/L in tap water and zero in other water samples.

3.2. Preparation of I_2 Antigen

50 ml of I_2 strain were prepared in Embryonated eggs and divided into five cryo vials and kept in a deep freezer. I_2 was used as antigen in HI test.

3.3. Assessment of immune response

Five groups of chicks were vaccinated by LaSota vaccine prepared in artesian well (group A), shallow well (group B), tap water (group C), surface water (group D) and bottled water (group E) the result of HI test was calculated using Geometric Mean.

3.3.1. Group A

The geometric mean HI titers for group A were $\log_2$ 2.78, 4.41, 4.39, 4.38, 3.52 respectively (Figure 5).

3.3.2. Group B

The geometric mean HI titers for group B were $\log_2$ 3.52, 4.89, 4.28, 3.32, 3.26 respectively (Figure 6).

3.3.3. Group C

The geometric mean HI titers for group C were $\log_2$ 4.54, 6.38, 4.52, 3.95, 3.63 respectively (Figure 7).

3.3.4. Group D

The geometric mean HI titers for group D were $\log_2$ 2.49, 3.95, 4.94, 3.14, 2.88 respectively (Figure 8).

3.3.5. Group E

The geometric mean HI titers for group E were $\log_2$ 5.05, 6.47, 4.39, 3.95, 3.52 respectively (Figure 9).

3.4. Statistical analysis

The multiple comparisons between five water sources from Khartoum State as analyzed using statistical method was based on observed means as shown in Table 2. The mean difference is significant at the 0.05 level. The difference between the treatment groups was found to be highly significant ($p < 0.01$). We found the comparison between water groups in column (I) with the other groups in column (J). Comparison between the five groups of vaccinated chicks and time interval shows high significance of the relation between group and weeks interval (Table 3).

Fig. (1): Turbidity of five water sources commonly used to prepare poultry vaccines

Turbidity N.T.U
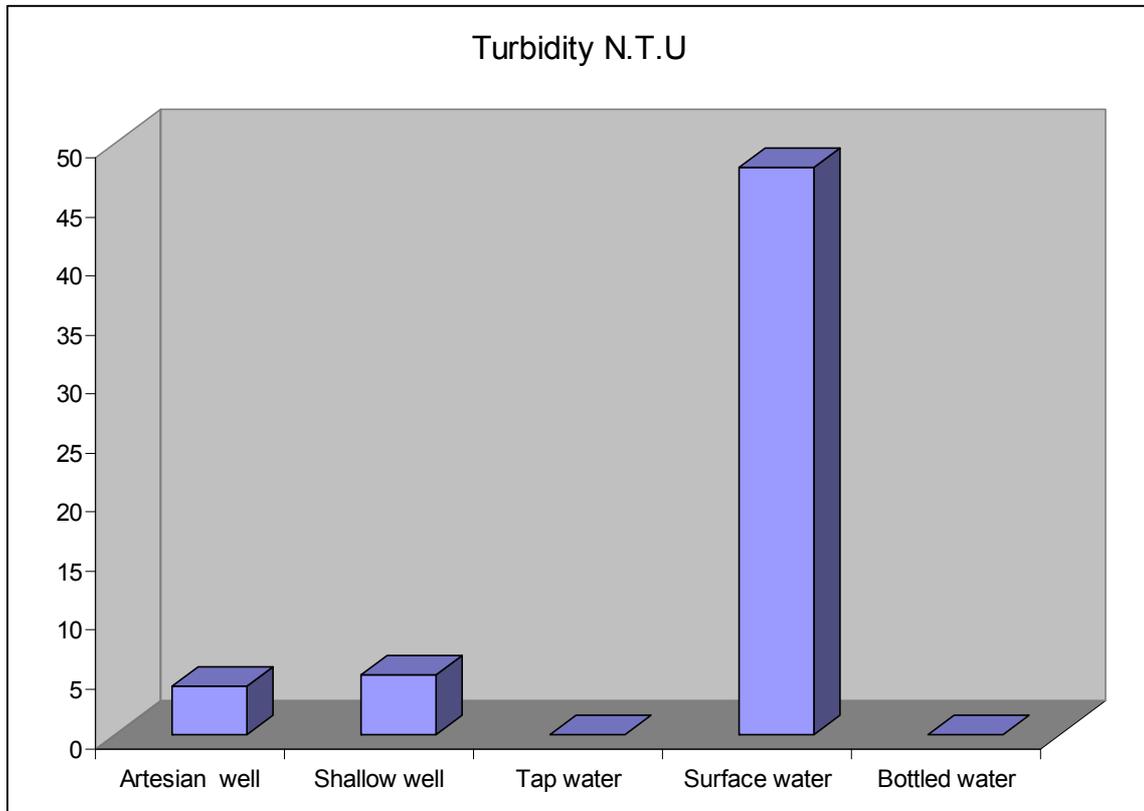
Fig. (2): pH levels of five water sources commonly used to prepare poultry vaccines

PH
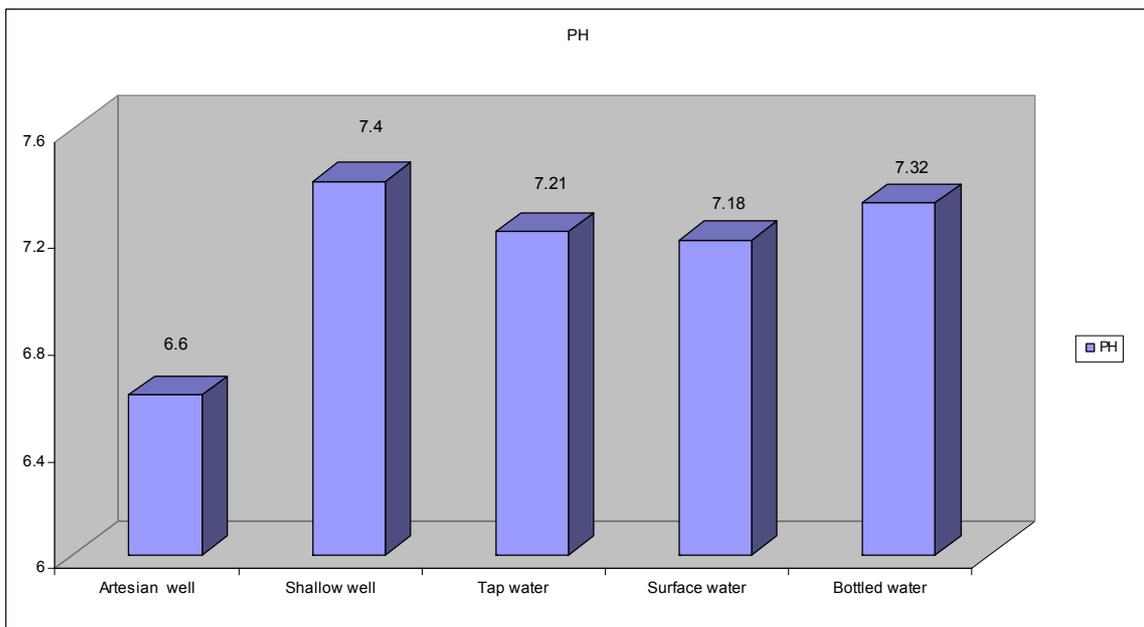
Fig. (3): TDS and Conductivity results of five water sources commonly used to prepare poultry vaccines
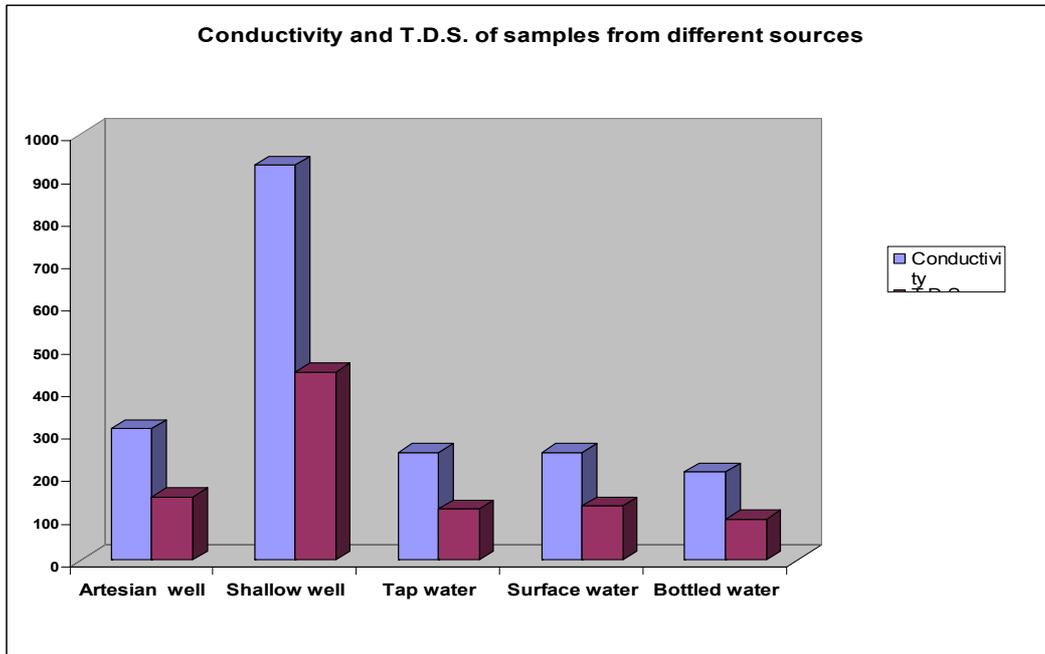
Fig. (4): TSS of five water sources commonly used to prepare poultry vaccines
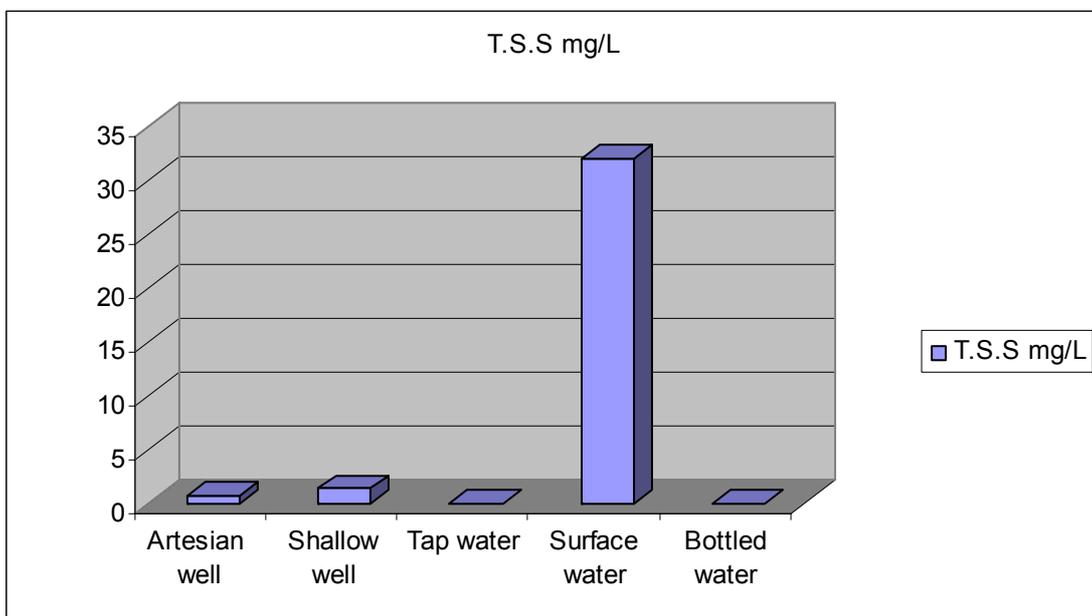
Fig. (5) Antibody titres in sera collected from experimental chicks vaccinated with LaSota vaccine prepared in artesian well
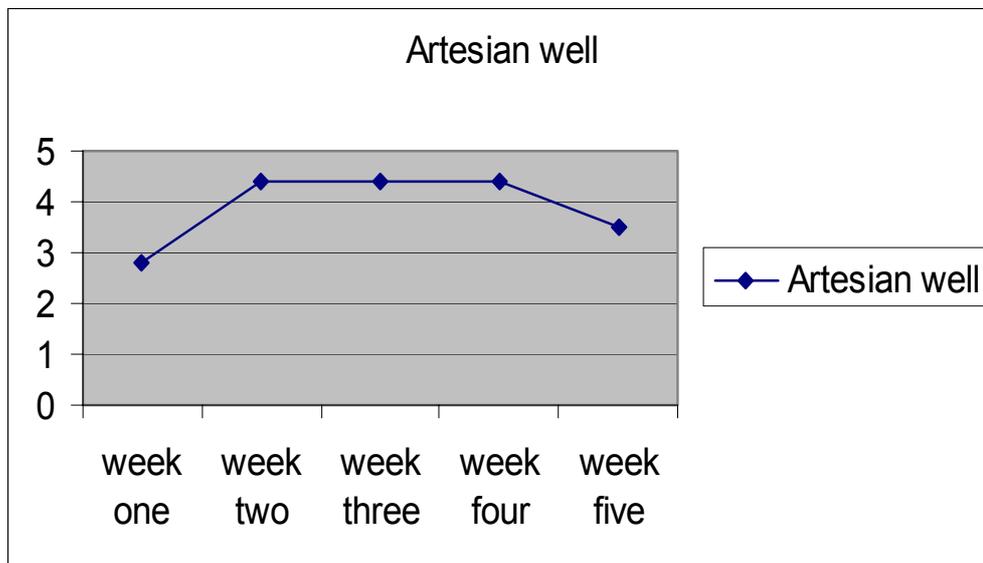
Fig. (6) Antibody titers in sera collected from experimental chicks vaccinated with LaSota vaccine prepared in shallow well
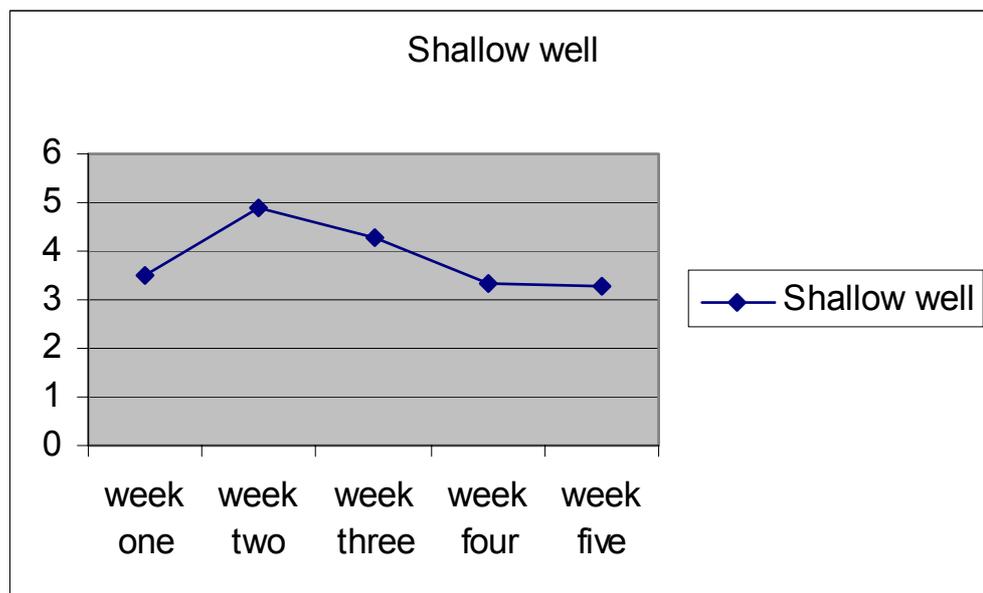
Fig. (7) Antibody titers in sera collected from experimental chicks vaccinated with LaSota vaccine prepared in tap water
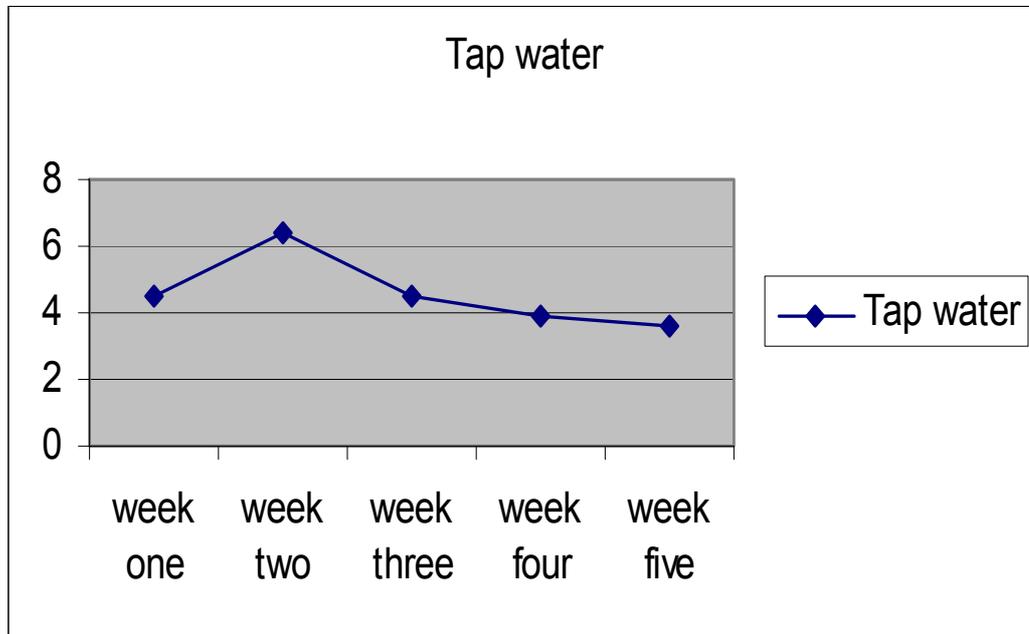
Fig. (8) Antibody titers in sera collected from experimental chicks vaccinated with LaSota vaccine prepared in surface water
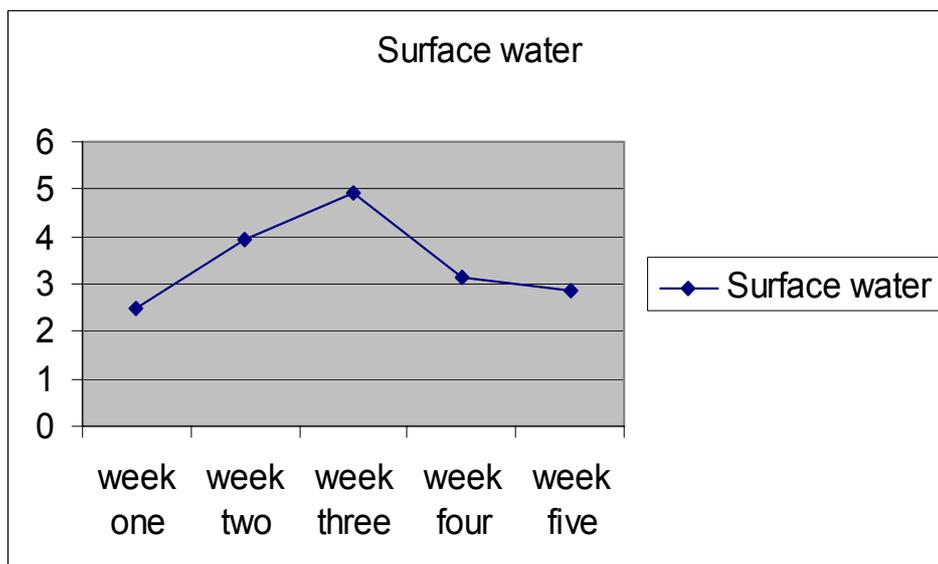
Fig. (9) Antibody titers in sera collected from experimental chicks vaccinated with LaSota vaccine prepared in bottled water
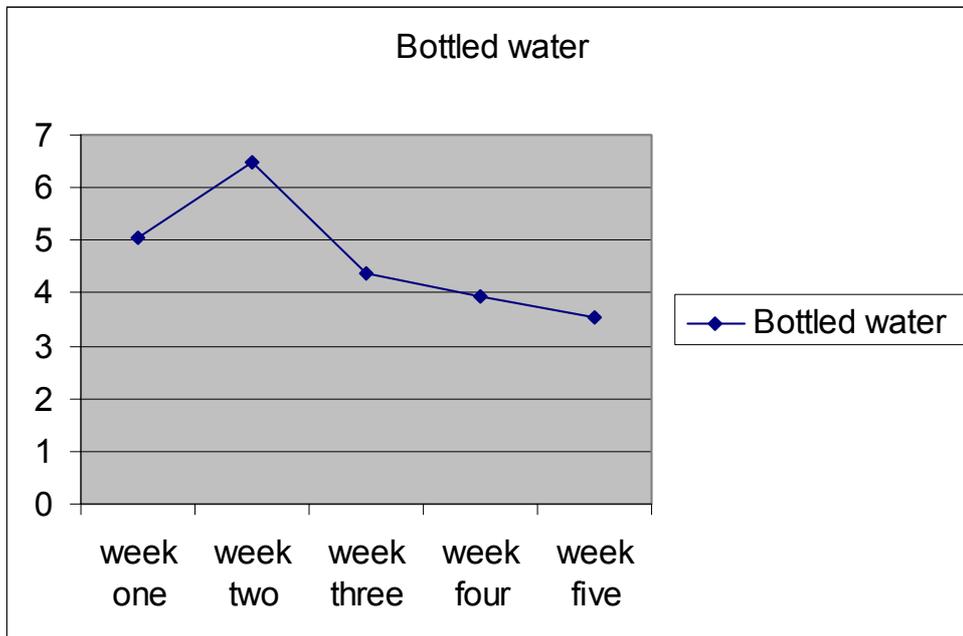
Fig. (10) Antibody titers in sera collected from different groups of Chicks vaccinated using water sources commonly used in poultry farms.
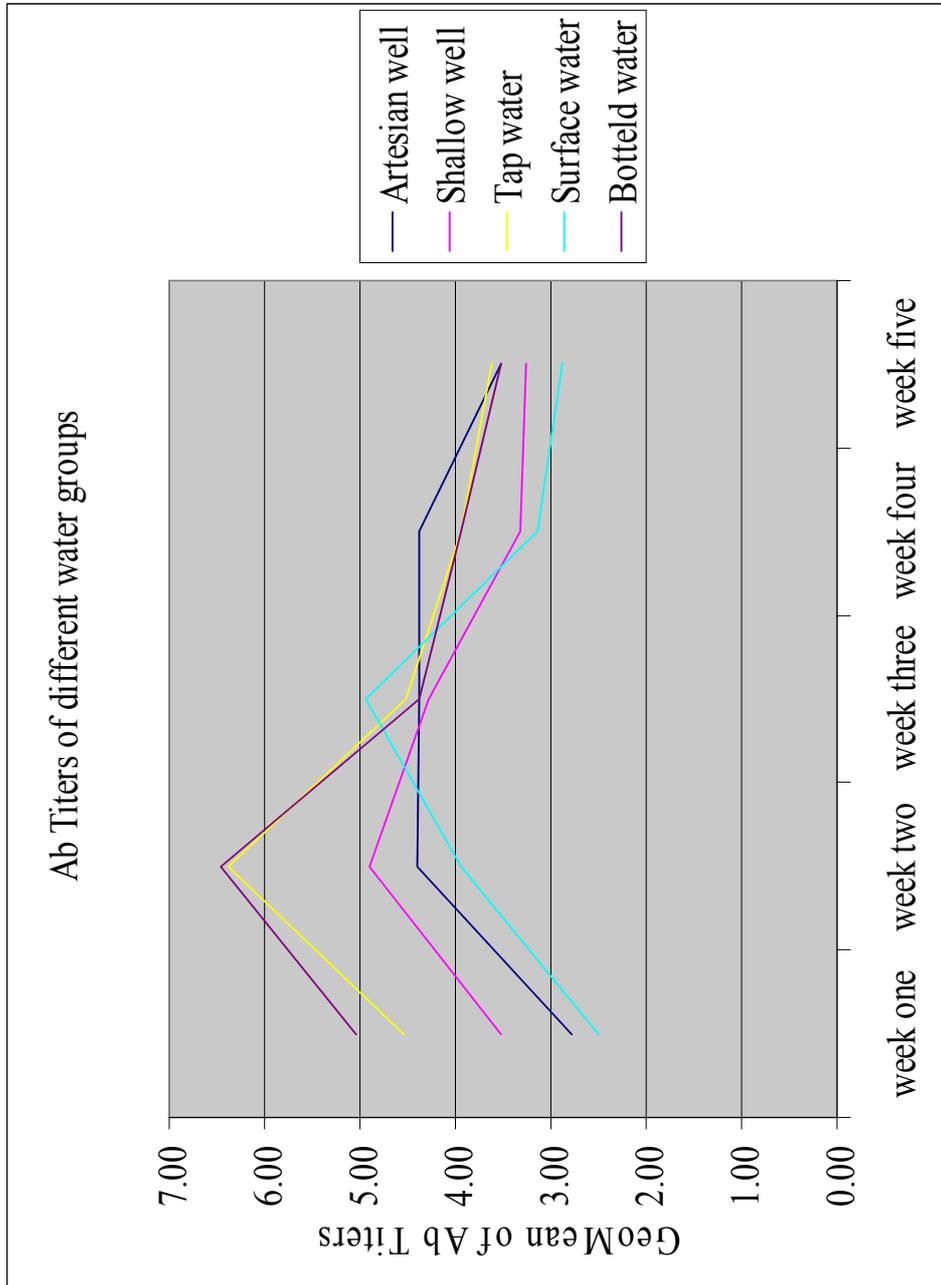
Table (1): Physical analysis of five water resources commonly used as diluents for poultry vaccines collected from different areas in Khartoum state

Water sample	PH	Conductivity	T.D.S+ mg/L	T.S.S++ mg/L	Turbidity N.T.U	Salinity	Chlorine mg/L
Artesian well	6.6	309	148	0.8	4	0.1	-
Shallow well	7.4	928	442	1.4	5	0.1	-
Tap water	7.21	253	120	0	0	0.1	0.2
Surface water	7.18	253	127	32	48	0.1	-
Bottled water	7.32	207	96	0	0	0.1	-

+ T.D.S Total dissolved solids   ++ T.S.S Total suspended solids

Table (2): Multiple comparisons between five water sources commonly used as diluents in poultry vaccines from Sudan as analyzed by LSD table

Multiple Comparisons

Dependent Variable: Titer

LSD

(I) Group treatment	(J) Group treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Artisian well	Shallow well	.0000	.13341	1.000	-.2625	.2625
	Tap water	-.7193*	.13438	.000	-.9838	-.4549
	Surface water	.3771*	.14614	.010	.0896	.6647
	Bottled water	-.7857*	.13341	.000	-1.0482	-.5232
Shallow well	Artisian well	.0000	.13341	1.000	-.2625	.2625
	Tap water	-.7193*	.13438	.000	-.9838	-.4549
	Surface water	.3771*	.14614	.010	.0896	.6647
	Bottled water	-.7857*	.13341	.000	-1.0482	-.5232
Tap water	Artisian well	.7193*	.13438	.000	.4549	.9838
	Shallow well	.7193*	.13438	.000	.4549	.9838
	Surface water	1.0965*	.14703	.000	.8071	1.3858
	Bottled water	-.0664	.13438	.622	-.3308	.1981
Surface water	Artisian well	-.3771*	.14614	.010	-.6647	-.0896
	Shallow well	-.3771*	.14614	.010	-.6647	-.0896
	Tap water	-1.0965*	.14703	.000	-1.3858	-.8071
	Bottled water	-1.1629*	.14614	.000	-1.4504	-.8753
Bottled water	Artisian well	.7857*	.13341	.000	.5232	1.0482
	Shallow well	.7857*	.13341	.000	.5232	1.0482
	Tap water	.0664	.13438	.622	-.1981	.3308
	Surface water	1.1629*	.14614	.000	.8753	1.4504

Based on observed means.

*. The mean difference is significant at the .05 level.

Table (3): Comparison between the five groups of vaccinated chicks and time interval

Tests of Between-Subjects Effects

Dependent Variable: Titer

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	747.168[a]	39	19.158	32.561	.000
Intercept	6002.418	1	6002.418	10201.516	.000
GROUP	65.275	4	16.319	27.735	.000
WEEKS	574.498	7	82.071	139.486	.000
GROUP * WEEKS	90.848	28	3.245	5.514	.000
Error	278.894	474	.588		
Total	7428.000	514			
Corrected Total	1026.062	513			

a. R Squared = .728 (Adjusted R Squared = .706)

CHAPTER FOUR
DISCUSSION

The poultry industry has largely grown, from backyard operations that provided supplemental income for the family, to a vertically integrated industry. Therefore, many studies targeting to improve this industry are mainly in the increasing of production levels or in the prevention from poultry diseases. The search for appropriate Newcastle disease control is one of the strategies in extensive poultry systems and it depends on vaccination protocols. Drinking water administration has many advantages such as low costing, and that it is easier, faster and needs few labors particularly in extensive systems. However, this method can cause vaccination failure because of poor water quality. Therefore, analysis of water sources in a particular area is of paramount importance to determine the best source to prepare poultry vaccines.

In the present study the results of testing five water sources, namely artesian well, shallow well, tap water, surface water and bottled water revealed that turbidity and TSS showed great variability between sources and divided the five water sources into two main groups; the untreated water group includes artesian well, shallow well and surface water; the other is the treated water group which includes tap and bottled water. These two groups represent the different levels of turbidity and TSS mainly in surface water. High turbidity levels may be due to high concentrations of particulate matter and microorganisms and that also means increase in total suspended solids (sluis, 2002) mentioned that turbid water reduce the efficacy of vaccines, medicines and other water additives. Turbid water has great effect in reducing immune response by multi steps of inflammation and CMI processes. Results of TDS and conductivity showed highest level in well's water; shallow and artesian.

Blake and Hess (2001) related the increase of electrical conductivity to the increase of inorganic substances TDS which show high levels in ground water. On the other hand we found the lowest pH level in the artesian well water. (Morris, 2002) mentioned that well's water had a low pH level from 6.5-8.5 and this is an acceptable range for poultry performance.

The only chlorinated water source among the five water sources used in the present study is tap water. Chlorination is commonly used in row water to reduce bacterial contamination (Blake and Hess, 2001). Therefore, no chlorine was found in row water sources (artesian well, shallow well and surface water) as well as bottled water. Bottled water used ozone method to treat water against bacterial contamination. Although chlorinated water is hazardous to vaccines and can severely reduce the amount of vaccine virus presented to birds (Cargill, 2006); the chlorine level in tap water in Khartoum State determined in the present study was within the recommended levels. The general recommendations are to have a level of 0.2- 0.5 mg/L at the bird drinker (Blake and Hess, 2001).

The result of testing sera collected from chicks at day 1, 6 and 12 of age (first before vaccination) showed higher maternal antibodies titer of $\log_2 3.86$, 3.25 and 1.74 respectively. Accordingly, we selected chicks at day 14 of age for the vaccine trials. The LaSota NDV vaccine was prepared in different water sources and gave to five groups of chicks. Results of geo means HI titers were significantly better in group E which was vaccinated using bottled water as diluent. The Ab level started to rise from the first week after vaccination ($\log_2 5.05$) and it reached the peak on the second week ($\log_2 6.47$) then declined to reach ($\log_2 3.52$) at week five. All vaccinated groups showed highest HI titers on the second week

except group D which gave the weaker immune response that in contrast to the 2nd week in the rest of vaccinated groups.

Results of testing Ab levels in chicks vaccinated using bottled and tap water as diluents showed a similarity in immune response in the fourth week after vaccination but overall better immune response was detected when bottled water was used.

Antibody levels as detected by HI test was more than 3 $\log_2$ which is protective according to Spradbrow *et al*., (2002) in all vaccination groups except group D.

The present study revealed that bottled water is the best water source used to prepare drinking water vaccines in Khartoum followed by tap water. Shallow well, artesian well and surface water gave poor immune response and therefore, are not recommended to be used in preparation of poultry vaccines.

According to the present study the order of the five water sources in terms of resulting in better immune response could be bottled water, tap water, shallow well, artesian well and surface water.

CONCLUSIONS

1. Water sources commonly used to prepare poultry vaccines in Khartoum State varied greatly in their physical properties and quality.

2. High quality water represented by treated water (bottled and tap water) produce efficient and better immunity when used as diluent in comparison with row water sources represented by shallow well, artesian well and surface water.

RECOMMENDATIONS

1. Using of high quality water in drinking vaccine preparation produce better immune response in the absence of other factors that affect vaccine efficacy.

2. The best water sources used to prepare poultry vaccines are bottled water and tap water.

3. Surface water, artesian well and shallow well should not be used to prepare ND vaccines.

4. More studies should be done to examine water sources used to prepare poultry vaccines in different parts of the country other than Khartoum State.

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ملخص الأطروحة

أجريت هذه الدراسة لتحديد تأثيرالماء من مصادر مختلفة فى مزارع الدواجن بولاية الخرطوم علي قوة و فعالية لقاح النيوكاسل (لاسوتا) المذاب فى هذه المياه وذلك بأستخدام إختبار التلازن الدموي وتثبيط التلازن الدموي.

تم إختيار خمسة مصادر من المياه المستخدمة في مزارع الدواجن بولاية الخرطوم ، وهذه المصادر هى : بئر إرتوازية بمنطقة السرحة – محلية امدرمان ، بئرسطحية بمنطقة السقاي ، شبكة تنقية المياه بشمبات ، مياه سطحية من ترعة تتغذي من النيل بمنطقة الجريف ومياه معدنية تباع تجارياً.

أخذت عينة من الماء من كل مصدر وأجريت سبعة اختبارات علي هذه العينات لتحديد نوعية وجودة هذه المياه وهذه الإختبارات هي: العكر ، مجموع المواد الذائبة ،والأس الهيدروجيني ، القدرة علي التوصيل ، مجموع المواد المذيبة ، الكلورين و نسبة الملوحة. وأظهرت نتائج تحليل العكر إختلافات كبيرة بين أنواع المياه (بئر ارتوازية ، بئر سطحية ، مياه شبكة التنقية ، مياه الترعة والمياه المعدنية) على النحو التالى 4 ، 5 ، 0 ، 48 و 0 علي التوالي ، والمواد الذائبة علي النحو التالي: 148، 442، 120، 127، 96 ملجم/لترعلي التوالي. نتائج الأس الهيدروجيني كانت 6.6، 7.4، 7.21، 7.18، 7.32 علي التوالي ، ونتائج المواد المذيبة هي: 0.8 ، 1.4 ، 0، 32، 0 ملجم/لترعلي التوالي، اما نتائج القدرة علي التوصيل فكانت 207، 253، 253، 928، 359 علي التوالي ، بينما كانت نسبة الملوحة 0.1 وهي متساوية في كل العينات.

ولدراسة كفاءة اللقاح المذاب في المياه اعلاه اجريت تجربة علي كتاكيت بياض عمر يوم ، تم تقسيم الكتاكيت علي خمسه مجموعات بكل مجموعة عدد 40 كتكوت ، وفي عمر 14 يوم تم تطعيمهم بلقاح النيوكاسل (لاسوتا) المذاب في مياه بئر ارتوازية وسميت المجموعة (أ)، كما تم تطعيم المجموعة الثانية باللقاح المذاب في مياه بئر سطحية وسميت المجموعة (ب)، اما المجموعة (ج) فقد تم تطعيمها باستخدام اللقاح المذاب فى مياه الشبكة، وطعمت المجموعة (د) باللقاح المذاب في مياه الترعة ، اما الجموعة (هـ) فقد تم تطعيمها باللقاح المذاب فى المياه المعدنية.

بعد اسبوع من التطعيم تم جمع عينات السيرم من كل مجموعة واستمر اخذ السيرم اسبوعياً لمدة خمسة اسابيع وتم اجراء اختبار التلازن الدموي وتثبيط التلازن الدموي لتحديد مناعة كل مجموعة وبحساب معايير الاجسام المضادة بواسطة الوسط الهندسي اوضحت التجربة ان المجموعة (هـ) اعطت نتائج مناعية جيدة من الاسبوع الأول حتي الخامس علي النحو التالي: 5.05، 6.47، 4.39، 3.95، 3.52 علي التوالي؛ وكانت المجموعة (ج) ثاني افضل مجموعة بمعايير اسبوعية: 4.54، 6.38، 4.52، 3.95، 3.63 علي التوالي ، وكانت نتائج المجموعتين (أ) و (ب) متقاربة، بينما إختلفت نتائج المجموعة (د) عن نتائج بقية المجموعات وهي: 2.49، 3.95، 4.94، 3.14، 2.88 علي التوالي.

خلصت الدراسة بأن هنالك اختلافات كبيرة في نوعية وجودة المياه المستخدمة بمزارع الدواجن والتي بدورها تؤثر علي فعالية وكفاءة اللقاحات ؛ لذلك يمكن إستخدام المياه المعدنية أو مياه شبكة التنقية في تطعيم الدواجن بلقاح النيوكاسل بولاية الخرطوم