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Newcastle Disease in Ethiopia: A Review on Epidemiology, Diagnosis, Control and Other Methods

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Abstract: Livestock production is a major component of Ethiopian economy. Among livestock sector poultry production system is one that will assist in poverty alleviation and the improvement of food security in developing countries. Among viral disease, Newcastle disease (ND) is one of the most economically important disease which affect the poultry industry worldwide. The economic losses due to Newcastle disease are due to high mortality and morbidity and decreased production of eggs from layer and production of eggs of low quality from breeder flocks. The treatment cost along with extra management of poultry flocks during course of the disease also enhances the economic losses due to (ND). The Newcastle disease virus has capability to infect wide range of poultry birds including domestic, commercial layer, broiler and breeder flocks and is responsible for high morbidity and mortality. The ND is prevalent in domestic (rural) and commercial flocks and is responsible for heavy economic losses in developing countries like Ethiopia. Virulent strains of ND are avian paramyxovirus type1 (APMV-1) of the genus Avulavirus belonging to the family Paramyxoviridae. There are nine serotypes of avian paramyxoviruses designated APMV-I to APMV-9. The virus strains have three distinct pathotypes based on their virulence factor: lentogenic, mesogenic, or velogenic. The general approaches to the control of Newcastle disease are hygiene and vaccination.

Key words: Newcastle disease · Pathogenicity · Avulavirus · Poultry · Vaccination

INTRODUCTION

Livestock production is a major component of Ethiopian economy. In developing countries like Ethiopia, livestock production goes well beyond food production; sales of livestock and their products provide immediate cash income to farmers and foreign exchange for the countries [1]. Among livestock sector poultry production system is one that will assist in poverty alleviation and the improvement of food security in developing countries. Poultry production is important for food security and poverty alleviation in developing countries where it is mainly under the traditional small-scale system [2].

Most poultry improvement programs in developing countries including Ethiopia have been directed towards the introduction of specialized exotic breeds or cross breeding and management intensification. Genetic improvement could be achieved either through selection and controlled mating or by introducing exotic chickens [3].

There are many challenges to village chicken production including diseases, poor nutrition and predation [4]. The major constraint to productions of village chickens in many developing countries are bacterial and other viral disease, internal and external parasites.

Among viral disease, Newcastle diseases one of the most economically important disease which affect the poultry industry worldwide. In many tropical and subtropical countries, virulent strains of Newcastle disease virus (NDV) are endemic [5]. Strains of NDV are capable of causing 100% mortality in unprotected flocks. The disease is caused by a paramyxovirus. Outbreaks of ND are unpredictable and discourage villagers from paying proper attention to the husbandry and welfare of their chickens. NDV may persist in undispersed chicken faeces for more than six months but under village conditions the virus can survive outside the host for more than one month.

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Newcastle disease virus (NDV) classified into three distinct pathotypes based on their virulence factor: lentogenic, mesogenic and velogenic[6]. The most virulent (velogenic) isolates are further sub-divided into neurotropic and viscerotropic. All velogenic viruses are virulent but not all virulent strains are velogenic. Velogenic Newcastle disease virus is considered to be endemic in village chickens in most of the developing countries and epidemics of the disease result in great economic losses [7]. The disease is listed by the World Health Organization for Animals (OIE) as important for avian species. The detection of virus in a specific geographical location leads to trade restriction and embargos. Mesogenic and velogenic viruses found only in poultry species. Virulent strains, typically mesogens and velogens are endemic in the majority of Asia, Africa and the Middle East, as well as parts of Central and South America in domestic poultry species [6]. The objective of this review is to understand the Newcastle disease causative agent, pathogenicity, clinical sign and how to prevent and control the Newcastle disease, which concerned with the currently published or reported research. Recently, the disease which decreases the development of poultry production for industry is the infectious diseases, among infection disease Newcastle is the one which causes economical lose of poultry and its product.

Description of the Virus and Infection Kinetics: Newcastle disease virus (NDV) is an avian paramyxovirus serotype 1(APMV-1) viruses, which belongs to order Mononegavirales, family Paramyxoviridae, sub-family Paramyxovirinae, genus Avulavirus [8].NDV is a single-stranded, non-segmented, negative-sense, enveloped RNA virus which is capable of infecting more than 250 species of birds [9]. The family Paramyxoviridae is divided into two subfamilies Paramyxovirinae and Pneumovirinae [10]. The subfamily Paramyxovirinae has five genera: Rubulavirus, which include the mumps virus, mammalian para-influenza 2 and 4; Respirovirus containing mammalian para-influenza viruses 1 and 3; Morbillivirus, measles, distemper and rinderpest; Henipavirus, formed from the Nipah and Hendra viruses; and Avulavirus, formed from NDV and other avian paramyxoviruses [10]. Nine serogroups of avian paramyxoviruses have been recognized: APMV-1 to APMV-9 [11]. Among these, NDV (APMV-1) remains the most important pathogen for poultry, but APMV-2, APMV- 3, APMV-6 and APMV-7 are known to cause disease in poultry. Avian avulavirus strains are phylogenetically classified into class I and class II. Class II are further differentiated into separate genotypes based on genetic and geographic variations.

The genome of NDV contains six open reading frames (ORF) which encode the nucleoprotein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin-neuraminidase (HN) and the large protein (L) [12].It was shown that NDV virulence is dependent on the amino acid sequence at the cleavage site encoded by the F gene. Highly virulent strains of NDV can be discriminated from low virulent strains by the presence of a multibasic amino acid motif at the proteolytic cleavage site of the fusion (F) proteinNDV possesses two surface proteins that are important to the identification and behavior of the virus. Hemagglutinin/neuraminidase (HN) and fusion (F) protein. HN is important in the attachment, release of the virus from the host cells and serologic identification. Fusion (F) protein has a crucial role in the pathogenesis of the disease.

Transmission and Clinical Signs: The mode of transmission route of NDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, newly introduced birds, selling and giving away sick birds, egg to hatching chick and contacts with contaminated feed, water, equipment and clothing [13]. The spread of virus is usually attributed to the movement of chickens through chicken markets and traders. Newcastle disease is very contagious and is easily spread from one bird to another. ND can be transmitted from one place to another via people, vehicles, animals, baskets cages and infected products (egg shells, feathers, bones intestines, etc [14]. Infected birds shed virus in aerosol, respiratory discharge and faeces. Infected birds start to excrete virus during the incubations period and continue to excrete virus for a varying but limited time during convalescence [15]. The virus is relatively stable outside of a host and in the environment, thus making fomite transmission a possibility. Infectious virus has been found to survive 7 days in the summer, 14 days during the spring and 30 days in the winter in poultry houses that were contaminated by infected birds [16].

The incubation period of NDV is 2-15 days post exposure. Gallinaceous birds (chickens, turkeys, grouse, pheasants and partridges) shed infectious virus for up to 1-2 weeks following infection; however, psittacine birds (parrots, parakeets and macaws) have demonstrated the capacity to shed infectious virus for several months to

1 year following infection, primarily via respiratory secretions and feces [17] It is likely that the mortality rate for chicks born with virulent NDV infections would be very high, especially as maternal antibody levels waned.

The clinical signs in birds infected with NDV vary greatly from very high morbidity and mortality to asymptomatic carriers. The severity of an infection is dependent on factors like the virulence and tropism of the virus, host species, age of host, immune status, other diseases and environmental conditions [18]. The major clinical symptoms are gasping, coughing, sneezing and rales, tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhoea, depression and inappetence, partial or complete drop in egg production and an increased production of deformed eggs.

Clinical signs vary considerably based on the virulence and tropism of the NDV involved, the species of bird, the age of host, the immune status of the host and environmental conditions. Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhoea, depression and inappetence, partial or complete drop in egg production and an increased production of deformed eggs [18]. Strains of NDV have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens [19]. These are:

Viscerotropic Velogenic: Characterized by an acute lethal infection of chickens of all ages, Hemorrhagic lesions of the digestive tracts are frequently present. Obvious depression, inappetence, substantial drop in egg production, increased respiration, a profuse greenish-yellow diarrhoea that rapidly leads to dehydration and collapse, swollen heads and cyanotic combs. Mortality can be up to 90% and infected birds usually die within one or two days. Birds that survive the initial phase often develop nervous signs.

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

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

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

Asymptomatic Enteric: A form that usually consists of a subclinical enteric infection.

Epidemiology/Geographical Distribution

Occurrence: Newcastle disease has worldwide distribution events on a global scale in all poultry raising countries. Virulent Newcastle disease virus(VNDV) strains are endemic in many poultry raising countries of Asia, the middle EAST, Africa and Central and South America [20]. Other countries, including the USA and Canada, are free of those strains in poultry and maintain that status with import restrictions and eradication by destroying infected poultry [21]. Cormorants, pigeons and imported psittacine species are more commonly infected with VNDV and have also been sources of VNDV infections of poultry. NDV strains of low virulence are prevalent in poultry and wild birds, especially waterfowl. Infection of domestic poultry with lentogenic NDV contributes to lower productivity. NDV is infective for almost all avian species, both domestic and wild. Domestic Chickens/poultry are highly susceptible to infection with Newcastle disease virus, including the pigeon variant of APMV-1 [13]. NDV is heat stable when compared with most of paramyxovirus. It remain infectious in bone marrow and muscle of slaughtered chicken at least six month at -20°C and for up to four month in refrigerator temperature and also infectious virus may survive for months at room temperature in eggs laid by infected hens and for over year at 4°C [22].

Higher prevalence of ND is reported during dry season than wet season. Human activity and increased turnover in the chicken markets during dry season could leads to outbreaks of ND that have been attributed to high prevalence during dry season [23, 24]. As studies reported on Newcastle disease there is a higher significant difference in ND prevalence between local and cross breeds of chickens. Highest prevalence's are recorded in cross breeds of chickens than local breed [25]. The low altitudes do have higher prevalence than the mid and high [26]. The prevalence of ND varies among years in Ethiopia.

Pathogenicity: One of the most characteristic properties of different strains of ND virus has been their great variation in pathogenicity for chickens [27]. The pathaogenicity of NDV strains varies greatly with and/or within the host. Chickens are highly susceptible but Turkeys, ducks and geese may be infected and show few or no clinical signs. In chickens, the pathogenicity of NDV is determined by the Strain of the virus, the dose and route of administration, age of the chicken and environmental conditions. In general, the younger the chicken, the more acute disease which may experience sudden deaths without major clinical signs. Older diseased birds may be more protracted and with characteristic clinical signs. Breed or genetic stock appears to have very little effect on the susceptibility of chickens to the disease [28].

Diagnosis: A wide variety of diagnostic tools have been developed to determine pathogen identity. These methods are often used serially [6].As signs of clinical disease in chickens vary widely and diagnosis may be complicated further by the different responses to infection by different hosts, clinical signs alone do not present a reliable basis for diagnosis of ND. However, the characteristic signs and lesions associated with the virulent pathotypes will give rise to strong suspicion of the disease. Samples collected for virus isolations are from recently dead birds or moribund birds that have been killed humanely. Samples from dead birds should consist of oro-nasal swabs, lung, kidneys, intestine (including contents), spleen, brain, liver and heart tissues [19]. Samples from live birds should include both tracheal or oropharyngeal and cloacal swabs. Samples should be taken in the early stages of the disease.

Pathogenicity Index: The extreme variation in virulence of different NDV isolates and the widespread use of live vaccines means that the identification of an isolate as APMV-1 from birds showing clinical signs does not confirm a diagnosis of ND [19]. Mean death time (MDT) is a frequently used diagnostic which involves inoculating samples into embryonated eggs and determining the time in hours required to kill the chicken embryo [29]. The intracerebral pathogenicity index in 1-day-old chicks (ICPI) and the intravenous pathogenicity index in 6-week-old chickens (IVPI) involve the weighted scoring of clinical signs following an intracerebral or intravenous injection, respectively. ICPI is the test of choice for NDV whereas IVPI is used only occasionally and never for official purposes. Samples collected from sick and dying birds can be inoculated into embryonated eggs and further differentiation can be undertaken [9].

Although conventional diagnosis has proven adequate for the control of ND in the past, it does present some problems. These tests are labors intensive, challenges associated with obtaining SPF eggs, day-old chicksand time consuming [30]. In addition, the use of animals in this way is becoming increasingly unacceptable with the development of actual or potential alternatives [31].Various diagnostic methods like haemagglutination inhibition (HI) test, indirect haemagglutination (IHA) test, virus neutralization test (VNT), enzyme linked immunosorbent assay (ELISA), fluorescent antibody technique (FAT), plaque reduction neutralization test (PRNT) and agar gel immunodiffusion test (AGIDT) are used limitedly to detect NDV. Molecular techniques like reverse transcriptase polymerase chain reaction (RT-PCR) have been frequently used all over the world to detect viruses from the field samples. Hemagglutinin inhibition (HI) assay or an enzyme-linked immunosorbent assay (ELISA) can be used to evaluate the presence of NDV specific antibodies and antigen in the host [19] Serological diagnostics do not allow for the differentiation of antibodies resultant from exposure to a vaccine strain as compared to a virulent NDV isolate; therefore, they are not tremendously efficacious for the determination of NDV as the cause of a specific outbreak because of rampant vaccination. Now RT-PCR is the most exclusively used method to detect AIVs and NDVs. RT-PCR assay is more sensitive, specific and less labor intensives as compare to other conventional methods[30].

Isolation and Identification of Agent

Virus Isolation of NDV: Sampling from live birds of any species for virus isolation should consist of both cloacal swabs (faeces) and tracheal swabs, regardless of the clinical signs. From dead birds intestines, intestinal contents and tracheas should be sampled, together with organs and tissues obviously affected or associated with the clinical signs, example the brain if nervous signs are present [19].

Samples should be placed in phosphate buffered isotonic saline containing antibiotics at PH 7.0-7.4 (checked after the addition of antibiotics); faeces and minced tissues as 10-20% W/V suspensions. The exact mixture of antibiotics does not appear to be critical and may be varied to meet local conditions. However, high concentrations are usually necessary, especially for faeces and cloacal swabs [14].

The specific pathogen free viable 10 day-old embryonated chicken eggs are inoculated through allantoic cavity route for NDV. The embryos died after 24 hrs of incubation chilled at 4°C for 1- 2 hours. Allantoic fluid is collected and tested by slide HA test. Those manifesting HA-positive (clumping of RBC) is collected, as a source of ND virus.

Culture System: Virulent ND viruses can be propagated in many cell culture systems and viruses of low virulence can be induced to replicate in some of them. It is possible to use primary cell cultures or even cell lines for routine isolation of NDV. The embryonated chicken egg, however, represents an extremely sensitive and convenient vehicle for the propagation of NDV and is used almost universally in diagnosis. Embryonated chicken eggs should be obtained from a specific pathogen free (SPF) flock and incubated for 9-10 days at 37°C before use. If SPF eggs cannot be obtained, eggs from a flock free of NDV antibodies should be used. NDV strains in eggs containing yolk antibodies can be propagated, but the virus titer is usually greatly reduced and such eggs should be avoided for diagnostic use.

Serological Methods: Antibodies to NDV may be detected in poultry sera by a variety of tests including single radial immune diffusion, single radial hemolysis, Hemagglutination, hemagglutination Inhibition (HI) test, agar gel precipitin, VN in chick embryos and plaque neutralization [13]. HA activity detected in bacteriologically sterile fluids harvested from inoculated eggs may be due to the presence of any of the ten subtypes of APMV (including NDV) or 16 haemagglutinin subtypes of influenza A viruses or Nonsterile fluid could contain bacterial HA. At present, the HI test is most widely used for detecting antibodies to APMV-1 in birds while the use of commercial ELISA kits to assess post-vaccination antibody levels is common. Usually chicken antiserum that has been prepared against one of the strains of NDV is used [19].

Haemagglutination and Haemagglutination Inhibition Test: Chicken sera rarely give nonspecific positive reactions in the HI test and any pretreatment of the sera is unnecessary. Sera from species other than chickens may sometimes cause agglutination of chicken red blood cells (RBCs), so this property should first be determined and then removed by adsorption of the serum with chicken RBCs. This is done by adding 0.025 ml of packed chicken RBCs to each 0.5 ml of antisera, shaking gently and leaving for at least 30 minutes; the RBCs are then pelleted by centrifugation at 800 *g* for 2-5 minutes and the adsorbed sera are decanted.

Enzyme Linked Immunosorbent Assay (ELISA): There are a variety of commercial ELISA kits available and these are based on several different strategies for the detection of NDV antibodies, including indirect, sandwich and blocking or competitive ELISAs using MAbs. The HI test and ELISA may measure antibodies to different antigens; depending on the system used ELISAs may detect antibodies to more than one antigen while the HI test is probably restricted to those directed against the HN protein. However, comparative studies have demonstrated that the ELISAs are reproducible and have high sensitivity and specificity; they have been found to correlate well with the HI test [32]. Conventional ELISAs have the disadvantage that it is necessary to validate the test for each species of bird for which they are used. Competitive ELISAs may not recognise all strains of APMV-1 if they use MAb known for their specificity for single epitopes.

Differential Diagnosis of Newcastle Disease: Differential diagnosis is the process of differentiating Newcastle disease with other disease which share similar signs or symptoms. The disease which have similar clinical sign with Newcastle disease are as follow: Fowl cholera, highly pathogenic avian influenza, Laryngotracheitis, Fowl pox (diphtheritic form), Psittacosis (psittacinebirds), Mycoplasmosis, Infectious bronchitis, Aspergillosis, Also management errors such as deprivation of water, lack of or nutritionally deficient feed and poor ventilation. Some important thumb rules to differentiate the two in an ordinary laboratory are: The avian influenza virus canshaemagglutinate rabbit erythrocytes, whereas ND virus does not. Avian influenza virus does not produce disease in pigeons, whereas ND virus can. It is also differentiated from infectious laryngotrachites, fowl cholera and coryza [20].

VACCINE: Vaccines are being used to control and prevent ND. Currently, many inactivated and live ND vaccines are available around the world [33; 34]. There are three types of commercially available vaccines for ND: Live lentogenic, live mesogenic and inactivated [35]. Chickens and turkeys are immunized against New-castle disease. Live virus vaccines are administered by variety

of routes and schedules from hatching till grow-out. Killed virus oil emulsion vaccines are administered parentally prior to the onset of egg production. The effectiveness of ND vaccines in the control of the disease depends on the virulence of the field strain, the type and state of the vaccine, the immunological state of the birds and the method of the vaccine application [36].

NDV vaccine is usually given at 10 and 35 days of age and repeated every three months. The commonly used vaccines in Ethiopia are hatchiner's B1 and lasota, which, are produced at the National veterinary institute (NVI), Debrezeit [28].

Prevention and Control The general approaches to the control of Newcastle disease are hygiene and vaccination, especially in the control of ND in semi-intensive systems where birds are confined within a fenced yard or house. Hygienic measureincludes such as cleaning, disinfection, limiting access to wild birds/biosecurity, minimizing stress on birds, quarantine, vector control, proper management and personal hygiene of the farm staff [37]. Vaccination in combination with appropriate hygiene measures remains the most effective way of controlling ND. Vaccination against VND would result in immunity against infection and replication of the virus. ND vaccination usually protects the bird from the more serious consequences of disease, but virus replication and shedding may still occur [38].

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