NEWCASTLE DISEASE STANDARD OPERATING PROCEDURES:
1. OVERVIEW OF ETIOLOGY AND ECOLOGY

FAD PReP

Foreign Animal Disease Preparedness & Response Plan

United States Department of Agriculture

United States Department of Agriculture • Animal and Plant Health Inspection Service • Veterinary Services

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The Foreign Animal Disease Preparedness and Response Plan (FAD PReP) Standard Operating Procedures (SOPs) provide operational guidance for responding to an animal health emergency in the United States.

These draft SOPs are under ongoing review. This document was last updated in **December 2013.** Please send questions or comments to:

National Preparedness and Incident Coordination
Veterinary Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 41
Riverdale, Maryland 20737
Telephone: (301) 851-3595 Fax: (301) 734-7817
E-mail: FAD.PReP.Comments@aphis.usda.gov

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Disease

Virulent Newcastle disease virus, in domestic poultry, is referred to in the United States as Newcastle Disease (ND).

Mortality and Morbidity

In domestic poultry, ND consistently causes high mortality and morbidity rates approaching 100% in unvaccinated flocks. Surviving birds may suffer permanent neurological damage such as twisted necks or head tremors.

Susceptible Species

All avian species have demonstrated some level of susceptibility. Humans are also susceptible to ND.

Zoonotic Potential?

Yes, but not a significant threat to public health. In humans, conjunctivitis is the most often clinical sign of disease observed.

Reservoir

Chickens, cormorants, pigeon and other migratory waterfowl are considered natural reservoirs of all pathogenicity levels of ND (domestic poultry populations within the U.S. are considered free of virulent isolates).

Transmission

Direct exposure to infected birds, excrement, respiratory or other secretions.

Persistence in the Environment

Viable in ambient environments for longer periods; may survive indefinitely in a frozen environment.

Animal Products and By-Products

Virus can persist in eggs and poultry meat products.

1.1 Introduction

Newcastle disease (ND) is an infectious viral disease that affects at least 250 species of birds in 27 orders.[1] There are many different clinical signs of ND; signs expressed depend on the virulence of the virus strain, in addition to the susceptibility of the bird species and the immunity of the bird infected. Newcastle disease virus (NDV) is commonly classified according to disease severity with strains being defined as virulent (vNDV) or of low virulence (loNDV). Only infections of NDV (see Section 1.3.5.1) meeting the World Organization for Animal Health (OIE) definitions are reportable to the OIE.

Prior to the ability to easily sequence NDV strains, it was common to define strains by the clinical signs observed in chickens upon infection. The three pathotypes are: lentogenic (low virulence), mesogenic, and velogenic (both virulent). Virulent NDV is further classified as neurotropic and viscerotropic. The neurotropic subtype is commonly associated with neurologic and respiratory symptoms; and the viscerotropic subtype is commonly associated with hemorrhagic lesions in the intestinal tract, but may also cause neurological signs.

Newcastle Disease (ND), as examined here, refers to infections in poultry species with virulent NDV (vNDV). There is currently no evidence of ND circulation in domestic poultry flocks in the United States.

Morbidity and mortality rates vary according to order and species, affecting domestic poultry most acutely. While ND is zoonotic, the transference to humans typically manifests as conjunctivitis, rarely progressing to mild, influenza-like symptoms.

1.1.1 Further Information

This document is intended to be an overview, focusing on vNDV in domestic poultry, which in the United States is referred to as ND. Additional resources on ND are listed in Attachment 1.A. In addition to this document, information can also be found in the *ND Response Plan: The Red Book* and the vNDV Case Definition. These documents are available on the APHIS public website (http://www.aphis.usda.gov/animal_health/emergency_management) or on the Animal and Plant Health Inspection Service (APHIS) Intranet (http://inside.aphis.usda.gov/vs/em/fadprep.shtml, for APHIS employees.

1.1.2 Goals

As a preparedness goal, APHIS will provide etiology and ecology summaries for ND, and update these summaries at regular intervals.

As a response goal, the Unified Command and stakeholders will have a common set of etiology and ecology definitions and descriptions, to ensure proper understanding of ND when establishing or revising goals, objectives, strategies, and procedures.

[1] Center for Food Security and Public Health (CFSPH), Iowa State University & Institute for International Cooperation in Animal Biologics. 2008. "Newcastle Disease." http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=newcastle-disease&lang=en.

1.2 Purpose

The purpose of this document is to provide responders and stakeholders with a common understanding of the disease agent.

1.3 Etiology

1.3.1 Name

The virus responsible for causing ND is "a member of the family Paramyxoviridae in the genus Avulavirus."[2] NDV is also known as avian paramyxovirus, of serotype 1 (APMV-1). Only infections with the virulent APMV-1 (or vNDV) are responsible for ND. Strains of NDV that are of low virulence (loNDV) are often used as vaccines to prevent disease and death from ND.

1.3.2 Virus Characteristics

According to the International Committee on Taxonomy of Viruses (ICTV), this disease has the following characteristics:

- Family: Paramyxoviridae
- Genus: Avulavirus
- Serotypes: One
- Classes: Two
- Genotypes: At least nine identified; classification list is growing
- Isolates/Strains: Hundreds positively identified; classification list is growing
    - Throughout the world, novel strains of the virus are identified as affecting species previously thought to be resistant.[3]

1.3.3 Morphology

According to ICTV, ND virions measure approximately 150–200 nanometers (nm) in diameter, and 1,000–10,000 nm in length. Surface projections on the envelope are spaced widely apart, and haemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins are equally distributed on the surface, embedded in a lipid bilayer. This lipid bilayer is comprised of the HN and the F glycoproteins. The capsid/nucleocapsid is elongated with a helical symmetry; the nucleocapsid is not segmented.[4]

1.3.4 Genus Characteristics

This genus has the following characteristics:

[2] World Organization for Animal Health (OIE). 2009. OIE Technical Disease Card. Newcastle Disease. http://www.oie.int/.

[3] Center for Food Security and Public Health (CFSPH), Iowa State University & Institute for International Cooperation in Animal Biologics. 2008. "Newcastle Disease Fact Sheet." http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=newcastle-disease&lang=en.

[4] International Committee on Taxonomy of Viruses (ICTV). 2011. ICTV Database. http://www.ictvdb.org/ICTVdB/index.htm.

- Two surface proteins are important to the identification and behavior of the virus: HN and the F protein.
- HN is "important in the attachment and release of the virus from the host cells in addition to its serologic identification."[5]
- The F protein is vital to the virulence and pathogenesis of the disease.

1.3.5 ND Virus Types

There are three major pathotypes based on virulence: lentogenic (categorized as low virulence viruses), mesogenic and velogenic (virulent viruses). The virulent viruses are further classified as either neurotropic or viscerotropic. Even under controlled laboratory conditions, these pathotype groupings may not be obvious; low virulence and virulent, based on diagnostics, is a more appropriate way to classify NDV. Only NDV with virulent fusion cleavage sites fit the U.S. definition of ND.

Subtypes are classified into strains, which are described by a number of characteristics, including type, host, place of first isolation, strain number, year of isolation, and antigenic subtype.

1.3.5.1 NDV: OIE Identification

The OIE defines vNDV as the following:

> For the purposes of the *Terrestrial Code*, Newcastle disease (ND) is defined as an infection of poultry caused by a virus (NDV) of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence:
>
> a. The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks (*gallus gallus*) of 0.7 or greater; or
>
> b. Multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term 'multiple basic amino acids' refers to at least three arginine or lysine residues between residues 113 and 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterization of the isolated virus by an ICPI test.
>
> In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene, 113–116 corresponds to residues -4 to -1 from the cleavage site.[6]

1.3.5.2 NDV: U.S. Identification

The United States defines ND as the following:

> Newcastle disease is an acute, rapidly spreading, and usually fatal viral infection of poultry

[5] United States Animal Health Association (USAHA). 2008. Foreign Animal Diseases. 7th ed. Boca Raton, FL: Boca Publications Group.

[6] World Organization for Animal Health (OIE). 2013. Terrestrial Animal Health Code. Chapter 10.9. Newcastle Disease. http://www.oie.int.

caused by an avian paramyxovirus serotype 1 that meets one of the following criteria for virulence: The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks (*Gallus gallus)* of 0.7 or greater; or multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term "multiple basic amino acids" refers to at least three arginine or lysine residues between residues 113 and 116. In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene; 113-116 corresponds to residues -4 to -1 from the cleavage site. Failure to demonstrate the characteristic pattern of amino acid residues as described above may require characterization of the isolated virus by an ICPI test. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.[7]

1.4 Ecology

1.4.1 Susceptible Species

Evidence suggests all avian species are susceptible to infection with ND viruses, including:

- Chickens
- Turkeys
- Cormorants
- Migratory waterfowl
- Parrots
- Pigeons
- A wide variety of other birds, including shorebirds and penguins.

ND is particularly threatening to domestic poultry. Under non-experimental conditions, there is no evidence to suggest ND affects mammalian hosts, with humans being the sole exception.

1.4.2 Reservoirs

NDV, particularly the loNDV, is frequently isolated from free-living wild birds. Migratory waterfowl and Charadriiformes (shorebirds) can be infected with loNDV and vNDV. It is possible for them to be infected with and shed NDV in saliva and feces without showing any signs of illness. NDV strains are found worldwide and have been isolated from over 250 species.[8] The relative significance of different wild bird reservoirs is unknown, however, some evidence suggest that some bird species such as cormorants and pigeons may represent a major potential source of infection for domestic birds, particularly poultry. There is no evidence of ND generation in a reservoir.

[7] From 9 CFR 82.1.

[8] Center for Food Security and Public Health (CFSPH), Iowa State University & Institute for International Cooperation in Animal Biologics. 2008. "Newcastle Disease Fact Sheet." http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=newcastle-disease&lang=en.

1.4.3 Distribution

Endemic vNDV is present in much of the world, including Asia, Africa, Central and South America, and regions of Mexico. An outbreak of vNDV in the United States has not occurred since 2003.[9, 10]

1.4.4 Introduction and Transmission of ND Virus

Contact with infected domestic birds, and occasionally wild birds or wild waterfowl, is the most likely mode of introduction of ND into a poultry population. Many species of migratory waterfowl carry the loNDV sub-clinically, which makes it difficult to track. As many wild birds are migratory, they are able to spread the disease and make containment difficult. In addition, upon transmission to domestic poultry, a loNDV may mutate into a vNDV. However, only two documented outbreaks worldwide have been caused by loNDV that originated from wild birds and mutated into vNDV as the virus circulated in poultry.[11,12]

Because of the ND reservoir in wild birds and the ability of this virus to mutate, minimizing contact between domestic and wild birds is fundamental to preventing ND infection in the U.S. domestic poultry population. Live-bird markets are considered another method by which sub-clinically infected birds may transmit the virus to susceptible birds, who then act as carriers of NDV; these carriers may then, in turn, return to their originating farms and transmit the virus.

A vNDV is usually transmitted to domestic birds through direct contact with feces, feathers, eggshells, or respiratory secretions from infected domestic birds. Transmission of the virus can also result from contact with birds smuggled from areas that have vNDV endemic in the environment, as is seen in Mexico.[13] Also, the movement of contaminated fomites, people, clothing, boxes, equipment, egg trays, and vehicles increases the potential for susceptible birds to be exposed to vNDV.

Vaccination against ND has been used within the U.S. for a number of years.[14] There is evidence that immunization may help mitigate infection within contained bird populations; however, this offers no guarantee against infection, morbidity, and mortality within the domestic poultry population at large. Study of the actual benefits and results of herd immunity remains active research.[15]

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[9] Pedersen, J.C., D.A. Senne, P.R. Woolcock, H. Kinde, D.J. King, M.G. Wise, B. Panigrahy, and B.S. Seal. 2004. "Phylogenetic Relationships among Virulent Newcastle Disease Virus Isolates from the 2002-2003 Outbreak in California and Other Recent Outbreaks in North America." *Journal of Clinical Microbiology*, 42(5), 2329–2334.

[10] USDA-APHIS-VS-CEAH National Surveillance Unit; Draft Case Definition for Virulent Newcastle Disease Virus, 2011.

[11] Alexander, D.J., Campbell, G., Manvell, R.J., Collins, M.S., Parsons, G., and McNulty, M.S. 1992. "Characterisation of an antigenically unusual virus responsible for two outbreaks of Newcastle disease in the Republic of Ireland in 1990." *Vet Record,* 130(4), 65-68.

[12] Gould, A.R., Kattenbelt, J.A., Selleck, P., Hansson, E., Della-Porta, A.,Westbury, H.A. 2001. "Virulent Newcastle disease in Australia: molecular epidemiological analysis of viruses isolated prior to and during the outbreaks of 1998-2000" *Virus Research*, 77(1), 51-60.

[13] Perozo, F., Merino, R., Afonso, C.L., Villegas, P., Calderon, N. 2008. "Biological and phylogenetic characterization of virulent Newcastle disease virus circulating in Mexico." *Avian Diseases,* 52(3), 472-9.

[14] Hitchner, S.B. 2004. "History of Biological Control of Poultry Diseases in the U.S.A." Avian Diseases. 48(1), 1-8.

[15] Van Boven, M., Bouma, A., Fabri, T.H.F., Katsma, E. Hartog, L., Koch, G. 2008. "Herd Immunity to Newcastle Disease Virus in Poultry by Vaccination." *Avian Pathology,* 37(1), 1-5.

1.4.5 Incubation and Infectious Periods

Incubation periods for ND are variable depending on the host species. The OIE *Terrestrial Animal Health Code* (2013) gives the incubation period for ND as 21 days.[16] ND incubation periods vary depending on the strain of the virus; age, health, and species of infected birds; and other environmental factors. Most commonly, after natural exposure the period can be from two to five days with the average being five to six days.[17]

It is possible for a bird to shed the virus before and after the appearance of clinical signs. Depending on the species of bird, virus shedding can last anywhere from one week to a year.

1.4.6 Morbidity and Mortality in Birds

ND affects many species of birds, though the rates of morbidity and mortality and the variety and appearance of clinical symptoms vary from species to species. In domestic poultry, ND causes high morbidity and mortality rates, approaching 100 percent in unvaccinated flocks. Raptors are usually somewhat resistant, while ducks, waterfowl, parrots, and geese infected with ND may not show clinical signs. In many cases, the first sign of ND in a flock is abnormal and sudden mortality. In vaccinated populations, such as layers with high antibody titers, a decrease in egg production may be seen three to four weeks after infection, and misshapen and lighter in color than normal eggs may be produced (Figure 1-1).[18] Lower morbidity and mortality can be seen in vaccinated flocks. Well-vaccinated birds still can be infected with a vNDV, but may not show clinical signs of the disease while shedding the virulent virus in saliva and feces. The rate of death will depend on the immunity of the birds and the virulence of the NDV isolate. Surviving birds often suffer permanent neurological damage, such as twisted necks or head tremors.

Figure 1-1. Misshapen Eggs

Source: Dr. P. Miller

[16] World Organization for Animal Health (OIE). 2013. Terrestrial Animal Health Code. Chapter 10.9. Newcastle Disease. http://www.oie.int.

[17] Alexander, D.J. and D.A. Senne. 2008. Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In Diseases of Poultry, 12th ed. Y.M. Saif. et al. (ed.). Blackwell Publishing, Ames, Iowa.

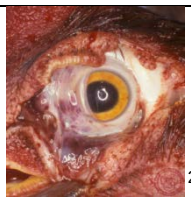
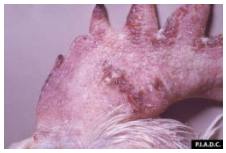
[18] Miller, P.J., Decanini, E.L., Afonso, C.L. 2010. "Newcastle Disease: Evolution of genotypes and the related diagnostic challenges. Infection, Genetics, and Evolution." *Infection, Genetics, and Evolution* ,10 (1), 26-35. http://ddr.nal.usda.gov/bitstream/10113/44041/1/IND44408321.pdf.

1.4.6.1 Clinical Signs

Infection in birds can give rise to a wide variety of clinical signs that may vary according to the host, strain of virus, host immune status, presence of any secondary exacerbating organisms, and environmental conditions.

Table 1-1 gives a description of some of the most basic clinical symptoms; visuals are supplied where applicable.

Table 1-1. Physical Symptoms of Poultry with ND

Subtype	Symptom description	Visual
General, regardless of diagnosed subtype	- apathy, depression (at right) - lack of movement - lack of appetite - reduced egg production - green or watery stool - respiratory difficulty (i.e. coughing and gasping) - conjunctivitis (at right)	 [19]
Viscerotropic	- internal and external hemorrhages (seen on comb at right) - swelling of the head and neck	 [20]  [21]
Neurotropic	- partial wing paralysis (may also see with mesogenic strains) - involuntary muscle tremors (at right) - stiff or twisted head or neck	 [22]

1.4.7 ND in Humans

ND is a zoonotic disease, though not one that poses a significant threat to public health. Human infection via exposure to infected birds can cause mild conjunctivitis and influenza-like

[19] Cornell University College of Veterinary Medicine: http://partnersah.vet.cornell.edu/avian-atlas/taxonomy/term/562.

[20] Center for Food Security and Public Health (CFSPH), Iowa State University & Institute for International Cooperation in Animal Biologics. 2008. "Newcastle Disease Fact Sheet." http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=newcastle-disease&lang=en.

[21] Center for Food Security and Public Health (CFSPH), Iowa State University & Institute for International Cooperation in Animal Biologics. 2008. "Newcastle Disease Fact Sheet." http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=newcastle-disease&lang=en.

[22] Cornell University College of Veterinary Medicine: http://partnersah.vet.cornell.edu/avian-atlas/taxonomy/term/562.

symptoms, and in severe cases, can lead to some lasting impairment of vision.[23] Individuals most likely to become infected are those working in the poultry industry or in laboratories; evidence has linked past human ND infection with lack of correct eyewear while working with commercial poultry. Although there is no evidence to indicate that the virus is contagious from one human to another, responder groups and vaccination crews should comply with the appropriate biosecurity and safety measures, including the use of personal protective equipment (PPE). Transmission of ND through the consumption of properly cooked poultry products has not been reported. Immuno-suppressed individuals are urged to take extra care to avoid exposure.[24]

1.5 Environmental Persistence of ND Viruses

While the "epidemiology of APMV-1 is incompletely understood,"[25] substantive information exists regarding its environmental persistence. ND can survive "for several weeks in a warm and humid environment... [and] indefinitely in frozen material."[26] When using agents to inactivate virus, it is critical that the manufacturer's directions for the correct concentration of the solution, and the time needed for complete inactivation to occur, be followed. In addition, appropriate PPE (goggles, glove, and respirator) should be used.

- *Temperature*: ND is "inactivated by being heated at 56°C (132.8°F) for 3 hours, or 60°C (140°F) for 30 minutes."[27]
- *pH*: Inactivated by acidic pH of ≤ 2.
- *Chemicals*: "Ether sensitive; inactivated by formalin, phenolics and oxidising agents (e.g. Virkon®); chlorhexidine, sodium hypochlorite (6%)."[28]
- *Disinfectants*: Multi-purpose disinfectants, such as Virkon®, ether, or formalin will inactivate ND virus particulate.[29]
- *Survival*: Survival of the aerosolized virus and long distance transmission are still subject to further study; aerosolized survival is likely dependent on humidity and a number of other environmental factors.[30] Other reports indicate that the virus is destroyed by dehydration and exposure to ultraviolet rays.[31]

[23] Swayne, D.E. and King, D.J. 2003. "Avian Influenza and Newcastle Disease." *JAVMA,* 222(11), 1534-1540.

[24] Goebel, S.J., Taylor, J., Barr, B.C., Kiehn, T.E., Castro-Malaspina, H.R., Hedvat, C.V., Rush-Wilson, K.A., Kelly, C.D., Davis, S.W., Samsonoff, W.A., Hurst, K.R., Behr, M.J., Masters, P.S. 2007. "Isolation of avian paramyxovirus 1 from a patient with a lethal case of pneumonia." *J Virol,* 81(22),12709-14. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2168997/.

[25] World Organization for Animal Health (OIE). 2009. OIE Technical Disease Card. Newcastle Disease. http://www.oie.int.

[26] USDA-APHIS-VS-CEAH National Surveillance Unit; Draft Case Definition for Virulent Newcastle Disease Virus, 2011.

[27] World Organization for Animal Health (OIE). 2009. OIE Technical Disease Card. Newcastle Disease. http://www.oie.int.

[28] USDA-APHIS-VS-CEAH National Surveillance Unit; Draft Case Definition for Virulent Newcastle Disease Virus, 2011.

[29] USDA-APHIS-VS-CEAH National Surveillance Unit; Draft Case Definition for Virulent Newcastle Disease Virus, 2011.

[30] Center for Food Security and Public Health (CFSPH), Iowa State University & Institute for International Cooperation in Animal Biologics. 2008. "Newcastle Disease Fact Sheet." http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=newcastle-disease&lang=en.

[31] USDA-APHIS-VS-CEAH National Surveillance Unit; Draft Case Definition for Virulent Newcastle Disease Virus, 2011.

1.5.1 In Eggs and Egg Products

The OIE recommends the following times and temperatures for the inactivation of ND virus in eggs and egg products (Table 1-2). [32]

Table 1-2. Inactivation of ND in Eggs and Egg Products

Type of product	Core temperature (°C)	Time
Whole egg	55.0	2,521 seconds
Whole egg	57.0	1,596 seconds
Whole egg	59.0	674 seconds
Liquid egg white	55.0	2,278 seconds
Liquid egg white	57.0	986 seconds
Liquid egg white	59.0	301 seconds
10% salted yolk	55.0	176 seconds
Dried egg white	57.0	50.4 hours

Source: OIE Terrestrial Animal Health Code, 2013.

The OIE *Terrestrial Animal Health Code (2013)* states that the listed temperature achieves a 7-log kill. Where scientifically documented, variances from these times and temperatures may also be suitable when they achieve the inactivation of the virus.

1.5.2 In Meat

The OIE recommends the following times and temperatures for inactivation of ND virus in meat (Table 1-3). Where scientifically documented, variances from these times and temperatures may also be suitable when they achieve the inactivation of the virus.[33]

Table 1-3. Inactivation of ND in Meat

Product	Core temperature (°C)	Time
Poultry meat	65.0	39.8 seconds
	70.0	3.6 seconds
	74.0	0.5 seconds
	80.0	0.03 seconds

Source: OIE Terrestrial Animal Health Code, 2013.

[32] World Organization for Animal Health (OIE). 2013. Terrestrial Animal Health Code. Chapter 10.9. Newcastle Disease. http://www.oie.int.

[33] World Organization for Animal Health (OIE). 2013. Terrestrial Animal Health Code. Chapter 10.9. Newcastle Disease. http://www.oie.int.

1.5.3 In Carcasses

ND viruses can survive in bird carcasses for several days at ambient temperatures and a few weeks at refrigeration temperatures. Titers in carcasses will vary depending on the strain of the virus, species of bird, and time of death in relation to clinical stage of infection. Burying, incineration, and composting are alternatives for disposal.[34]

[34] World Organization for Animal Health (OIE). 2009. OIE Technical Disease Card. Newcastle Disease. http://www.oie.int.

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Attachment 1.B Abbreviations

AMPV-1	avian paramyxovirus, type 1
APHIS	Animal and Plant Health Inspection Service
CFSPH	Center for Food Security and Public Health
F	fusion
FAD PReP	Foreign Animal Disease Preparedness and Response Plan
HN	Hemagglutinin-neuraminidase
ICPI	intracerebral pathogenicity index
ICTV	International Committee on Taxonomy of Viruses
loNDV	NDV of low virulence
ND	Newcastle Disease
NDV	Newcastle Disease virus
nm	nanometers
OIE	World Organization for Animal Health
PPE	personal protective equipment
rRT-PCR	real-time reverse transcriptase polymerase chain reaction
USDA	United States Department of Agriculture
vNDV	virulent Newcastle disease virus