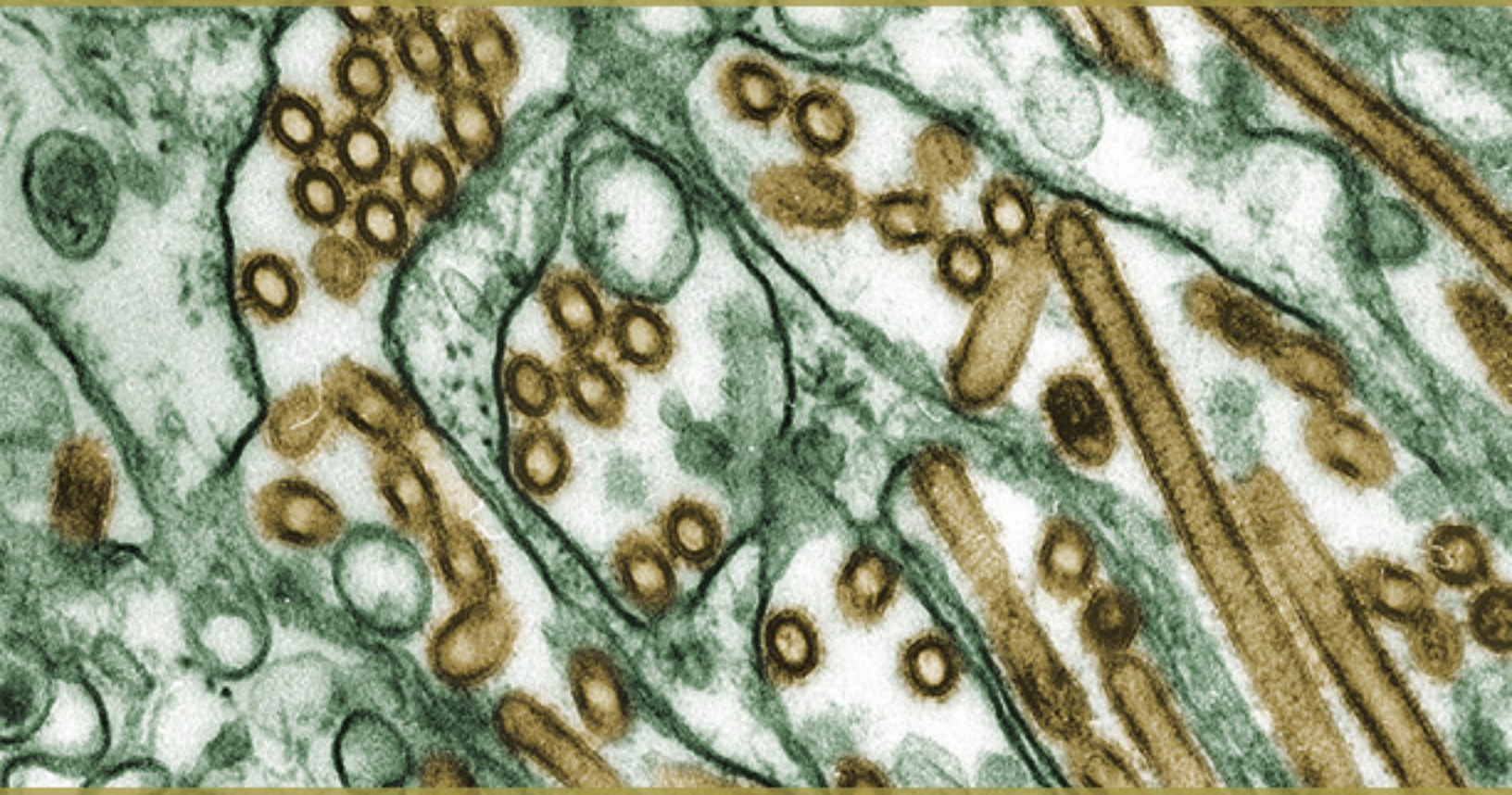
Animal Virology



Roxanne Rossi

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Chapter 1

Animal Virology

The study of animal viruses is important from a veterinary viewpoint and many of these viruses cause diseases that are economically devastating. Many animal viruses are also important from a human medical perspective. The emergence of the SARS virus in the human population, coming from an animal source, highlights the importance of animals in bearing infectious agents; avian influenza viruses can directly infect humans. In addition research into animal viruses has made an important contribution to our understanding of viruses in general, their replication, molecular biology, evolution and interaction with the host.

Foot-and-Mouth Disease Virus

Foot-and-mouth disease virus (FMDV) is the prototypic member of the Aphthovirus genus in the Picornaviridae family. This picornavirus is the etiological agent of an acute systemic vesicular disease that affects cattle worldwide, foot-and-mouth disease. FMDV is a highly variable and transmissible virus. It enters the body through inhalation. Soon after infection, the single stranded positive RNA that constitutes the viral genome is efficiently translated using a cap-independent mechanism driven by the internal ribosome entry site element (IRES). This process occurs concomitantly with the inhibition of cellular protein synthesis, caused by the expression of viral proteases. Processing of the viral polyprotein is achieved cotranslationally by viral encoded proteases, giving rise to the different mature viral proteins. Viral RNA as well as viral proteins interact with different components of the host cell, acting as key determinants of viral pathogenesis. In depth knowledge of the molecular basis of the viral cycle is needed to control viral pathogenesis and disease spreading.

Pestiviruses

Pestiviruses account for important diseases in animals such as Classical swine fever (CSF) and Bovine viral diarrhea / Mucosal disease (BVD/MD). According to the current O.I.E. list CSF and BVD/MD are notifiable diseases and eradication programs are administered in many countries worldwide. The molecular biology of pestiviruses shares many similarities and peculiarities with the human hepaciviruses. Genome organisation

and translation strategy are highly similar for the members of both genera. One hallmark of pestiviruses is their unique strategy to establish persistent infection during pregnancy. Persistent infection with pestiviruses often goes unnoticed; for BVDV frequently nonhomologous RNA recombination events lead to the appearance of genetically distinct viruses that are lethal to the host.

==Arteriviruses== BY KRUNAL BADANI In 1996, the family *Arteriviridae* was included within the order *Nidovirales*. Arteriviruses are small, enveloped, animal viruses with an icosahedral core containing a positive-sense RNA genome. The family includes equine arteritis virus (EAV), porcine reproductive and respiratory syndrome virus (PRRSV), lactate dehydrogenase elevating virus (LDV) of mice and simian haemorrhagic fever virus (SHFV). Three of these viruses were first discovered and characterized in the 1950/60s, whereas PRRSV was first isolated in Europe and in North America in the early 1990s. The arteriviruses are highly species specific, but share many biological and molecular properties, including virion morphology, a unique set of structural proteins, genome organization and replication strategy, and the ability to establish prolonged or true persistent infection in their natural hosts. However, the epidemiology and pathogenesis of the infection caused by each virus is distinct, as are the diseases they cause.

Coronaviruses

Coronavirus (CoV) genome replication takes place in the cytoplasm in a membrane-protected microenvironment, and starts with the translation of the genome to produce the viral replicase. CoV transcription involves a discontinuous RNA synthesis (template switch) during the extension of a negative copy of the subgenomic mRNAs. The requirement for basepairing during transcription has been formally demonstrated in arteriviruses and CoVs. CoV N protein is required for coronavirus RNA synthesis, and has RNA chaperone activity that may be involved in template switch. Both viral and cellular proteins are required for replication and transcription. CoVs initiate translation by cap-dependent and cap-independent mechanisms. Cell macromolecular synthesis may be controlled after CoV infection by locating some virus proteins in the host cell nucleus. Infection by different coronaviruses cause in the host alteration in the transcription and translation patterns, in the cell cycle, the cytoskeleton, apoptosis and coagulation pathways, inflammation, and immune and stress responses. The balance between genes up- and down-regulated could explain the pathogenesis caused by these viruses. Coronavirus expression systems based on single genome constructed by targeted recombination, or by using infectious cDNAs, have been developed. The possibility of expressing different genes under the control of transcription regulating sequences (TRSs) with programmable strength, and engineering tissue and species tropism indicates that CoV vectors are flexible. CoV based vectors have emerged with high potential for vaccine development and, possibly, for gene therapy.

Influenza

Influenza is caused by RNA viruses of the family Orthomyxoviridae and affects birds and mammals.

Avian Influenza

Wild aquatic birds are the natural hosts for a large variety of influenza A viruses. Occasionally viruses are transmitted from this reservoir to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. Proteolytic activation of the hemagglutinin is an important determinant for pathogenicity and adaptation of the receptor binding specificity of the hemagglutinin and adaptation of the polymerase to new hosts play important roles in interspecies transmission.

==Bluetongue Virus==BY KRUNAL BADANI Bluetongue virus (BTV), a member of Orbivirus genus within the Reoviridae family causes serious disease in livestock (sheep, goat, cattle). Partly due to this BTV has been in the forefront of molecular studies for the last three decades and now represents one of the best understood viruses at the molecular and structural levels. BTV, like the other members of the family is a complex non-enveloped virus with seven structural proteins and a RNA genome consisting of 10 double-stranded (ds) RNA segments of different sizes. It has been possible to determine the complex nature of the virion through 3D structure reconstructions (diameter ~ 800 Å); the atomic structure of proteins and the internal capsid (~ 700 Å, the first large highly complex structure ever solved); the definition of the virus encoded enzymes required for RNA replication; the ordered assembly of the capsid shell and the protein sequestration required for it; and the role of host proteins in virus entry and virus release. These areas are important for BTV replication but they also indicate the pathways that may be used by related viruses, which include viruses that are pathogenic to man and animals, thus providing the basis for developing strategies for intervention or prevention.

Porcine Circoviruses

Porcine Circoviruses (PCV) are the smallest viruses replicating autonomously in eukaryotic cells. The virions are non-enveloped and spherical with a diameter of 16-18 nm and the covalently closed and single-stranded DNA genomes comprise less than 1800 nucleotides. The genomes encode only two major open reading frames. The gene products Rep, Rep' and Cap are involved in viral replication, regulation of transcription and capsid formation. Due to their highly limited coding capacity, circoviruses are supposed to rely principally on the host's machinery for synthesis of macromolecules. Two types of PCV are known, which differ with respect to their pathogenicity. Porcine circovirus type 1 (PCV1) is not linked with a disease, while porcine circovirus type 2 (PCV2) is the etiological agent of Postweaning Multisystemic Wasting Syndrome (PMWS), a new emerging and multifactorial disease in swine. PCV1 and PCV2 show a high degree of sequence homology and a similar genomic organisation; nevertheless, the basis of the distinct pathogenicity has not yet been unravelled.

Herpesviruses

Herpesviruses are highly successful pathogens infecting animals and man. Although there is a wide variety of different herpesviruses with different biological characteristics, they have in common basic properties such as morphology of the virion, highly regulated transcription and establishment of latency. In animal virology the most important herpesviruses belong to the Alphaherpesvirinae. Research on pseudorabies virus, the causative agent of Aujeszky's disease in pigs, has pioneered animal disease control with genetically modified vaccines. PrV is now extensively studied as a model for basic processes during lytic herpesvirus infection, and for unravelling molecular mechanisms of herpesvirus neurotropism, whereas bovine herpesvirus 1, the causative agent of bovine infectious rhinotracheitis and pustular vulvovaginitis, is analyzed to elucidate molecular mechanisms of latency. The avian infectious laryngotracheitis virus is phylogenetically distant from these two viruses and serves to underline similarity and diversity within the Alphaherpesvirinae.

African Swine Fever Virus

African swine fever virus (ASFV) is a large double-stranded DNA virus which replicates in the cytoplasm of infected cells and is the only member of the Asfarviridae family. In common with other viral haemorrhagic fevers, the main target cells for replication are those of monocyte, macrophage lineage. The virus causes a haemorrhagic fever with high mortality rates in pigs, but persistently infects its natural hosts, warthogs, bushpigs and soft ticks of the Ornithodoros species with no disease signs. The virus encodes enzymes required for replication and transcription of the genome, including elements of a base excision repair system, structural proteins and many proteins that are not essential for replication in cells but have roles in virus survival and transmission in its hosts. Virus replication takes place in perinuclear factory areas. Assembly of the icosahedral capsid occurs on modified membranes from the endoplasmic reticulum. Products from proteolytically processed polyproteins form the core shell between the internal membrane and the nucleoprotein core. An additional outer membrane is gained as particles bud from the plasma membrane. The virus encodes proteins that inhibit signalling pathways in infected macrophages and thus modulate transcriptional activation of immune response genes. In addition the virus encodes proteins which inhibit apoptosis of infected cells to facilitate production of progeny virions. Viral membrane proteins with similarity to cellular adhesion proteins modulate interaction of virus-infected cells and extracellular virions with host components.

Lentivirus

Lentiviruses comprise a genus of diverse viruses in the *Retroviridae* family which are united in their ability to infect and persist in macrophages. Infections are characterized by immune system dysfunctions following sometimes lengthy incubation periods. The viruses in this genus include primate lentiviruses such as HIV as well as animal lentiviruses including equine infectious anemia virus (EIAV). A feature of lentiviruses is their ability to hijack macrophages so that they are simultaneously involved in the

dissemination and control of virus spread throughout the host, leading to disease induction and/or transmission to a new host. Despite the devastating infections that lentiviruses cause, they also have enormous potential as research tools due to their ability to integrate into the host genome and are being exploited for use as delivery vehicles in gene therapy.

Flaviviruses

Flaviviruses constitute a family of linear, single-stranded RNA(+) viruses. Flaviviruses include the West Nile virus, dengue virus, Tick-borne Encephalitis Virus, Yellow Fever Virus, and several other viruses. Many flavivirus species can replicate in both mammalian and insect cells. Most flaviviruses are arthropod borne and multiply in both vertebrates and arthropods.

Paramyxoviruses

Paramyxoviruses are a diverse family of non-segmented negative strand RNA viruses that include many highly pathogenic viruses affecting humans, animals, and birds. In recent years the advent of reverse genetics has led to a greater understanding of the genomics, molecular biology and viral pathogenesis. Paramyxoviruses cause a range of diseases in animal species: canine distemper virus (dogs), phocine distemper virus (seals), cetacean morbillivirus (dolphins and porpoises) Newcastle disease virus (birds) and rinderpest virus (cattle). Some paramyxoviruses such as the henipaviruses are zoonotic pathogens, occurring naturally in an animal host, but also able to infect humans.

Hendra and Nipah Virus

Over the past decade, the previously unknown paramyxoviruses Hendra virus (HeV) and Nipah virus (NiV) have emerged in humans and livestock in Australia and Southeast Asia. Collectively they are known as henipaviruses. Both viruses are contagious, highly virulent, and capable of infecting a number of mammalian species and causing potentially fatal disease. Due to the lack of a licensed vaccine or antiviral therapies, HeV and NiV are designated as biosafety level 4 agents. The genomic structure of both viruses is that of a typical paramyxovirus. However, due to limited sequence homology and little immunological cross-reactivity with other paramyxoviruses, HeV and NiV have been classified into a new genus within the family Paramyxoviridae named Henipavirus.

Insect viruses

Viruses that are pathogenic to insects cause millions of dollars worth of damage to industries such as sericulture, apiculture and aquaculture (e.g. infection of honeybees and silk worms). On the other hand, viruses that are pathogenic to insect pests can be exploited as biological control agents. Some insect viruses, e.g. baculovirus, have been commercially exploited for use as gene expression and delivery vectors in both insect and mammalian cells.

Interferon

Interferons (IFNs) play pivotal roles in shaping the immune responses in mammals and are particularly important for the control of viral infections and cell growth, and immune regulation. These proteins rapidly induce an "anti-viral state" in cells that surround infected cells. In order to survive, viruses have evolved multiple strategies to evade the anti-viral effects of IFNs. Elucidating the molecular and cellular biology of the virus-interferon interaction is key to understanding issues such as viral pathogenesis, latency, and the development of novel antivirals.

Chapter 2

Avian Influenza

Avian influenza, sometimes **avian flu**, and commonly **bird flu**, refers to "influenza caused by viruses adapted to birds." Of the greatest concern is **highly pathogenic avian influenza** (**HPAI**).

"Bird flu" is a phrase similar to "swine flu," "dog flu," "horse flu," or "human flu" in that it refers to an illness caused by any of many different strains of influenza viruses that have adapted to a specific host. All known viruses that cause influenza in birds belong to the species *influenza A virus*. All subtypes (but not all strains of all subtypes) of influenza A virus are adapted to birds, which is why for many purposes avian flu virus *is* the influenza A virus (note that the "A" does *not* stand for "avian").

Adaptation is non-exclusive. Being adapted towards a particular species does not preclude adaptations, or partial adaptations, towards infecting different species. In this way strains of influenza viruses are adapted to multiple species, though may be preferential towards a particular host. For example, viruses responsible for influenza pandemics are adapted to both humans and birds. Recent influenza research into the genes of the Spanish flu virus shows it to have genes adapted to both birds and humans; with more of its genes from birds than less deadly later pandemic strains.

While its most highly pathogenic strain (H5N1) had been spreading throughout Asia since 2003, Avian Influenza reached Europe in 2005, and the Middle East as well as Africa the following year.

Genetics

Genetic factors in distinguishing between "human flu viruses" and "avian flu viruses" include:

PB2: (RNA polymerase): Amino acid (or residue) position 627 in the PB2 protein encoded by the PB2 RNA gene. Until H5N1, all known avian influenza viruses had a Glu at position 627, while all human influenza viruses had a Lys.
HA: (hemagglutinin): Avian influenza HA bind alpha 2-3 sialic acid receptors while human influenza HA bind alpha 2-6 sialic acid receptors. Swine influenza viruses have

the ability to bind both types of sialic acid receptors. Hemagglutinin is the major antigen of the virus against which neutralizing antibodies are produced and influenza virus epidemics are associated with changes in its antigenic structure. This was originally derived from pigs, and should technically be referred to as "Pig Flu"

Subtypes

There are many subtypes of avian influenza viruses, but only some strains of four subtypes have been highly pathogenic in humans. These are types H5N1, H7N3, H7N7 and H9N2.

Contraction/Spreading of Avian Influenza

Most human contractions of the Avian flu are a result of either handling dead infected birds or from contact with infected fluids. While most wild birds mainly have only a mild form of the H5N1 strain, once domesticated birds such as chickens or turkeys are infected, it could become much more deadly because the birds are often within close contact of one another. There is currently a large threat of this in Asia with infected poultry due to low hygiene conditions and close quarters . Although it is easy for humans to become infected from birds, it's much more difficult to do so from human to human without close and lasting contact.

Spreading of H5N1 from Asia to Europe is much more likely the cause of both legal and illegal poultry trades than dispersing through wild bird migrations, being that in recent studies, there were no secondary rises in infection in Asia when wild birds migrate south again from their breeding grounds. Instead, the infection patterns followed transportation such as railroads, roads, and Country borders, suggesting poultry trade as being much more likely. While there have been strains of Avian Flu to exist in the United States, such as Texas in 2004, they have been extinguished and have not been known to infect humans.

Examples of avian influenza A virus strains:

HA subtype designation	NA subtype designation	Avian influenza A viruses
H1	N1	A/duck/Alberta/35/76(H1N1)
H1	N8	A/duck/Alberta/97/77(H1N8)
H2	N9	A/duck/Germany/1/72(H2N9)
H3	N8	A/duck/Ukraine/63(H3N8)
H3	N8	A/duck/England/62(H3N8)
H3	N2	A/turkey/England/69(H3N2)
H4	N6	A/duck/Czechoslovakia/56(H4N6)

H4	N3	A/duck/Alberta/300/77(H4N3)
H5	N3	A/tern/South Africa/300/77(H4N3)
H5	N4	A/jyotichinara/Ethiopia/300/77(H6N6)
H5	N9	A/turkey/Ontario/7732/66(H5N9)
H5	N1	A/chick/Scotland/59(H5N1)
H6	N2	A/turkey/Massachusetts/3740/65(H6N2)
H6	N8	A/turkey/Canada/63(H6N8)
H6	N5	A/shearwater/Australia/72(H6N5)
H6	N1	A/duck/Germany/1868/68(H6N1)
H7	N7	A/fowl plague virus/Dutch/27(H7N7)
H7	N1	A/chick/Brescia/1902(H7N1)
H7	N3	A/turkey/England/639H7N3)
H7	N1	A/fowl plague virus/Rostock/34(H7N1)
H8	N4	A/turkey/Ontario/6118/68(H8N4)
H9	N2	A/turkey/Wisconsin/1/66(H9N2)
H9	N6	A/duck/Hong Kong/147/77(H9N6)
H9	N6	A/duck/Hong Kong/147/77(H9N6)
H9	N8	A/manishsurpur/Malawi/149/77(H9N8)
H9	N7	A/turkey/Scotland/70(H9N7)
H10	N8	A/quail/Italy/1117/65(H10N8)
H11	N6	A/duck/England/56(H11N6)
H11	N9	A/duck/Memphis/546/74(H11N9)
H12	N5	A/duck/Alberta/60/76/(H12N5)
H13	N6	A/gull/Maryland/704/77(H13N6)
H14	N4	A/duck/Gurjev/263/83(H14N4)
H15	N9	A/shearwater/Australia/2576/83(H15N9)

Influenza pandemic

Pandemic flu viruses have some avian flu virus genes and usually some human flu virus genes. Both the H2N2 and H3N2 pandemic strains contained genes from avian influenza viruses. The new subtypes arose in pigs coinfected with avian and human viruses and were soon transferred to humans. Swine were considered the original "intermediate host" for influenza, because they supported reassortment of divergent subtypes. However, other

hosts appear capable of similar coinfection (e.g., many poultry species), and direct transmission of avian viruses to humans is possible. The Spanish flu virus strain may have been transmitted directly from birds to humans. In spite of their pandemic connection, avian influenza viruses are noninfectious for most species. When they are infectious they are usually asymptomatic, so the carrier does not have any disease from it. Thus while infected with an avian flu virus, the animal doesn't have a "flu". Typically, when illness (called "flu") from an avian flu virus *does* occur, it is the result of an avian flu virus strain adapted to one species spreading to another species (usually from one bird species to another bird species). So far as is known, the most common result of this is an illness so minor as to be not worth noticing (and thus little studied). But with the domestication of chickens and turkeys, humans have created species subtypes (domesticated poultry) that can catch an avian flu virus adapted to waterfowl and have it rapidly mutate into a form that kills in days over 90% of an entire flock and spread to other flocks and kill 90% of *them* and can only be stopped by killing every domestic bird in the area. Until H5N1 infected humans in the 1990s, this was the only reason avian flu was considered important. Since then, avian flu viruses have been intensively studied; resulting in changes in what is believed about flu pandemics, changes in poultry farming, changes in flu vaccination research, and changes in flu pandemic planning.

H5N1 has evolved into a flu virus strain that infects more species than any previously known flu virus strain, is deadlier than any previously known flu virus strain, and continues to evolve becoming both more widespread and more deadly causing Robert G. Webster, a leading expert on avian flu, to publish an article titled "The world is teetering on the edge of a pandemic that could kill a large fraction of the human population" in *American Scientist*. He called for adequate resources to fight what he sees as a major world threat to possibly billions of lives. Since the article was written, the world community has spent billions of dollars fighting this threat with limited success.

Vaccines have been formulated against several of the avian H5N1 influenza varieties. Vaccination of poultry against the ongoing H5N1 epizootic is widespread in certain countries. Some vaccines also exist for use in humans, and others are in testing, but none have been made available to civilian populations, nor produced in quantities sufficient to protect more than a tiny fraction of the Earth's population in the event that an H5N1 pandemic breaks out. The World Health Organization has compiled a list of known clinical trials of pandemic influenza prototype vaccines, including those against H5N1.

H5N1

The highly pathogenic influenza A virus subtype H5N1 virus is an emerging avian influenza virus that has been causing global concern as a potential pandemic threat. It is often referred to simply as "bird flu" or "avian influenza" even though it is only one subtype of avian influenza causing virus.

H5N1 has killed millions of poultry in a growing number of countries throughout Asia, Europe and Africa. Health experts are concerned that the co-existence of human flu viruses and avian flu viruses (especially H5N1) will provide an opportunity for genetic material to be exchanged between species-specific viruses, possibly creating a new virulent influenza strain that is easily transmissible and lethal to humans.

Since the first H5N1 outbreak occurred in 1987, there has been an increasing number of HPAI H5N1 bird-to-human transmissions leading to clinically severe and fatal human infections. However, because there is a significant species barrier that exists between birds and humans, the virus does not easily cross over to humans, though some cases of infection are being researched to discern whether human to human transmission is occurring. More research is necessary to understand the pathogenesis and epidemiology of the H5N1 virus in humans. Exposure routes and other disease transmission characteristics such as genetic and immunological factors, that may increase the likelihood of infection, are not clearly understood.

On January 18, 2009, a 27-year-old woman from eastern China died of bird flu, Chinese authorities said, making her the second person to die from the deadly virus at that time. Two tests on the woman were positive for H5N1 avian influenza, said the ministry, which did not say how she might have contracted the virus.

Although millions of birds have become infected with the virus since its discovery, 306 humans have died from the H5N1 in twelve countries according to WHO data as of February 2, 2011.

The avian flu claimed at least 300 humans in Azerbaijan, Cambodia, China, Egypt, Indonesia, Iraq, Laos, Nigeria, Pakistan, Thailand, Turkey, and Vietnam. Epidemiologists are afraid that the next time such a virus mutates, it could pass from human to human; however, the current A/H5N1 virus does not transmit easily from human to human. If this form of transmission occurs, another pandemic could result. Thus disease-control centers around the world are making avian flu a top priority. These organizations encourage poultry-related operations to develop a preemptive plan to prevent the spread of H5N1 and its potentially pandemic strains. The recommended plans center on providing protective clothing for workers and isolating flocks to prevent the spread of the virus.

The Thailand outbreak of avian flu caused massive economic losses especially among poultry workers. Infected birds were culled and sacrificed. The public lost confidence with the poultry products, thus decreasing the consumption of chicken products. This also elicited a ban from importing countries. There were however, factors which aggravated the spread of the virus which includes bird migration, cool temperature (increases virus survival) and several festivals at that time.

In March 2011, one of the student from Gadjah Mada University, Indonesia has discovered an Avian Influenza H5N1 virus vaccine by using extract of Phaleria Macrocarpa (Mahkota Dewa) fruit. The vaccinne from the fruit consists of Saponin compound which effectiveness up to 87 percent to inhibit the development and growth of the virus. It cost Rp.75,000 ($8.00) per 100 doses and will be cheaper than current vaccine at around Rp200,000 ($23.00) per 100 doses. It will be presented at Japan Amstecs international seminar on March 19 to 20, 2011.

In domestic animals

Several domestic species have been infected with and shown symptoms of H5N1 viral infection including cats, dogs, ferrets, pigs,and birds.

Birds

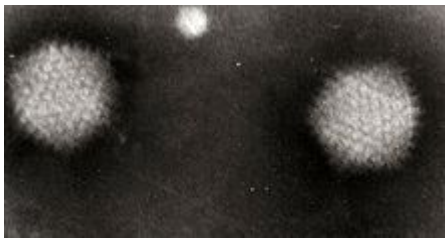
Attempts are made in the United States not to minimize the presence of highly pathogenic avian influenza (HPAI) in poultry in thorough routine surveillance of poultry flocks in commercial poultry operations. Detection of a HPAI virus may result in immediate culling of the flock. Less pathogenic viruses are controlled by vaccination, which is done primarily in turkey flocks (ATCvet codes: QI01AA23 for the inactivated fowl vaccine, QI01CL01 for the inactivated turkey combination vaccine).

Chapter 3

Agamid Adenovirus and Avian paramyxovirus

Agamid adenovirus

Adenoviruses



Transmission electron micrograph of two adenovirus particles

Virus classification

Group:	Group I (dsDNA)
Family:	*Adenoviridae*
Genus:	*Atadenovirus*
Species:	**Agamid adenovirus 1** Wellehan

Agamid adenovirus (Agamid AdV1) is a type of virus in the *Adenoviridae* family. The virus is widespread in captive populations of *Pogona vitticeps*, known commonly as the Inland Bearded Dragon, in the United States. Other countries with confirmed cases are Australia, Japan, Germany, The Netherlands, Belgium, UK and Central America (El Salvador). It is often discovered in association with other infections, and causes increased juvenile mortality and adult deaths.

History

The first detection of adenovirus-like particles in Bearded Dragons was reported from New Zealand in 1982 (Julian and Durham, 1985). Since then multiple studies have come out about the virus. University of Florida seems to lead with the most detailed and up to date reports, however University of Illinois is also known to be involved in research of the virus.

Diversity of agamid adenoviruses

Data have recently been published on the diversity of Agamid adenovirus 1 in the United States**.**

Agamid adenovirus 1 has also been identified in bearded dragons in Austria:

- Agamid atadenovirus ben
- Agamid atadenovirus wall

Infection and consequences

In a study published by the Journal of Virology, intranuclear inclusions, or infected cells, were found in the intestinal mucosa, hepatocytes, and bile ducts. Even where an Agamid shows no signs of infection, many are believed to be sub-clinically infected, or carriers of the virus. Although they show no signs they can infect others. It is known that the virus is transferable through fecal-oral contact, however it is speculated that it can be passed in ways as of current research are unknown. Agamid Adenovirus is becoming widespread in the United States, with several breeders admitting infection and shutting down their current projects. However some breeders are still not convinced of the dangers of Agamid Adenovirus, thus they do not test, they could be selling Adenovirus positive offspring. A report from Louisiana State University found that baby and juvenile Bearded Dragons have a high mortality rate associated with this virus.

The following is quoted from Cheri Smith's compilation of Adenovirus symptoms: "Any animal that is suspected of having this virus should be isolated, never breed and great care taken when handling between animals. All animals in contact with another that is suspected of having it or confirmed should be isolated from each other, never bred, certainly never sold to others that may unknowingly start the cycle again with other animals. One confirmed case had a couple with an ill animal that brought it to a breeder to look at and he followed all safety precautions, only to find the couple handling some of his babies while he was looking at theirs, 10 days later his entire clutch was ill and dying, it passes that easily! Another breeder at a show in NY, returned from the show and had babies dying that tested positive within 2 weeks (since that time, 2 other breeders that attend the same show have also lost their colony of dragons to the virus)"

In 2005 it was noted by Cheri Smith that "Sibling clutches have been tested and some are positive, some are negative in the same clutch. This leads to the theory that some are

infected when the eggs pass through the cloaca and pick up viral particles or some are infected before they are shelled when others are spared."

Symptoms

Symptoms of Agamid Adenovirus 1 in bearded dragons are variable, and range from asymptomatic infection to enteritis to death. It is probable that there is a relationship between dose of infection and clinical disease. Coinfections with other agents such as Isospora amphibolouri, a coccidia, and dependovirus, a genus of Parvovirus may play roles in clinical presentation of disease. Husbandry factors such as temperature range, diet, population density, and other stressors also are likely to play significant roles in clinical presentation.

Testing

- Electron Microscopy or 'EM' testing is available through the University of Illinois, College of Veterinary Medicine, Center for Microscopic Imaging (CMI)
- Polymerase chain reaction or 'PCR' based testing is available through the University of Florida- this laboratory requires samples to be submitted by a veterinarian.

Avian paramyxovirus

Avian paramyxovirus	
Virus classification	
Group:	Group V ((-)ssRNA)
Order:	*Mononegavirales*
Family:	*Paramyxoviridae*
Genus:	*Avulavirus*
Species:	***Avian paramyxovirus***

Introduction

Avian paramyxoviruses 1 through 9 are multiple unique serotypes of virus in the genera *Avulavirus*. Newcastle disease virus is another well characterized species within the same genus and is named APMV-1. Currently, avian paramyxoviruses (APMV) consist of nine distinct known serotypes and the numbers will increase due to isolation of new unknown serotypes. The APMVs are separated into distinct serotypes using Hemagglutination assay and Neuraminidase assay. All the APMVs hemagglutinate chicken RBCs except for APMV-5 which does not hemagglutinate any species RBC making it unique among

the APMVs. APMV-6 is also unique to the presence of SH gene between F and HN genes.

Virology

APMV contain 6–10 tandemly linked genes that encode at least 7 and as many as 12 different proteins. The gene arrangement is 3'Leader-N-P-M-F-HN-L-Trailer-5'for all the serotypes except for APMV-6 which has a unique SH gene between F and HN. The 3′ and 5′ ends of the genome contain short respective extragenic ‘leader’ and ‘trailer’, regions. The following are the important proteins produced nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a fusion glycoprotein (F), an attachment glycoprotein that in the case of the APMVs is a hemagglutinin-neuraminidase (HN), and a large polymerase protein (L) The viral RNA polymerase begins transcription at the 3′ end and proceeds downstream in a sequential manner generating individual mRNAs encompass by gene-start (GS) and gene-end (GE) signals that flank each gene. The genome is transcribed sequentially from N to L with reduction in expression levels along its length. Non-coding intergenic sequences (IGS) are present between gene boundaries and are not copied into mRNAs. N encodes nucleocapsid protein that associates with the genomic RNA forming the nucleocapsid. M encodes the Matrix protein required for viral assembly. HN and F form the viral coat, and are required for viral entry into cells and also determine the antibody response. The phosphoprotein P is a cofactor for L. The atomic structure is now available for two of them, F and HN.

Revisiting the rule of 'six'

The viral Genome consists of a RNA with negative Polarity. The length of the RNA is unusually constant and within the kinds very similar. That amount to about 13 KB (Genus Metapneumovirus) to 18 KB (Genus Henipavirus), with most paramyxoviruses usually around 15 KB. Regarding the length of individual members of the Subfamily Paramyxovirinae more exactly, then this follows a regularity unusual with viruses, the divisibility by the number of 6: e.g. Mumpsvirus 15.384 NT, Newcastle Disease virus 15,156 NT. This multiples of the number of 6 are justified in a special mechanism of the RNA with these viruses.

Signature domains of avian paramyxoviruses

Fusion cleavage site

[APMV F protein cleavage site APMV-5 GKRKKR ¯ F APMV-1(Avirulent) ...QG. ¯ L APMV-1(Virulent) ...Q.. ¯ . APMV-2 D..A.. ¯ . APMV-3 AR.RG. ¯ L APMV-4 ADIQP. ¯ . APMV-6 PA.EP. ¯ L APMV-7 TLPSS. ¯ . APMV-8 .Y.QT. ¯ L APMV-9 RI.EG. ¯ I

Genebank accession numbers

Accession numbers for complete genome sequence submitted in genebank are as follows.

APMV-1, AF077761. APMV-2, EU338414. APMV-3, EU403085. APMV-4KR, EU877976. APMV-4HK, FJ177514. APMV-5, GU206351. APMV-6TW, NC 003043. APMV-6HK, EU622637. APMV-6FE, EF569970. APMV-7, FJ231524. APMV-8DEL, FJ215863. APMV-8WAK, FJ215864. APMV-9, EU910942. APMV-10 (Proposed), HM755888.

Avian Paramyxovirus - Type 1 (APMV-1)

Newcastle disease virus (NDV) strain Texas GB is a highly virulent neurotropic virus that is used as a standard vaccine challenge virus in the U.S. In this study, the complete genome sequence of strain Texas GB was determined and compared with the complete genome sequences of other NDV strains. The genome is 15,186 nucleotides (nt) long and consists of six genes in the order of 3'leader-N-P-M-F-HN-L-5'trailer. The genome contains a 55-nt leader sequence at the 3' end and a 114-nt trailer sequence at the 5' end. The intergenic sequences are 2, 1, 1, 31, and 47 nt between N/P, P/M, M/F, F/HN, and HN/L genes, respectively. The putative cleavage site of fusion protein showed amino acid sequence of R-R-Q-K-R downward arrow F in position 112 to 117, which corresponds to those of virulent NDV strains. The phylogenetic analysis showed that strain Texas GB is closely related to the neurovirulent mesogenic strain Beaudette C (BC) and to NDV viruses isolated in China and Egypt than to other strains of NDV.

Avian Paramyxovirus - Type 2 (APMV-2)

The complete RNA genome sequence of avian paramyxovirus (APMV) serotype 2, strain Yucaipa isolated from chicken has been determined. With genome size of 14,904 nucleotides (nt), strain Yucaipa is consistent with the "rule of six" and is the smallest virus reported to date among the members of subfamily Paramyxovirinae. The genome contains six non-overlapping genes in the order 3'-N-P/V-M-F-HN-L-5'. The genes are flanked on either side by highly conserved transcription start and stop signals and have intergenic sequences varying in length from 3 to 23nt. The genome contains a 55nt leader sequence at 3' end and a 154nt trailer sequence at 5' end. Alignment and phylogenetic analysis of the predicted amino acid sequences of strain Yucaipa proteins with the cognate proteins of viruses of all of the five genera of family Paramyxoviridae showed that APMV-2 strain Yucaipa is more closely related to APMV-6 than APMV-1.

Avian Paramyxovirus - Type 3 (APMV-3)

The complete genome sequence was determined for prototype parakeet/Netherlands/449/75 strain of avian paramyxovirus (APMV) serotype 3. The genome is 16,272 nucleotides (nt) in length, consisting of six non-overlapping genes in the order of 3'-N-P/V/W-M-F-HN-L-5', with intergenic regions of 31-63nt. APMV-3 genome follows the "rule of six" and is the largest among the avian paramyxoviruses reported to date, with a trailer region of 707nt, the longest in the family Paramyxoviridae. The cleavage site of F protein, A-R-P-R-G-R downward arrowL, does not conform to the preferred cleavage site of the ubiquitous cellular protease furin. Therefore, exogenous protease was needed for replication in vitro. Alignment and phylogenetic analysis of the

predicted amino acid sequences of strain Netherlands proteins with the cognate proteins of viruses of all of the five genera of family Paramyxoviridae showed that APMV-3 strain Netherlands is more closely related to APMV-1 than APMV-6.

Avian Paramyxovirus - Type 4 (APMV-4)

Avian paramyxoviruses (APMVs) are frequently isolated from domestic and wild birds throughout the world. All APMVs, except avian metapneumovirus, are classified in the genus Avulavirus of the family Paramyxoviridae. At present, the APMVs of genus Avulavirus are divided into nine serological types (APMV 1-9). Newcastle disease virus represents APMV-1 and is the most characterized among all APMV types. Very little is known about the molecular characteristics and pathogenicity of APMV 2-9. As a first step towards understanding the molecular genetics and pathogenicity of APMV-4, we have sequenced the complete genome of APMV-4 strain duck/Hong Kong/D3/75 and determined its pathogenicity in embryonated chicken eggs. The genome of APMV-4 is 15,054 nucleotides (nt) in length, which is consistent with the "rule of six". The genome contains six non-overlapping genes in the order 3'-N-P/V-M-F-HN-L-5'. The genes are flanked on either side by highly conserved transcription start and stop signals and have intergenic sequences varying in length from 9 to 42 nt. The genome contains a 55 nt leader region at 3' end. The 5' trailer region is 17 nt, which is the shortest in the family Paramyxoviridae. Analysis of mRNAs transcribed from the P gene showed that 35% of the transcripts were edited by insertion of one non-templated G residue at an editing site leading to production of V mRNAs. No message was detected that contained insertion of two non-templated G residues, indicating that the W mRNAs are inefficiently produced in APMV-4 infected cells. The cleavage site of the F protein (DIPQR downward arrowF) does not conform to the preferred cleavage site of the ubiquitous intracellular protease furin. However, exogenous proteases were not required for the growth of APMV-4 in cell culture, indicating that the cleavage does not depend on a furin site. Phylogenic analysis of the nucleotide sequences of viruses of all five genera of the family Paramyxoviridae showed that APMV-4 is more closely related to the APMVs than to other paramyxoviruses, reinforcing the classification of all APMVs in the genus Avulavirus of the family Paramyxoviridae.

Avian Paramyxovirus - Type 5 (APMV-5)

Avian paramyxoviruses (APMV) consist of nine known serotypes. The genomes of representatives of all APMV serotypes except APMV type 5 have recently been fully sequenced. Here, we report the complete genome sequence of the APMV-5 prototype strain budgerigar/Kunitachi/74. APMV-5 Kunitachi virus is unusual in that it lacks a virion hemagglutinin and does not grow in the allantoic cavity of embryonated chicken eggs. However, the virus grew in the amniotic cavity of embryonated chicken eggs and in twelve different established cell lines and two primary cell cultures. The genome is 17,262 nucleotides (nt) long, which is the longest among members of genus Avulavirus, and encodes six non-overlapping genes in the order of 3′N-P/V/W-M-F-HN-L-5′ with intergenic regions of 4–57 nt. The genome length follows the ‘rule of six’ and contains a 55-nt leader sequence at the 3′end and a 552 nt trailer sequence at the 5′ end. The

phosphoprotein (P) gene contains a conserved RNA editing site and is predicted to encode P, V, and W proteins. The cleavage site of the F protein (G-K-R-K-K-R↓F) conforms to the cleavage site motif of the ubiquitous cellular protease furin. Consistent with this, exogenous protease was not required for virus replication in vitro. However, the intracerebral pathogenicity index of APMV-5 strain Kunitachi in one-day-old chicks was found to be zero, indicating that the virus is avirulent for chickens despite the presence of a polybasic F cleavage site.Phylogenetic analysis of the sequences of the APVM-5 genome and proteins versus those of the other APMV serotypes showed that APMV-5 is more closely related to APMV-6 than to the other APMVs. Furthermore, these comparisons provided evidence of extensive genome-wide divergence that supports the classification of the APMVs into nine separate serotypes. The structure of the F cleavage site does not appear to be a reliable indicator of virulence among APMV serotypes 2–9. The availability of sequence information for all known APMV serotypes will facilitate studies in epidemiology and vaccinology.

Avian Paramyxovirus - Type 6 (APMV-6)

Complete genome sequences were determined for two strains of avian paramyxovirus serotype 6 (APMV-6): the prototype Hong Kong (HK) strain and a more recent isolate from Italy (IT4524-2). The genome length of strain HK is 16236 nucleotide (nt), which is the same as for the other two APMV-6 strains (FE and TW) that have been reported to date, whereas that of strain IT4524-2 is 16230 nt. The length difference in strain IT4524-2 is due to a 6-nt deletion in the downstream untranslated region of the F gene. All of these viruses follow the "rule of six". Each genome consists of seven genes in the order of 3'N-P-M-F-SH-HN-L5', which differs from other APMV serotypes in containing an additional gene encoding the small hydrophobic (SH) protein. Sequence comparisons revealed that strain IT4524-2 shares an unexpectedly low level of genome nt sequence identity (70%) and aggregate predicted amino acid (aa) sequence identity (79%) with other three strains, which in contrast are more closely related to each other with nt sequence 94-98% nt identity and 90-100% aggregate aa identity. Sequence analysis of the F-SH-HN genome region of two other recent Italian isolates showed that they fall in the HK/FE/TW group. The predicted signal peptide of IT4524-2 F protein lacks the N-terminal first 10 aa that are present in the other five strains. Also, the F protein cleavage site of strain IT4524-2, REPR downward arrow L, has two dibasic aa (arginine, R) compared to the monobasic F protein cleavage site of PEPR downward arrow L in the other strains. Reciprocal cross-hemagglutination inhibition (HI) assays using post-infection chicken sera indicated that strain IT4524-2 is antigenically related to the other APMV-6 strains, but with 4- to 8-fold lower HI tiers for the test sera between strain IT4524-2 and the other APMV-6 strains. Taken together, our results indicated that the APMV-6 strains represents a single serotype with two subgroups that differ substantially based on nt and aa sequences and can be distinguished by HI assay.

Avian Paramyxovirus - Type 7 (APMV-7)

The complete genome sequence of avian paramyxovirus serotype 7 (APMV-7) prototype strain dove/Tennessee/4/75 was determined. The genome size is 15,480 nucleotides (nt)

long and follows the "rule of six". The genome contains six non-overlapping genes in the order of 3'-N-P/V/W-M-F-HN-L-5'. The 3'-leader and 5'-trailer sequences of the genome are 55 and 127nt long, respectively. The first 12nt of the leader and trailer sequences are complementary to each other. The viral genes are flanked by highly conserved gene-start (GS) and gene-end (GE) transcription signals, and in addition the 3'-leader sequence contains a sequence ((35)AAUUAUUUUUU(45)) that is identical to the GE signal present at two of the genes. The genes are separated by intergenic sequences (IGS) ranging between 11 and 70nt. The phosphoprotein (P) gene contains a conserved RNA editing site (3'-UUUUUCCC-5') presumed to be involved in the production of V and W proteins. The viral fusion (F) protein has a single basic amino acid at the putative cleavage site ((101)TLPSSR; however, the virus did not require exogenous protease for in vitro replication. The virus grew in only a few established cell lines, indicating a restricted host range. Sequence alignment and phylogenetic analysis of the predicted amino acid sequence of APMV-7 proteins with the cognate proteins of the viruses of all five genera of the family Paramyxoviridae showed that APMV-7 is more closely related to APMV-2, -6, -8 than to APMV-1, -3, -4 and -9. The mean death time in embryonated chicken eggs was found to be more than 144h, indicating APMV-7 to be avirulent for chickens.

Avian Paramyxovirus - Type 8 (APMV-8)

Complete consensus genome sequences were determined for avian paramyxovirus type 8 (APMV-8) strains goose/Delaware/1053/76 (prototype strain) and pintail/Wakuya/20/78. The genome of each strain is 15,342 nucleotides (nt) long, which follows the "rule of six". The genome consists of six genes in the order of 3'-N-P/V/W-M-F-HN-L-5'. The genes are flanked on either side by conserved transcription start and stop signals, and have intergenic regions ranging from 1 to 30nt. The genome contains a 55nt leader region at the 3'-end and a 171nt trailer region at the 5'-end. Comparison of sequences of strains Delaware and Wakuya showed nucleotide identity of 96.8% at the genome level and amino acid identities of 99.3%, 96.5%, 98.6%, 99.4%, 98.6% and 99.1% for the predicted N, P, M, F, HN and L proteins, respectively. Both strains grew in embryonated chicken eggs and in primary chicken embryo kidney cells, and 293T cells. Both strains contained only a single basic residue at the cleavage activation site of the F protein and their efficiency of replication in vitro depended on and was augmented by, the presence of exogenous protease in most cell lines. Sequence alignment and phylogenic analysis of the predicted amino acid sequence of APMV-8 strain Delaware proteins with the cognate proteins of other available APMV serotypes showed that APMV-8 is more closely related to APMV-2 and -6 than to APMV-1, -3 and -4.

Avian Paramyxovirus - Type 9 (APMV-9)

The complete genome consensus sequence was determined for avian paramyxovirus (APMV) serotype 9 prototype strain PMV-9/domestic Duck/New York/22/78. The genome is 15,438 nucleotides (nt) long and encodes six non-overlapping genes in the order of 3'-N-P/V/W-M-F-HN-L-5' with intergenic regions of 0-30 nt. The genome length follows the "rule of six" and contains a 55-nt leader sequence at the 3' end and a

47-nt trailer sequence at the 5' end. The cleavage site of the F protein is I-R-E-G-R-I downward arrowF, which does not conform to the conventional cleavage site of the ubiquitous cellular protease furin. The virus required exogenous protease for in vitro replication and grew only in a few established cell lines, indicating a restricted host range. Alignment and phylogenetic analysis of the predicted amino acid sequences of APMV-9 proteins with the cognate proteins of viruses of all five genera of family Paramyxoviridae showed that APMV-9 is more closely related to APMV-1 than to other APMVs. The mean death time in embryonated chicken eggs was found to be more than 120h, indicating APMV-9 to be avirulent for chickens.

Avian Paramyxovirus - Type 10 (APMV-10) (Proposed)

The biological, serological, and genomic characterization of a paramyxovirus recently isolated from rockhopper penguins (Eudyptes chrysocome) suggested that this virus represented a new avian paramyxovirus (APMV) group, APMV10. This penguin virus resembled other APMVs by electron microscopy; however, its viral hemagglutination (HA) activity was not inhibited by antisera against any of the nine defined APMV serotypes. In addition, antiserum generated against this penguin virus did not inhibit the HA of representative viruses of the other APMV serotypes. Sequence data produced using random priming methods revealed a genomic structure typical of APMV. Phylogenetic evaluation of coding regions revealed that amino acid sequences of all six proteins were most closely related to APMV2 and APMV8. The calculation of evolutionary distances among proteins and distances at the nucleotide level confirmed that APMV2, APMV8, and the penguin virus all were sufficiently divergent from each other to be considered different serotypes. We propose that this isolate, named APMV10/penguin/Falkland Islands/324/2007, be the prototype virus for APMV10. Because of the known problems associated with serology, such as antiserum cross-reactivity and one-way immunogenicity, in addition to the reliance on the immune response to a single protein, the hemagglutinin-neuraminidase, as the sole base for viral classification, we suggest the need for new classification guidelines that incorporate genome sequence comparisons (J Virol. 2010 Nov;84(21):11496-504. Epub 2010 Aug 11)().

Chapter 4

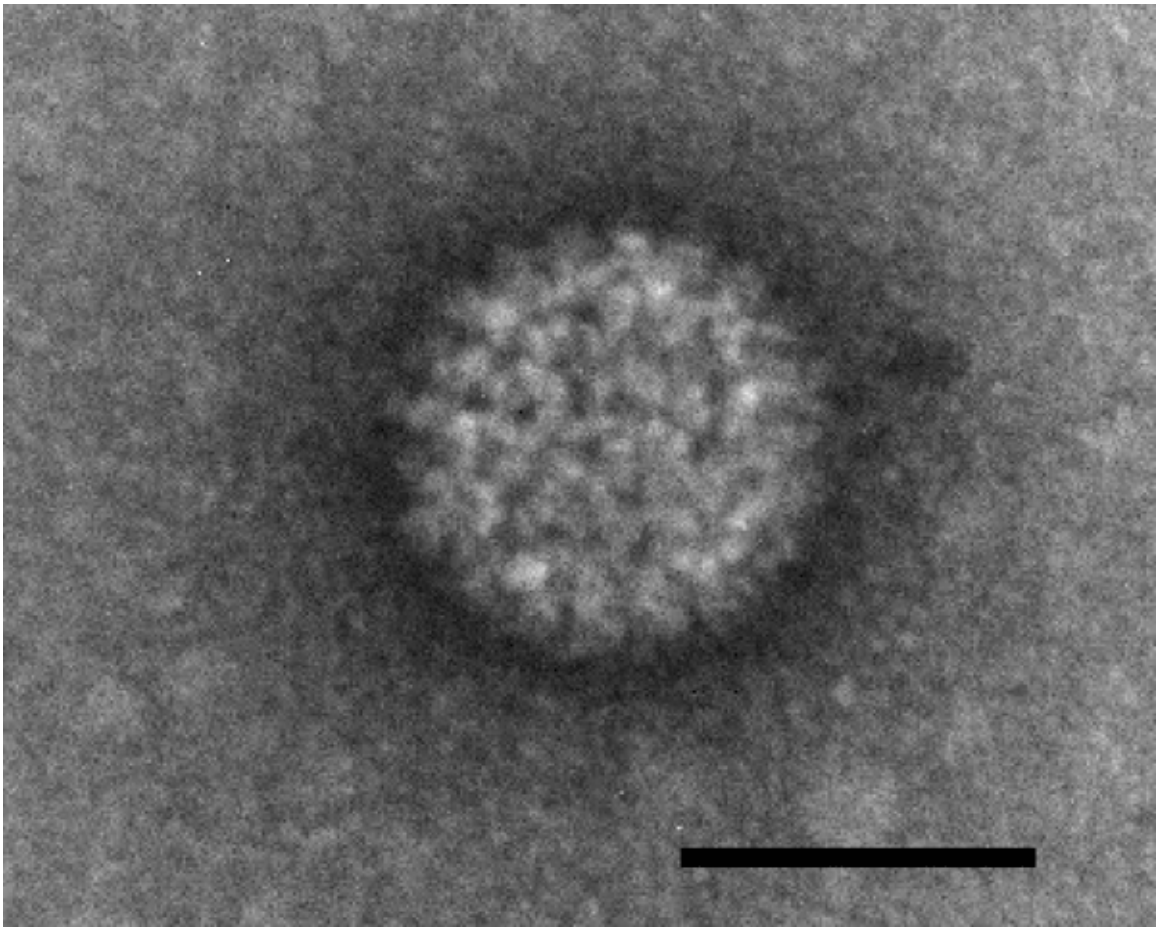
Bluetongue Disease

Bluetongue virus
Virus classification
Group: Group III (dsRNA)
Family: Reoviridae
Genus: *Orbivirus*
Species: Bluetongue virus, BTV

Bluetongue disease or **catarrhal fever** is a non-contagious, non-zoonotic, insect-borne, viral disease of ruminants, mainly sheep and less frequently cattle, goats, buffalo, deer, dromedaries and antelope. It is caused by the **Bluetongue virus** (**BTV**).

There are no reports of human transmission. Although the tongues of human patients with some types of heart disease may be blue, this sign is not related to bluetongue disease.

Pathogen and vector



Bluetongue virus particle

Bluetongue is caused by the pathogenic virus, Bluetongue virus (BTV), of the genus *Orbivirus*, is a member of the Reoviridae family. There are 25 serotypes. It is transmitted by a midge, *Culicoides imicola* and other culicoids.

Bluetongue virus

Bluetongue virus causes serious disease in livestock (sheep, goats, cattle and deer). Partly due to this BTV has been in the forefront of molecular studies for last three decades and now represents one of the best understood viruses at the molecular and structural levels. BTV, like the other members of the family is a complex non-enveloped virus with seven structural proteins and a RNA genome consisting of 10 double-stranded (ds) RNA segments of different sizes. Data obtained from studies over a number of years have defined the key players in BTV entry, replication, assembly and exit and have increasingly found roles for host proteins at each stage. Specifically, it has been possible to determine the complex nature of the virion through 3D structure reconstructions (diameter ~ 800 Å); the atomic structure of proteins and the internal capsid (~ 700 Å, the first large highly complex structure ever solved); the definition of the virus encoded

enzymes required for RNA replication; the ordered assembly of the capsid shell and the protein sequestration required for it; and the role of host proteins in virus entry and virus release. These areas are important for BTV replication but they also indicate the pathways that may be used by related viruses, which include viruses that are pathogenic to man and animals, thus providing the basis for developing strategies for intervention or prevention.

BTV is the type species of the genus *Orbivirus* within the family Reoviridae. The Reoviridae family is one of the largest families of viruses and includes major human pathogens (such as rotavirus) as well as other vertebrate, plant and insect pathogens. Like the other members of the family, Orbiviruses which encompass, besides BTV, the agents causing African horse sickness (AHSV) and epizootic hemorrhagic disease of deer (EHDV), have the characteristic double-stranded and segmented features of their RNA genomes. However, unlike the mammalian reoviruses, Orbiviruses comprising 14 serogroups, are vectored to a variety of vertebrates by arthropod species (for example, gnats, mosquitoes and ticks) and replicate in both hosts. BTV, the etiological agent of Bluetongue disease of animals, is transmitted by *Culicoides* species. In sheep BTV causes an acute disease with high morbidity and mortality. BTV also infects goats, cattle and other domestic animals as well as wild ruminants (e.g., blesbuck, white-tailed deer, elk, pronghorn antelope, etc.). The disease was first described in the late 18th century and was believed for many decades to be confined to Africa. However, to date BTV has been isolated in many tropical, subtropical and temperate zones and 24 serotypes have been identified from different parts of the world. Due to its economic significance BTV has been the subject of extensive molecular, genetic and structural studies. As a consequence it now represents one of the best characterised viruses.

Unlike the reovirus and rotavirus particles, the mature BTV particle is relatively fragile and the infectivity of BTV is lost easily in mildly acidic conditions. BTV virions (550S) are architecturally complex structures composed of 7 discrete proteins that are organised into two concentric shells, the outer and inner capsids, and a genome of 10 dsRNA segments. The outer capsid, which is composed of two major structural proteins (VP2 and VP5), is involved in cell attachment and virus penetration during the initial stages of infection. Shortly after infection, BTV is uncoated, i.e. VP2 and VP5 are removed, to yield a transcriptionally active 470S core particle which is composed of two major proteins VP7 and VP3, and the three minor proteins VP1, VP4 and VP6 in addition to the dsRNA genome. There is no evidence that any trace of the outer capsid remains associated with these cores, as has been described for reovirus. The cores may be further uncoated to form 390S subcore particles that lack VP7, also in contrast to reovirus. Subviral particles are probably akin to cores derived *in vitro* from virions by physical or proteolytic treatments that remove the outer capsid and causes activation of the BTV transcriptase. In addition to the seven structural proteins, three non-structural (NS) proteins, NS1, NS2, NS3 (and a related NS3A) are synthesised in BTV-infected cells. Of these, NS3/NS3A is involved in the egress of the progeny virus. The two remaining non-structural proteins, NS1 and NS2, are produced at high levels in the cytoplasm and are believed to be involved in virus replication, assembly and morphogenesis.

Current research

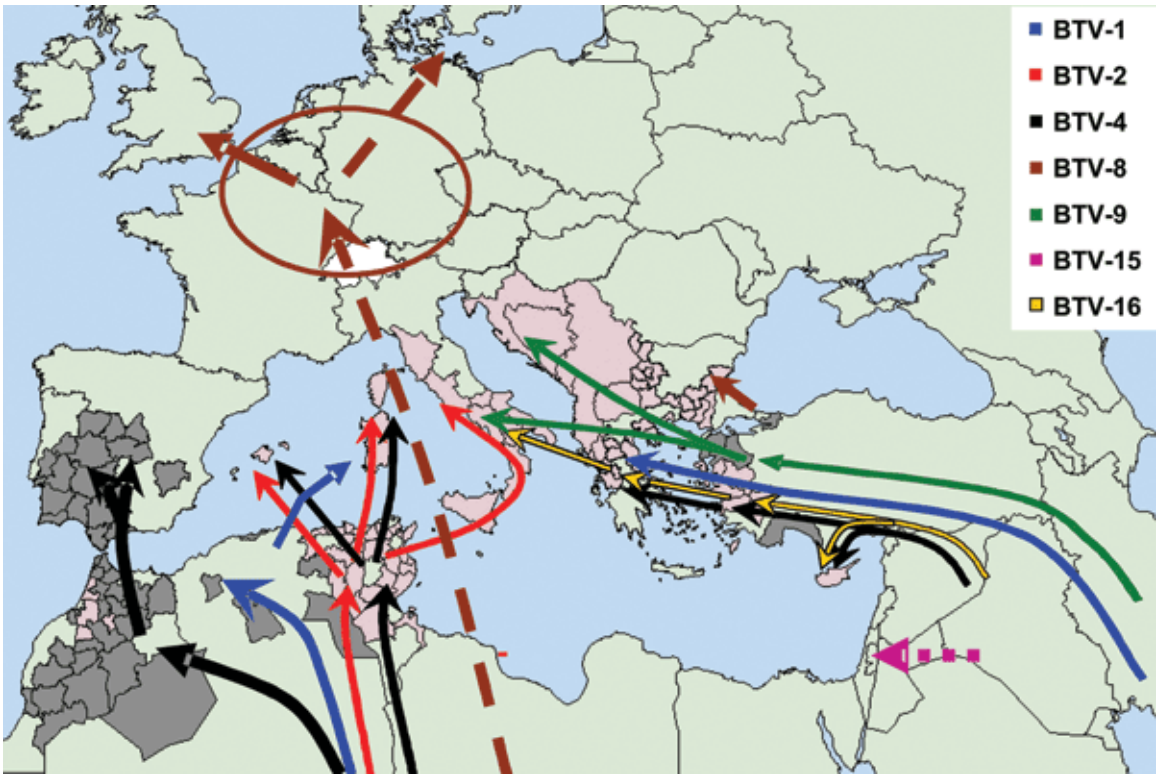
Bluetongue virus (BTV) is well characterized both genetically (the sequence was completed in 1989) and structurally. Understanding of the molecular biology of the virus and mapping the role of each protein in virus life cycle has benefited significantly through the availability of recombinant BTV proteins and sub-viral particles. In addition the structure of BTV proteins, core and virion particles have contributed greatly to understanding the mechanism of protein–protein interaction in the virus assembly pathway of BTV and other orbiviruses. Most importantly, information gained from these studies has laid sound foundation for the generation of safe BTV vaccines with the possibility of use in animals in the near future. Latterly, studies have concentrated on the fundamental mechanisms that are used by the virus to invade, replicate in and escape from susceptible host cells. Progress has been made in understanding the structure and entry of intact virus particles, the role of each enzymatic protein in the transcription complex, the critical interactions that occur between the viral non-structural proteins and viral RNA and the role of cellular proteins in non-enveloped virus egress.

Despite these advances, some critical questions remain unanswered for the BTV life cycle and a more complete understanding of the interactions between the virus and the host cell is required for these to be addressed. For example, although progress has been made in the identification of signals for the recruitment of BTV RNA segments into the virion assembly site in the host cell cytoplasm, it has not been possible yet to determine how exactly each genome segment is packaged into the progeny virus. It is also not apparent when and how these genome segments wrap around the polymerase complex once the RNA has been recruited. One of the major drawbacks of research with BTV and other members of Reoviridae has been the lack of availability of a suitable system for genetic manipulation of the virus. This has been a major obstacle in understanding the replication processes of these viruses. However, one of the recent developments in the field of BTV research has been to rescue live virus from transfection of BTV transcripts. There is no doubt that this will be soon extended to establish *in vitro* manipulative genetic system and will be utilized to address some of these remaining questions.

Very little is known of the intracellular trafficking of newly generated virions although there are some indications of involvement of the cytoskeleton, intermediate filaments and vimentin during BTV morphogenesis. Host–virus interactions during virus trafficking will be one of the future areas needing intense attention. Recent work has revealed unexpected and striking parallels between the entry and release pathways of BTV and pathways involved in entry and release of enveloped viruses. These parallels may be the result of an enveloped ancestor virus or because there are a limited number of cellular pathways that can be useful for the egress of large protein complexes from cells. It is notable that the NS3 glycoprotein of BTV is an integral membrane protein that is functionally involved in virus egress by bridging between the outer capsid protein VP2 and the cellular export machinery. Although no cell-free enveloped form of BTV has been isolated, budding of BTV particles from infected cells at the plasma membrane are quite apparent. The exact role of NS3 in this process and the role of host proteins

(Annexin II and p11, Tsg101 and MVB) and their contribution in the release of non-enveloped viruses, such as BTV, remains to be clarified.

Epidemiology



The molecular epidemiology of bluetongue virus (BTV) in Europe since 1998: routes of introduction of different serotypes and individual virus strains.

Bluetongue has been observed in Australia, the USA, Africa, the Middle East, Asia and Europe. Its occurrence is seasonal in the affected Mediterranean countries, subsiding when temperatures drop and hard frosts kill the adult midge vectors. Bluetongue has been spreading northward since October 1998, perhaps as a result of global warming, which may promote viral survival and vector longevity during milder winters. A significant contribution to the northward spread of Bluetongue disease has been the ability of Culicoides obsoletus and C.pulicaris to acquire and transmit the disease, both of which are spread widely throughout Europe. This is in contrast to the original C.imicola vector which is limited to North Africa and the Mediterranean. The relatively recent novel vector has facilitated a far more rapid spread than the simple expansion of habitats North through global warming. In August 2006, cases of bluetongue were found in the Netherlands, then Belgium, Germany, and Luxembourg. In 2007, the first case of bluetongue in the Czech Republic was detected in one bull near Cheb at the Czech-German border. In September 2007, the UK reported its first ever suspected case of the disease, in a Highland cow on a rare breeds farm near Ipswich, Suffolk. Since then the virus has spread from cattle to sheep in Britain. By October 2007 bluetongue had become a serious threat in Scandinavia and Switzerland and the first outbreak in Denmark was

reported. In autumn 2008, several cases were reported in the southern Swedish provinces of Småland, Halland, and Skåne, as well as in areas of the Netherlands bordering Germany, prompting veterinary authorities in Germany to intensify controls. Norway saw its first finding in February 2009, when cows at two farms in Vest-Agder in the south of Norway showed an immune response to bluetongue.

Although the disease is not a threat to humans the most vulnerable common domestic ruminants in the UK are cattle, goats and, especially, sheep.

Infection of the fetus

A puzzling aspect of the spread of serotype 8 BTV in northern Europe is the overwintering of the disease. Animals will recover between the end of the midge season in autumn and the beginning in spring, so it is believed that the virus somehow survives in overwintering midges. Researchers at the Institute for Animal Health (UK) has however offered an alternative hypothesis. Three cows that had recovered from bluetongue the previous autumn were exported from the Netherlands to Northern Ireland in January 2008. In February, these cows gave birth to calves that were found to be carriers of the disease. If BTV is capable of transplacental infection of the ruminant foetus, this would be a plausible way for it to overwinter. Midges will then spread the disease from the calves to other animals, starting a new season of infection. Based on this finding, it is advised to pay special attention to newborn animals in an effort to eradicate the disease.

It was previously believed that only special lab-raised BTV were capable of transplacental infection. Experiments on sheep in the 1970s showed that such infection would result in abortion or weak or deformed offspring, with some offspring carrying the virus in their bloodstream. Such damage to the offspring was also seen for the calves born in Northern Ireland.

Symptoms

Infected sheep.

A domestic yak infected with bluetongue virus. Tongue is swollen, cyanotic, and protruding from the mouth.

Major signs are high fever, excessive salivation, swelling of the face and tongue and cyanosis of the tongue. Swelling of the lips and tongue gives the tongue its typical blue appearance, though this sign is confined to a minority of the animals. Nasal symptoms may be prominent, with nasal discharge and stertorous respiration.

Some animals also develop foot lesions, beginning with coronitis, with consequent lameness. In sheep, this can lead to knee-walking. In cattle, constant changing of position of the feet gives bluetongue the nickname **The Dancing Disease**. Torsion of the neck (opisthotonos or torticollis) is observed in severely affected animals.

Not all animals develop symptoms, but all those that do lose condition rapidly, and the sickest die within a week. For affected animals which do not die, recovery is very slow, lasting several months.

The incubation period is 5–20 days, and all symptoms usually develop within a month. The mortality rate is normally low, but it is high in susceptible breeds of sheep. In Africa, local breeds of sheep may have no mortality, but in imported breeds it may be up to 90 percent.

In cattle, goats and wild ruminants infection is usually asymptomatic despite high virus levels in blood. Red deer are an exception, and in them the disease may be as acute as in sheep.

Treatment and prevention

There is no efficient treatment. Prevention is effected via quarantine, inoculation with live modified virus vaccine and control of the midge vector, including inspection of aircraft.

However, simple husbandry changes and practical midge control measures may help break the livestock infection cycle. Housing livestock during times of maximum midge activity (from dusk to dawn) may lead to significantly reduced biting rates. Similarly, protecting livestock shelters with fine mesh netting or coarser material impregnated with insecticide will reduce contact with the midges. The *Culicoides* midges that carry the virus usually breed on animal dung and moist soils, either bare or covered in short grass. Identifying breeding grounds and breaking the breeding cycle will significantly reduce the local midge population. Turning off taps, mending leaks and filling in or draining damp areas will also help dry up breeding sites. Control by trapping midges and removing their breeding grounds may reduce vector numbers. Dung heaps or slurry pits should be covered or removed, and their perimeters (where most larvae are found) regularly scraped.

Vaccine

Outbreaks in southern Europe have been caused by serotypes 2 and 4, and vaccines are available against these serotypes (ATCvet codes: QI04AA02 for sheep, QI02AA08 for cattle). However, the disease found in northern Europe (including the UK) in 2006 and 2007 has been caused by serotype 8. Vaccine companies Fort Dodge Animal Health (Wyeth), Merial and Intervet were developing vaccines against serotype 8 (Fort Dodge Animal Health has serotype 4 for sheep, serotype 1 for sheep and cattle and serotype 8 for sheep and cattle) and the associated production facilities. A vaccine for this is now available in the UK, produced by Intervet. Fort Dodge Animal Health has their vaccines available for multiple European Countries (vaccination will start in 2008 in Germany, Belgium, Switzerland, Spain and Italy).

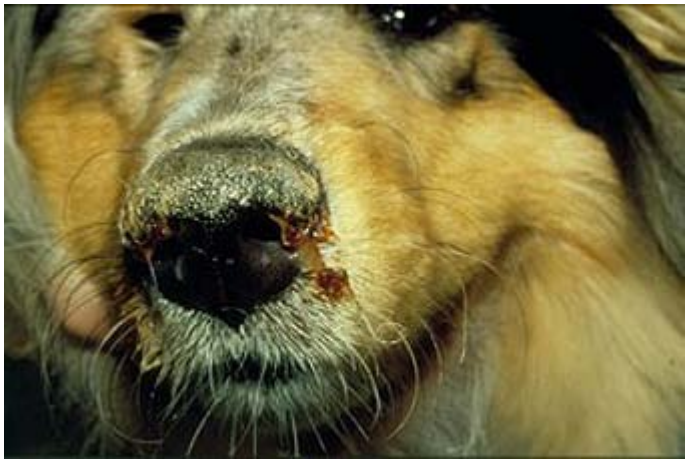
Related diseases

African horse sickness is related to Bluetongue and is spread by the same midges (*Culicoides* species). It can kill the horses it infects and mortality may go as high as 90% of the infected horses during an epidemic.

Chapter 5

Canine Distemper

Canine distemper virus	
Virus classification	
Group:	Group V ((-)ssRNA)
Order:	*Mononegavirales*
Family:	*Paramyxoviridae*
Genus:	*Morbillivirus*
Species:	***Canine Distemper Virus***



Dog infected with canine distemper. Note the purulent nasal discharge and hyperkeratotic nose.

Canine distemper is a viral disease that affects animals in the families Canidae, Mustelidae, Mephitidae, Hyaenidae, Ailuridae, Procyonidae, Pinnipedia, some Viverridae and Felidae (though not domestic cats; feline distemper or panleukopenia is a different virus exclusive to cats). It is most commonly associated with domestic animals such as dogs and ferrets, although it can infect wild animals as well. It is a single-stranded RNA

virus of the family paramyxovirus, and thus a close relative of measles and rinderpest. Despite extensive vaccination in many regions, it remains a major disease of dogs.

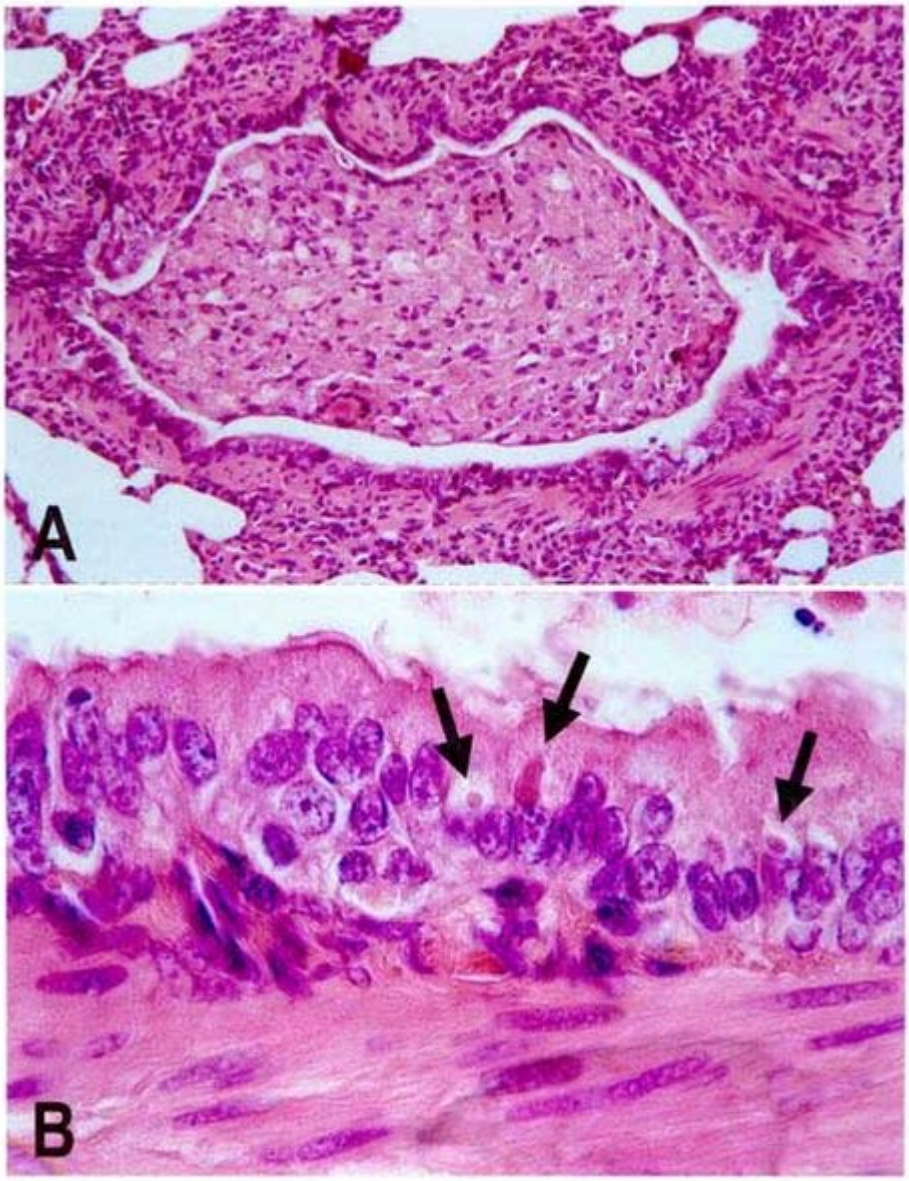
Etymology

The origin of the word *distemper* is from the Middle English *distemperen*, meaning to upset the balance of the humors, which is from the Old French *destemprer*, meaning to disturb, which is from the Vulgar Latin *distemperare*: Latin *dis-* and Latin *temperare*, meaning to not mix properly.

History

Although very similar to the measles virus, canine distemper virus (CDV) seems to have appeared more recently, with the first case described in 1905 by French veterinarian Henri Carré. It was first thought to be related to the plague and typhus, and was attributed to several species of bacteria. It now affects all populations of domestic dog and some populations of wildlife. A vaccine was developed in 1950, yet due to limited use, the virus remains prevalent in many populations. The domestic dog has largely been responsible for introducing canine distemper to previously unexposed wildlife, and now causes a serious conservation threat to many species of carnivores and some species of marsupials. The virus contributed to the near-extinction of the black-footed ferret. It also may have played a considerable role in the extinction of the Thylacine (Tasmanian tiger) and recurrently causes mortality among African wild dogs. In 1991, the lion population in Serengeti, Tanzania experienced a 20% decline as a result of the disease. The disease has also mutated to form phocid distemper virus, which affects seals.

Infection



A. Lung lesion in an African Wild Dog B. Viral inclusion bodies

Puppies from three to six months old are particularly susceptible. CDV spreads through aerosol droplets and through contact with infected bodily fluids including nasal and ocular secretions, feces, and urine 6–22 days after exposure. It can also be spread by food and water contaminated with these fluids. The time between infection and disease is 14 to 18 days, although there can be a fever from three to six days postinfection.

Canine distemper virus tends to orient its infection towards the lymphoid, epithelial, and nervous tissues. The virus initially replicates in the lymphatic tissue of the respiratory tract. The virus then enters the blood stream and infects the respiratory, gastrointestinal,

urogenital epithelium, central nervous system, and optic nerve. Therefore, the typical pathologic features of canine distemper include lymphoid depletion (causing immunosuppression and leading to secondary infections), interstitial pneumonia, encephalitis with demyelination, and hyperkeratosis of foot pads.

The mortality rate of the virus largely depends on the immune status of the infected dogs. Puppies experience the highest mortality rate, where complications such as pneumonia and encephalitis are more common. In older dogs that develop distemper encephalomyelitis, vestibular disease may present. Around 15% of canine inflammatory central nervous system diseases are a result of CDV.

Disease progression

The virus first appears in bronchial lymph nodes and tonsils two days after exposure. The virus then enters the blood stream on the second or third day. A first round of acute fever tends to begin around 3 to 8 days after infection, which is often accompanied by a low white blood cell count, especially of lymphocytes, as well as low platelet count. These signs may or may not be accompanied by anorexia, a runny nose, and discharge from the eye. This first round of fever typically recedes rapidly within 96 hours, and then a second round of fever begins around the 11th or 12th day and lasts at least a week. Gastrointestinal and respiratory problems tend to follow, which may become complicated with secondary bacterial infections. Inflammation of the brain and spinal cord otherwise known as encephalomyelitis is either associated with this, subsequently follows, or comes completely independent of these problems. A thickening of the footpads sometimes develops, and vesicularpustular lesions on the abdomen usually develop. Neurological symptoms typically are found in the animals with thickened footpads from the virus. About half of sufferers experience meningoencephalitis.

Gastrointestinal and respiratory symptoms

Commonly observed signs are a runny nose, vomiting and diarrhea, dehydration, excessive salivation, coughing and/or labored breathing, loss of appetite, and weight loss. When and if the neurological symptoms develop, incontinence may ensue.

Neurological symptoms

The symptoms within the central nervous system include a localized involuntary twitching of muscles or groups of muscles, seizures often distinguished by salivation, and jaw movements commonly described as “chewing gum fits,” or more appropriately as "distemper myoclonus." As the condition progresses, the seizures worsen and advance to grand mal convulsions, followed by death of the animal. The animal may also show signs of sensitivity to light, incoordination, circling, increased sensitivity to sensory stimuli such as pain or touch, and deterioration of motor capabilities. Less commonly, it may lead to blindness and paralysis. The length of the systemic disease may be as short as 10 days, or the start of neurological symptoms may not come until several weeks or months

later. Those few that survive usually have a small tic or twitch of varying levels of severity. With time, this tic will usually diminish somewhat in its severity.

Diagnosis

The above symptoms, especially fever, respiratory signs, neurological signs, and thickened footpads found in unvaccinated dogs strongly indicate canine distemper. However, several febrile diseases match many of the symptoms of the disease and only recently has distinguishing between canine hepatitis, herpes virus, parainfluenza and leptospirosis been possible. Thus, finding the virus by various methods in the dog's conjunctival cells gives a definitive diagnosis. In older dogs that develop distemper encephalomyelitis, diagnosis may be more difficult since many of these dogs have an adequate vaccination history.

The most reliable test to confirm distemper is a Brush Border slide/smear of the bladder transitional epithelium of the inside lining from the bladder, stained with Dif-Quick. These cells will always have inclusions. Inclusions in these cells which will stain a carmine red color and be para nuclear in the cytoplasm of infected cells. About 90% of the bladder cells will be positive for inclusions in the early stages of distemper. This is good for at least the first 21 days from onset of the disease. After this point, it gets harder to detect as the disease progresses further in the stages and the physical clinical signs will become quite obvious.

Prevention

There exist a number of vaccines against canine distemper for dogs (ATCvet code: QI07AD05 and combinations) and domestic ferrets (QI20DD01), which in many jurisdictions are mandatory for pets. The type of vaccine should be approved for the type of animal being inoculated, or else the animal could actually contract the disease from the vaccine. A dog who has eaten meat infected with rinderpest can also sometimes receive temporary immunity. Infected animals should be quarantined from other dogs for several months due to the length of time the animal may shed the virus. The virus is destroyed in the environment by routine cleaning with disinfectants, detergents, or drying. It does not survive in the environment for more than a few hours at room temperature (20–25 °C), but can survive for a few weeks in shady environments at temperatures slightly above freezing. It, along with other labile viruses, can also persist longer in serum and tissue debris.

Treatment

Until recently, canine distemper has been associated with a long history of pessimism with respect to treatment of infected animals and the disease was usually assumed to have a poor prognosis. Most care offered was only palliative, geared toward easing the suffering. Several factors had an important role in maintaining the status quo.

Misdiagnosis, miseducation, a lack of treatment, inadequate, or inappropriate treatment has historically created barriers and slowed the development of effective solutions to the disease. Even today, the needs of affected animals often go unrecognized until the disease reaches the nervous phase, and the distressed behavior and/or impaired functional state of the animal is more obvious and less responsive to treatment.

Research and funding for the most part have focused on vaccination rather than on finding a cure for distemper.

Another factor is the outdated theory that the injuries that occurred were the result of a strictly autoimmune reaction, the thought being that initially the canine distemper virus was introduced, but then subsequently eliminated. However, the cytokines continued to attack and damage healthy tissue in the absence of a current pathogen. Based on that faulty assumption, anti-inflammatory and immunosuppressive drugs have been prescribed by some veterinarians in an attempt to bring the effects of the condition under control.

It was later considered that the action of macrophages on infected nerve cells indicated that the autoimmune reaction was likely a direct consequence of the presence of the virus. Often, owners seek expert help only when the disease is in its advanced stages (nervous phase) and prescription anti-inflammatory drugs (which are usually corticosteroids) undermine the immune system of the animal, allow the proliferation of the virus, and the autoimmune reaction increases as a means of containment of infected cells.

The most successful treatments for canine distemper are adaptations of established treatments used for other diseases caused by similar viruses, such as ribavirin and vitamin A, which are used to treat measles, which is in the same viral genus (*Morbillivirus*), and interferon alpha, also used for the treatment of measles and a vaccine used to immunize birds against Newcastle disease, which is in the same viral family, but a different genus (*Paramyxoviridae* - AVULAVIRUS). However, there is absolutely no proof that this treatment works and most veterinary professionals believe it to be entirely invalid as a treatment protocol.cite

The first references to suggest effective treatments for similar viruses could be effective for canine distemper arose when studies found that canine distemper was a disease comparable to measles and infected animals could be used to develop new technologies for treatment of measles. The question of whether the reciprocal would be true was resolved when studies assessed the efficacy of traditional treatments for measles, which were then successfully applied to animals with distemper.

Initially, induction of high levels of vitamin A, used to treat measles (including being recommended by the World Health Organization), produced a 100% cure in animals experimentally infected. The infected group given no vitamin A supplementation all died. Currently, it is known that the direct inhibitive effect of retinoids (vitamin A and subproducts) on the replication of the measles virus is what confirms their choice as a treatment for canine distemper.

The confirmation of the effectiveness of vitamin A in the treatment of canines, especially dogs, is its ability to convert the vitamin A into nontoxic esters. This characteristic of carnivores is well known; the risk of hypervitaminosis due to the maintenance of high doses is quite low. For dogs, there is a benchmark to measure the risk: a national research study found it takes a dose of 300,000 IU/kg daily for thirty days before the first signs of hypervitaminosis appear, and sixty days of ingestion at this dosage to kill the animal. This dosage, 300,000 IU / kg, is sixty times greater than the toxic limit established for humans.

The mechanisms of action that explain its effectiveness in the treatment of distemper (and measles) remain unexplained. Some evidence points to an indirect action, such as confirming there is a reduction in the amounts of vitamin A during infection, pointing to the hypothesis that it is raw material for some mechanism of resistance to infection. That the anti-infective characteristic is not specific to vitamin A is a mystery; however, there was no doubt about its effectiveness, action mechanisms elucidated or not.

The adoption of ribavirin as a treatment for canine distemper followed the same steps as vitamin A; it was the principle used in cases of subacute sclerosing panencephalitis under measles. The first verification of the effectiveness occurred "in vitro," It was observed that the distemper virus is very susceptible to ribavirin, and 0.02 to 0.05 micromols are needed to induce its mechanism of error catastrophe and the inhibitory effect on virus replication by 50%.

The main concern in the use of ribavirin was the result of its interaction with the blood-brain barrier. As the brain is an immunologically privileged area, the concern was the capacity of ribavirin to overcome this barrier. In a study using mice with encephalitis due to measles, it was found that once the virus has become established in the nervous phase, the blood-brain barrier, fails in a way, reducing the restriction to the action of the ribavirin in these areas. The verification of all these results *in vivo* resulted in an effectiveness of 80% in animals that had already reached the nervous phase of viral infection. The application of ribavirin demands a close monitoring of the animal due the risk of leukopenia and the ingestion of long-chain tryglicerides (fats) are needed to better absorb the drug and for preservation of gastric tissues, which are quite susceptible to it.

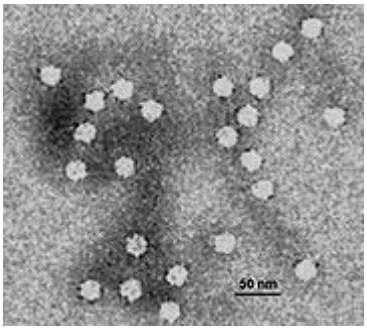
Canine distemper virus and Paget's disease

Paramyxoviruses, such as CDV, measles, respiratory syncytial virus, simian virus 5, and parainfluenza virus Type 3, have long been suspected as the causative agents of Paget's disease, a focal destructive disease of bone. Most studies, however, have pointed more directly at CDV and measles. A virus detection technique, *in situ* RT-PCR, has found CDV in 100% of Paget's disease samples, whereas other virus detection techniques have been less accurate.

Chapter 6

Canine Parvovirus

Canine parvovirus 2



Electron micrograph of canine parvovirus

Virus classification

Group: Group II (ssDNA)

Family: *Parvoviridae*

Genus: *Parvovirus*

Species: ***Canine parvovirus 2***

Canine parvovirus type 2 (**CPV2**, colloquially **parvo**) is a contagious virus mainly affecting dogs. The disease is highly contagious and is spread from dog to dog by direct or indirect contact with their feces. It can be especially severe in puppies that are not protected by maternal antibodies or vaccination. It has two distinct presentations, a cardiac and intestinal form. The common signs of the intestinal form are severe vomiting and dysentery. The cardiac form causes respiratory or cardiovascular failure in young puppies. Treatment often involves veterinary hospitalization. Vaccines can prevent this infection, but mortality can reach 91% in untreated cases. Canine parvovirus will not infect humans.

History

Parvovirus CPV2 is a relatively new disease that appeared in the late 1970s. It was first recognized in 1978 and spread worldwide in one to two years. The virus is very similar to feline panleukopenia (also a parvovirus); they are 98% identical, differing only in two amino acids in the viral capsid protein VP2. It is also highly similar to mink enteritis, and the parvoviruses of raccoons and foxes. The early belief was that the feline panleukopenia mutated into CPV2. It is possible that CPV2 is a mutant of an unidentified parvovirus (similar to feline parvovirus (FPV)) of some wild carnivore. A strain of CPV2b (strain FP84) has been shown to cause disease in a small percentage of domestic cats, although vaccination for FPV seems to be protective. CPV2, however, does not cause disease in cats and does so only mildly in mink and raccoons, and is a virus almost exclusively affecting canines.

Two more strains of canine parvovirus CPV2a and CPV2b were identified in 1979 and 1984 respectively. Most cases of canine parvovirus infection are believed to be caused by these two strains, which have replaced the original strain, and the present day virus is different from the one originally discovered although they are indistinguishable by most routine tests. A third type, CPV2c (a Glu-426 mutant), has been discovered in Italy, Vietnam, and Spain.

Virology

CPV2 is a non-enveloped single-stranded DNA virus. The name comes from the Latin *parvus*, meaning small, as the virus is only 20 to 26 nm in diameter. It has an icosahedral symmetry. The genome is about 5000 nucleotides long. CPV2 continues to evolve, and the success of new strains seems to depend on extending the range of hosts affected and improved binding to its receptor, the canine transferrin receptor. CPV2 has a high rate of evolution, possibly due to a rate of nucleotide substitution that is more like RNA viruses such as Influenzavirus A. In contrast, FPV seems to evolve only through random genetic drift.

CPV2 affects dogs, wolves, foxes, and other canids. CPV2a and CPV2b have been isolated from a small percentage of symptomatic cats and is more common than feline panleukopenia in big cats.

Previously it has been thought that the virus does not undergo cross species infection. However studies in Vietnam have shown that CPV2 can undergo minor antigenic shift and natural mutation to infect felids. Analyses of feline parvovirus (FPV) isolates in Vietnam and Taiwan revealed that more than 80% of the isolates were of the canine parvovirus type, rather than feline panleukopenia virus (FPLV). CPV2 may spread to cats easier than dogs and undergo faster rates of mutation within that species.

Variants

There are two types of canine parvovirus called canine minute virus (CPV1) and CPV2. CPV2 causes the most serious disease and affects domesticated dogs and wild canids. There are variants of CPV type 2 called CPV-2a, CPV-2b and CPV-2c. The antigenic patterns of 2a and 2b are quite similar to the original CPV type 2. Variant 2c however has a unique pattern of antigenicity. This has led to claims of ineffective vaccination of dogs, but studies have shown that the existing CPV vaccines based on CPV type 2b, provide adequate levels of protection against CPV type 2c. However, there are reports that outdated vaccines based on the old CPV-type 2 may not afford sufficient cross-protection against the type 2c variant.

Pathophysiology

There are two forms of CPV2: intestinal and cardiac. Puppies are most susceptible, but more than 80 percent of adult dogs show no symptoms. With severe disease, dogs can die within 48 to 72 hours without treatment by fluids and antibiotics. In the more common, less severe form, mortality is about 10 percent. Certain breeds, such as Rottweilers, Doberman Pinschers, and Pit bull terriers as well as other black and tan colored dogs may be more susceptible to CPV2 Along with age and breed, factors such as a stressful environment, concurrent infections with bacteria, parasites, and canine coronavirus increase a dog's risk of severe infection. Dogs who catch Parvovirus usually die from the dehydration it causes or secondary infection rather than the virus itself.

Intestinal form

Dogs become infected through oral contact with CPV2 in feces, infected soil, or fomites that carry the virus. Following ingestion, the virus replicates in the lymphoid tissue in the throat, and then spreads to the bloodstream. From there, the virus attacks rapidly dividing cells, notably those in the lymph nodes, intestinal crypts, and the bone marrow. There is depletion of lymphocytes in lymph nodes and necrosis and destruction of the intestinal crypts. Anaerobic bacteria that normally reside in the intestines can then cross into the bloodstream, a process known as translocation, and cause sepsis. The most common bacteria involved in severe cases are Clostridia, Campylobacter and salmonella species. This can lead to a syndrome known as Systemic inflammatory response syndrome(SIRS). SIRS leads to a range of complications such as hypercoagulability of the blood, endotoxaemia and acute respiratory distress syndrome(ARDS). Bacterial Myocarditis has also been reported secondarily to sepsis. Dogs with CPV are at risk of intussusception, a condition where part of the intestine prolapses into another part. Three to four days following infection, the virus is shed in the feces for up to three weeks, and the dog may remain an asymptomatic carrier and shed the virus periodically. The virus is usually more deadly if the host is concurrently infested with worms or other intestinal parasites.

Cardiac form

This form is less common and affects puppies infected in the uterus or shortly after birth until about 8 weeks of age. The virus attacks the heart muscle and the puppy often dies suddenly or after a brief period of breathing difficulty. On the microscopic level, there are many points of necrosis of the heart muscle that are associated with mononuclear cellular infiltration. The formation of excess fibrous tissue (fibrosis) is often evident in surviving dogs. Myofibers are the site of viral replication within cells. The disease may or may not be accompanied with the signs and symptoms of the intestinal form. However, this form is now rarely seen due to widespread vaccination of breeding dogs.

Even less frequently, the disease may also lead to a generalized infection in neonates and cause lesions and viral replication and attack in other tissues other than the gastrointestinal tissues and heart, but also brain, liver, lungs, kidneys, and adrenal cortex. The lining of the blood vessels are also severely affected, which lead the lesions in this region to hemorrhage.

Infection of the fetus

This type of infection can occur when a pregnant female dog is infected with CPV2. The adult may develop immunity with little or no clinical signs of disease. The virus may have already crossed the placenta to infect the fetus. This can lead to several abnormalities. In mild to moderate cases the pups can be born with neurological abnormalities such as cerebellar hypoplasia.

Signs and symptoms

Dogs that develop the disease show symptoms of the illness within 5 to 10 days. The symptoms include lethargy, vomiting, fever, and diarrhea (usually bloody). Diarrhea and vomiting result in dehydration and secondary infections can set in. Due to dehydration, the dog's electrolyte balance can become critically affected. Because the normal intestinal lining is also compromised, blood and protein leak into the intestines leading to anemia and loss of protein, and endotoxins escaping into the bloodstream, causing endotoxemia. Dogs have a distinctive odor in the later stages of the infection. The white blood cell level falls, further weakening the dog. Any or all of these factors can lead to shock and death. The first sign of CPV is lethargy. Usually the second symptoms would be loss of appetite or diarrhea followed by vomiting.

Diagnosis

Diagnosis is made through detection of CPV2 in the feces by either an EIA or a hemagglutination test, or by electron microscopy. PCR has become available to diagnose CPV2, and can be used later in the disease when potentially less virus is being shed in the feces that may not be detectable by EIA. Clinically, the intestinal form of the infection can sometimes be confused with coronavirus or other forms of enteritis. Parvovirus, however, is more serious and the presence of bloody diarrhea, a low white blood cell

count, and necrosis of the intestinal lining also point more towards parvovirus, especially in an unvaccinated dog. The cardiac form is typically easier to diagnose because the symptoms are distinct.

Prevention and decontamination

Prevention is the only way to ensure that a puppy or dog remains healthy because the disease is extremely virulent and contagious. The virus is extremely hardy and has been found to survive in feces and other organic material such as soil for over a year. It survives extremely cold and hot temperatures. The only household disinfectant that kills the virus is bleach.

Puppies are generally vaccinated in a series of doses, extending from the earliest time that the immunity derived from the mother wears off until after that passive immunity is definitely gone. Older puppies (16 weeks or older) are given 3 vaccinations 3 to 4 weeks apart. The duration of immunity of vaccines for CPV2 has been tested for all major vaccine manufacturers in the United States and has been found to be at least three years after the initial puppy series and a booster 1 year later.

A dog that successfully recovers from CPV2 sheds the virus for a few days. Ongoing infection risk is primarily from fecal contamination of the environment due to the virus's ability to survive many months in the environment. Neighbours and family members with dogs should be notified of infected animals so that they can ensure that their dogs are vaccinated or tested for immunity. The vaccine will take up to 2 weeks to reach effective levels of immunity; the contagious individual should remain in quarantine until other animals are protected.

Treatment

Survival rate depends on how quickly CPV is diagnosed, the age of the animal and how aggressive the treatment is. Treatment for severe cases that are not caught early usually involves extensive hospitalization, due to the severe dehydration and damage to the intestines and bone marrow. A CPV test should be given as early as possible if CPV is suspected in order to begin early treatment and increase survival rate if the disease is found.

Treatment ideally consists of crystalloid IV fluids and/or colloids, antinausea injections (antiemetics) such as metoclopramide, dolasetron, ondansetron and prochlorperazine, and antibiotic injections such as cefoxitin, metronidazole, timentin, or enrofloxacin. IV fluids are administered and antinausea and antibiotic injections are given subcutaneously, intramuscularly, or intravenously. The fluids are typically a mix of a sterile, balanced electrolyte solution, with an appropriate amount of B-complex vitamins, dextrose and potassium chloride. Analgesic medications such as buprenorphine are also used to counteract the intestinal discomfort caused by frequent bouts of diarrhea.

In addition to fluids given to achieve adequate rehydration, each time the puppy vomits or has diarrhea in a significant quantity, an equal amount of fluid is administered intravenously. The fluid requirements of a patient are determined by the animal's body weight, weight changes over time, degree of dehydration at presentation and surface area.

A blood plasma transfusion from a donor dog that has already survived CPV is sometimes used to provide passive immunity to the sick dog. Some veterinarians keep these dogs on site, or have frozen serum available. There have been no controlled studies regarding this treatment. Additionally, fresh frozen plasma and human albumin transfusions can help replace the extreme protein losses seen in severe cases and help assure adequate tissue healing.

Once the dog can keep fluids down, the IV fluids are gradually discontinued, and very bland food slowly introduced. Oral antibiotics are administered for a number of days depending on the white blood cell count and the patient's ability to fight off secondary infection. A puppy with minimal symptoms can recover in 2 or 3 days if the IV fluids are begun as soon as symptoms are noticed and the CPV test confirms the diagnosis. If more severe, depending on treatment, puppies can remain ill from 5 days up to 2 weeks. However, even with hospitalization, there is no guarantee that the dog will be cured and survive.

Unconventional treatments

There is no specific antiviral treatment for CPV. However, there have been anecdotal reports of oseltamivir (Tamiflu) reducing disease severity and hospitalization time in canine parvovirus infection. The drug may limit the ability of the virus to invade the crypt cells of the small intestine and decrease gastrointestinal bacteria colonization and toxin production. There is also anecdotal evidence suggesting that colloidal silver is effective at treating CPV although currently regulatory authorities are discouraging its use due to potential toxicity issues and lack of demonstrated efficacy. Lastly, recombinant feline interferon omega (rFeIFN-ω), produced in silkworm larvae using a baculovirus vector, has been demonstrated by multiple studies to be an effective treatment.

Canine Parvovirus Trial

In April 2009; IMULAN BioTherapeutics, LLC initiated a study to examine the effects of a new biologic for treatment of canine parvovirus. The study is expected to be completed in 2009 and will evaluate clinical signs and diagnostics (company website).

Prognosis

Untreated cases of CPV2 have a mortality rate approaching 91%. With aggressive therapy, survival rates may approach 80-95%.

Chapter 7

Equine Influenza

Equine influenza (**Horse flu**) is the disease caused by strains of Influenza A that are enzootic in horse species. Equine influenza occurs globally, and is caused by two main strains of virus: equine-1 (H7N7) and equine-2 (H3N8). The disease has a nearly 100% infection rate in an unvaccinated horse population with no prior exposure to the virus.

While equine influenza is historically not known to affect humans, the impact of an outbreak would have been devastating. Since people heavily relied upon horses for communication (postal service), military (cavalry) and general transport, the social and economic impact of widespread equine disease would have been devastating. However, in modern times the ramifications of equine influenza are most clear in the modern racing industry.

Characteristics

Equine influenza is characterized by a very high rate of transmission among horses, and has a relatively short incubation time of 1–5 days.

Horses with horse flu can run a fever, have a dry hacking cough, have a runny nose, and become depressed and reluctant to eat or drink for several days, but they usually recover in 2 to 3 weeks.

An 1872 report on equine influenza describes the disease as:

"An epizootic specific fever of a very debilitating type, with inflammation of the respiratory mucous membrane, and less frequently of other organs, having an average duration of ten to fifteen days, and not conferring immunity from a second attack in subsequent epizootics."

– James Law, Report of the Commissioner of Agriculture for the year 1872

Causes

Equine influenza is caused by several strains of the Influenza A virus endemic to horses. Viruses that cause equine influenza were first isolated in 1956. The viruses can cross the species-barrier to cause an epizootic disease in humans, and recently, in dogs.

The equine-1 virus affects heart muscle, while the equine-2 virus is much more severe and systemic.

The disease is primarily spread between infected horses. Exposure to infected waste materials (urine and manure) in stables leads to rapid spread of the disease.

History

A comprehensive report describing the disease - compiled in response to the 1872 outbreak of the disease in North America - provided a thorough examination of the history of the disease.

Early records

The report notes putative cases dating as far back as Hippocrates and Livius. Absyrtus, a Greek veterinarian from 330 CE, described a disease in the horse population having the general characters of influenza, which the report mentions as the earliest clear record of equine influenza in the lower animals.

The report notes the next recorded equine influenza case in 1299, the same year that a catarrhal epidemic affected Europe. Spanish records note cases in which "The horse carried his head drooping, would eat nothing, ran from the eyes, and there was hurried beating of the flanks. The malady was epidemic, and in that year one thousand horses died."

Prevalence of influenza is found in historic records in the centuries of the Middle Ages, but direct implication of horses is not always clear. Neither are recorded instances of record deaths among horses and other animals clear on the exact cause of death.

1872 American outbreak

An epizootic outbreak of equine influenza during 1872 in North America became known as "The Great Epizootic of 1872." The outbreak is known as the "most destructive recorded episode of equine influenza in history." The impact of the outbreak is marked as one of the major contributors to the Panic of 1873 in the United States.

The first cases of disease in pasture horses were in the townships of Scarborough, York, and Markham in Ontario, Canada. By October 1, 1872, the first case occurred in Toronto. It took only three days before all the street car horses and major livery-stables were

affected. By the middle of the month, Montreal, Detroit, and most of the Dominion of Canada and New England reported cases.

By the start of November Ohio, Massachusetts, and South Carolina were reporting cases. So was Chicago, Illinois. The contagion reached Florida and Louisiana by the end of November and Cuba on December 7. The height of the plague was December 14, when the Mexican government had to supply disease-free horses to the stricken United States. One major factor was that cities were not clean back in those days, which meant that germs spread all that much more quickly (especially through contaminated food and water).

The rate of infected horses approached 100%, and mortality rates ranged between 1% and 10%. Many horses were unable to stand in their stalls. Those that could stand coughed violently and were too weak to pull any loads or support riders.

The street railway industry ground to a halt in late 1872. Every aspect of American transportation was affected. Locomotives came to a halt as coal could not be delivered to power them, while fires in many major cities raged unchecked. One fire in Boston destroyed over 700 buildings (November 9-10 of that year). Indeed, many a fireman just stood there helpless and horror-stricken, for lack of any equipment to work with. Even the United States Army Cavalry was reduced to fighting on foot against the Apaches (as the plague had swept not only south to Mexico and Cuba, but also west to the Pacific Ocean within two months!), who likewise found their mounts too sick to do battle. The outbreak forced men to pull wagons by hand; while trains and ships full of cargo sat unloaded (perishables, such as milk, often became spoiled), tram cars stood idle and deliveries of basic community essentials (including food and clothing) were no longer being made. The Long Riders' Guild Academic Foundation founder CuChullaine O'Reilly said, "The Great Epizootic was the worst equestrian catastrophe in the history of the United States - and perhaps the world."

The Great Epizootic of 1872 was also a contributor to the Panic of 1873, which lasted six years; hence, it would be about seven years total before things were restored to normal operation.

2007 Australian outbreak

The continent/country of Australia had remained free of equine influenza until an outbreak in August 2007. While the virus was successfully contained and Australia has returned to its equine influenza-free status, the outbreak had significant effects to the country's racing industry.

Prevention

Prevention of equine influenza outbreaks are maintained through vaccines and hygiene procedures. Countries that are equine influenza-free will normally impose strict and rigorous quarantine measures.

Vaccines

Vaccines (ATCvet codes: QI05AA01 inactivated, QI05AD02 live, plus various combinations) are a major defense against the disease. Vaccination schedules generally require a primary course of vaccines, followed by booster shots. Standard schedules may not maintain absolutely foolproof levels of protection and more frequent administration is advised in high-risk situations.

The UK requires that horses participating in show events be vaccinated against equine flu, and a vaccination card must be produced; the FEI requires vaccination every 6 months.

Chapter 8

Feline Coronavirus

Feline coronavirus FCoV

Feline coronavirus (FCoV) is an ARN virus that can infect cats. It has 2 different forms:

1. An enteric one (intestinal) called FECV (Feline Enteric Coronavirus)
2. And one that causes the feline infectious peritonitis FIPV (Feline infectious peritonitis virus).

They are part of the coronavirus group 1, as well as the porcine gastroenteritis swine coronavirus (TGEV), the canine coronavirus (CCOV) and some human coronavirus.

The feline coronavirosis

The digestive form FECV

FECV virus is responsible for a gastrointestinal epithelial cells infection of the cat. This intestinal infection has few signs, and it is usually chronic. Then, the virus is excreted in the animal's feces (healthy carrier). This port can be searched out by Polymerase Chain Reaction or "PCR" (rectal sampling and PCR detection on it).

Cats living in groups can contaminate each other during visits to the litter tray. Some cats are resistant to the virus and have no infection (no carrying digestive). Others will be carriers of FECV some time. They may heal spontaneously, but acquired immunity is short, they are going to infect another time during a few weeks if they are living in a group with persistent excretory (healthy carriers). Some cats never heal and excretory remain permanently.

Passage from the FECV form to the FIPV one

Random errors replication in the enterocyte, sometimes the virus can mutate from FECV to FIPV.

More the cat group is big (n cats) and more the epidemiological risk of mutation (E) is high:

$E = (n^2)-n$

A house hosting 2 cats has a mutation risk = 2. If 4 kittens born in this house, the risk growth up from 2 to 34.

It's easy to understand, cats are permanently infected with a larger number of different strains of virus (as different from cats), visiting litter tray.

In the natural state cats are solitary animals, they don't sharing their areas (hunting area, rest area, area of defecation ...). Often domestic cats live in a group, it's a high epidemiological risk situation.

After this mutation, the FCoV acquires a tropism for the macrophages while losing the intestinal tropism.

The feline infectious peritonitis and the FIPV virus

In a cat group, the overcrowding and the risk of mutation (from FECV to FIPV) are risk factors for the development of cases of feline infectious peritonitis *'(FIP)'* . However, the FIP will mainly develop in cats whose immunity is low (younger kittens, old cats, immunosuppression due to viral — FIV (Feline immunodeficiency virus) and / or FeLV Feline leukemia virus) - stress including stress of separation and adoption).

Infection of macrophages by FIPV is responsible for a fatal granulomatous vasculitis, the FIP.

Therefore, FIP can occur in 2 factors are meeting: **(virus mutation) AND (cat field)**

- Mutation of the virus: virological factor related to the number of replication ...
- Field of cats related to its age, its genetics, its stress level, which determines the immune status and thus its ability (or not) to contain the infection at a low level.

There are 2 clinical forms of FIP *'(feline infectious peritonitis)'*:

1. An effusive form with effusion peritoneal fluid (= ascites), pleural and pericardial,
2. And a dry form.

Usually, the outcome is fatal, except for a few reported cases of healing with the feline omega interferon treatment.

Molecular aspects of the virus fusion to the host cell

The 2 forms of FCoV, the enteric one (FECV) and the FIP one (FIPV) have both from 2 different serotypes (with different antigens that cause different antibodies production of: serotype).

The FCoV serotype I (also called Type I) is most frequent: 80% of infections are due to type I FECV that could mutate to FIPV type I. Serotype I FCover Cultures are not easy, so studies about this serotype are few.

The FCoV serotype II (also called type II) are less frequent: FECV type II that can mutate to FIPV type II. FCoV type II is a recombinant virus type I with spikes genes (S protein) replacement from FCoV by the canine enteric coronavirus (CCOV)spikes. The type II cultures are easier, so we have many studies about this type II (though less common).

Model: "data about FCoV type II"

Virus fusion

FCoV is an RNA viruses that is included in the coronaviruses group 1. Coronaviruses are covered with several types of proteins "S proteins" (or E2) forming a crown of Spike to the virus surface. Coronaviruses take their name from the observation of this crown by electron microscopy

These spikes of Cov (group 1 and serotype II) are responsible for the infection power of the virus by binding him to a membrane receptor of the host cell: the Feline Amino peptidase N (fAPN).

The viral receptor: aminopeptidase N (APN)

fAPN (feline), hAPN (human) and pAPN (porcine) differ in some areas of N-glycosylation, that can explain:

- All strains of the coronavirus study group 1 (feline, porcine and human) can bind to the feline aminopeptidase N fapn but:
- The human coronavirus can bind to the human APN (HAPN) but not to the porcine form receptor (pAPN)
- The pig coronavirus can bind to the porcine APN (pAPN) but not the human form receptor (hAPN).

At the cellular level this facts can explain why the glycosylation level of enterocytes APN is important for the binding of virus to the receptor.

About viral spikes

The FECV spikes have a high affinity for enterocytes fAPN, while the mutant FIPV spikes have a high affinity for the macrophages ones.

During the viral replication cycle, spikes proteins have a maturation in the host cell golgi with a high mannose glycosylation.

This spike manno-glycosylation stage is indispensable for the acquisition of coronavirus infesting power.

Data about FCoV type I

The receptor?

In 2007, it is well established that serotype I do not work with the FCoV fapn receptor. The FCoV type I receptor still is unknown.

News about CoV receptor

- The human CoV SARS binds to the Angiotensin-converting enzyme ACE II. The ACE II is also called *'L-SIGN'*.
- Coronaviruses bind to macrophages via the "DC-SIGN". Sign-DC = Dendritic cell**.**

ACE and DC-SIGN are two trans-membrane receptors (mannose receptors) which can bind *'the plant lectins C-type mannose binding'*. DC-SIGN and ACE serve as retrovirus receptors.

- Aminopeptidase N has the same ability to interact with plant lectins C-type mannose-binding and also serves as a receptor for a retrovirus.
- Angiotensin-converting enzyme ACE, aminopetidase A and aminopeptidase N have cascading actions in the renin-angiotensin-aldosterone system, which suggests a common phylogenetic origin between these molecules.
- Some advanced studies have shown a high homology between the Aminopeptidase N and the Angiotensin-converting enzyme.

- It is likely that the unknown FCoV serotype I receptor is also of this receptor family that acting with the mannose binding lectins.

Role of mucus and glycocalix — Interactions between viruses and sialic acid

Sialic acid is a component of the complex sugar glycocalix, i.e. mucus protecting the gastrointestinal mucosa (but also respiratory one...). Sialic acid is an important facilitating fusion factor of any viruses to the host cell. This is very well detailed for the flu.

Extensive data also show that processes using sialic acid are directly involved in the interaction with receptors lectins.

About swine enteric coronavirus (group 1), it has been demonstrated that fusion to the enterocyte was through binding to the APN in the presence of sialic acid, the 2 elements are necessary.

About Felin coronavirus infections, it seems that the infection is sialic acid dependent.

Inhibition of the fusion: some studies (in vitro)

To inhibit the fusion of the virus to the cell, several solutions are possible:

1. modify glycosylation level of the viral spikes,
2. Change the level of glycosylation of fAPN,
3. Compete with the spikes, with molecules that will bind to fapn (occupation of the binding site),
4. Inhibit the binding depends on the sialic acid mucus.

- Experimentally the binding of FIPV (spike) in macrophages (fapn) is strongly inhibited by mannan(s) that compete with the fapn. With mannose, the inhibition is less than with Mannan-oligosaccharides.

- Some Molecules can inhibit glycosylation of spikes (monensin, tunicamycin ...) reduce or cancel the virus infesting power (action in the Golgi. The same is true for mannanases and mannosidase enzymes that cut mannose out of the spikes.

- The competition with spikes by other molecules having an affinity for fapn *'(common sugar recognition process)'* cancel or reduce the power of infesting CoV:

- Mannan binding Lectin:

-
  - ***plant Lectin***

1. Allium agglutinins

2. Urtica dioica agglutinins
3. Pradamycine A .../...

-
  - ***humoral lectin***

1. Ficoline
2. Collectine .../...

-Manno-Oligosaccharides (MOS) : source: yeast

- sialic acid :

Experimental sialic acid inhibition can decrease the avian and human coronavirus infectivity.

Protecting kittens through breastmilk

Kittens born from mother carrying FECV are protected from infection during their first weeks of life (until weaning food). Diane D. Addie advocates early weaning and segregation of kittens from their mother before they contaminate each (5 to 6 weeks). Kittens outside contamination, but are deprived of contact with their mother during their 2 months of life (an important educative time).

The initial protection of the kittens is very effective. We have to reflect about the different possible ways to do it.

Antibodies

It is widely accepted that passive protection is borne by the immunoglobulins nursery (antibodies) provided by the colostrum and the milk from the mother.

Several questions arise:

1. If this protection is only supported by maternal antibodies so why these antibodies do not protect the mother herself?
2. The kittens born to a mother's blood group B are removed from their mother for 24 hours (to avoid the Hemolytic disease of the newborn) and thus have no systemic passage of maternal antibodies. Why is it not described FCoV infection in these kittens more often than others?

Colostrum

Other molecules from colostrum and cat milk, could also bear this coverage:

- Lactoferrin,
- Lactoperoxidase,
- Lysozyme,
- Rich Proline polypeptide – PRP,
- alpha-lactalbumine,
- .../...

Lactoferrin has many properties that make it a very good candidate for this anti-coronavirus activity:

1. As CoV group I, it binds to APN
2. As the SARS CoV, it binds to enzymes convert angiotensin
3. It binds to DC-SIGN of macrophage,
4. The Lactoferrin anti-viral activity is sialic acid dependent.

The structures of the polypeptide chain and carbohydrate moieties of bovine lactoferrin (bLF) are well established. bLF consists of a 689-amino acid polypeptide chain to which complex and high-mannose-type glycans are linked (Pierce et al., 1991)

Other components

The colostrum and breast milk also contains:

1. Many oligosaccharides (glycan) responsible for anti-viral,
2. Many maternal immune cells,
3. Many cytokines (interferon ...); whose role by oro-mucosal route seems very important.
4. sialic acid: during lactation, it appears that neutralizing oligo-saccharides binding sialic acid decreases when it binds increasingly to glycoproteins. (The APN is a glycoprotein). The anti-viral effect of lactoferrin is increased by the removal of sialic acid.
5. Mannan binding lectins.

Other protective factors

Other assumptions may help to explain this resistance to FCoV infections by kittens.

- In the first weeks of life, APN could be immature because highly manno-glycosylated. The spikes of CoV could then not be bound.
- Factors breastmilk may inhibit the synthesis of fANP by enterocytes, as already described with fructose or sucrose.

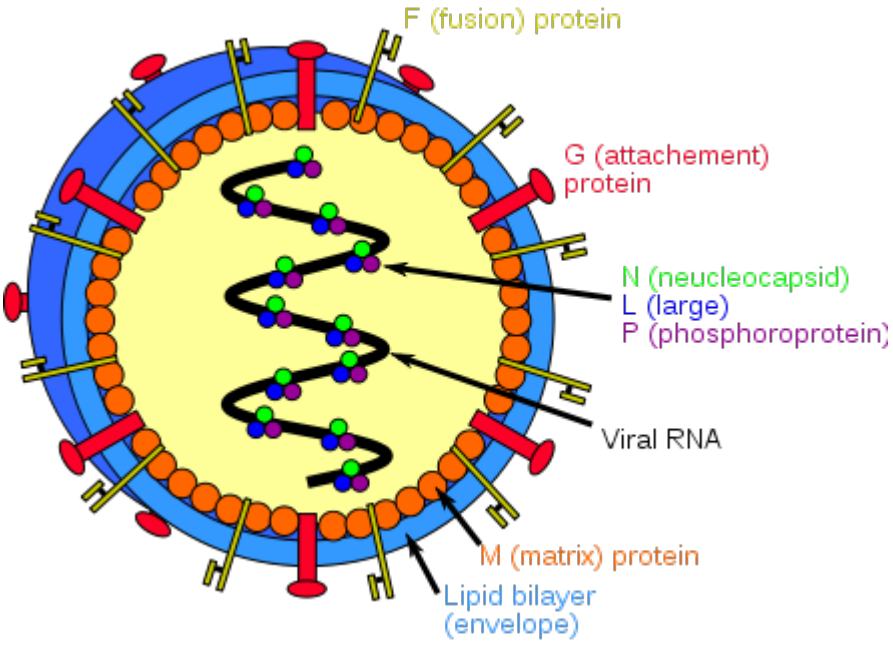
Chapter 9

Henipavirus

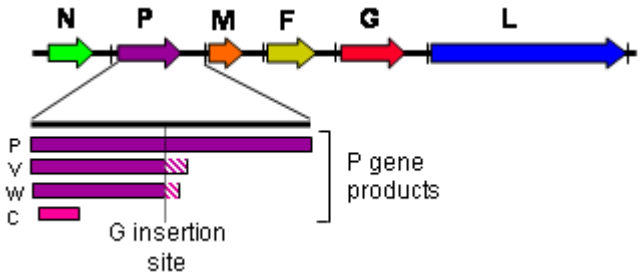
Henipaviruses
Virus classification
Group: Group V ((-)ssRNA)
Order: *Mononegavirales*
Family: *Paramyxoviridae*
Genus: ***Henipavirus***
Type species
Hendravirus
Species
Nipahvirus

Henipavirus is a genus of the family *Paramyxoviridae*, order *Mononegavirales* containing two members, **Hendravirus** and **Nipahvirus**. The henipaviruses are naturally harboured by Pteropid fruit bats (flying foxes) and are characterised by a large genome, a wide host range and their recent emergence as zoonotic pathogens capable of causing illness and death in domestic animals and humans.
In April 27, 1999, 257 cases of febrile encephalitis were reported to the Malaysian Ministry of Health (MOH), including 100 deaths. Laboratory results from 65 patients who died suggested recent Nipah virus infection..A highly fatal (case-fatality ratio 38%–75%), febrile human encephalitis in Malaysia and Singapore in 1999 and in Bangladesh during the winters of 2001, 2003, and 2004 has been detected which was caused by Nipah virus.. From February 2011, Nipah outbreak occurred at Hatibandha Upazila under Lalmonirhat district which is in northern part of Bangladesh. Till to date (7th February, 2011) there are 24 cases and 17 deaths.

Virus structure



Structure of henipaviruses



The henipavirus genome (3’ to 5’ orientation) and products of the P gene

Henipaviruses are ***pleomorphic*** (variably shaped), ranging in size from 40 to 600 nm in diameter. They possess a lipid membrane overlying a shell of viral matrix protein. At the core is a single helical strand of genomic RNA tightly bound to N (nucleocapsid) protein and associated with the L (large) and P (phosphoprotein) proteins which provide RNA polymerase activity during replication.

Embedded within the lipid membrane are spikes of F (fusion) protein trimers and G (attachment) protein tetramers. The function of the G protein is to attach the virus to the surface of a host cell via EFNB2, a highly conserved protein present in many mammals. The F protein fuses the viral membrane with the host cell membrane, releasing the virion contents into the cell. It also causes infected cells to fuse with neighbouring cells to form large, multinucleated syncytia.

Genome structure

As with all viruses in the *Mononegavirales* order, the Hendra virus and Nipah virus genomes are non-segmented, single-stranded negative-sense RNA. Both genomes are 18.2 kb in size and contain six genes corresponding to six structural proteins.

In common with other members of the *Paramyxovirinae* subfamily, the number of nucleotides in the henipavirus genome is a multiple of six, known as the 'rule of six'. Deviation from the rule of six, through mutation or incomplete genome synthesis, leads to inefficient viral replication, probably due to structural constraints imposed by the binding between the RNA and the N protein.

Henipaviruses employ an unusual process called RNA editing to generate multiple proteins from a single gene. The process involves the insertion of extra guanosine residues into the P gene mRNA prior to translation. The number of residues added determines whether the P, V or W proteins are synthesised. The functions of the V and W proteins are unknown, but they may be involved in disrupting host antiviral mechanisms.

Hendra virus

Emergence

Hendra virus (originally *Equine morbillivirus*) was discovered in September 1994 when it caused the deaths of fourteen horses, and a trainer at a training complex in Hendra, a suburb of Brisbane in Queensland, Australia.

The index case, a mare, was housed with 23 other horses after falling ill and died two days later. Subsequently, 19 of the remaining horses succumbed with 13 dying. Both the trainer and a stable hand were involved in nursing the index case and both fell ill within one week of the horse’s death with an influenza-like illness. The stable hand recovered while the trainer died of respiratory and renal failure. The source of virus was most likely frothy nasal discharge from the index case.

A second outbreak occurred in August 1994 (chronologically preceding the first outbreak) in Mackay 1000 km north of Brisbane resulting in the deaths of two horses and their owner. The owner assisted in necropsies of the horses and within three weeks was admitted to hospital suffering from meningitis. He recovered, but 14 months later developed neurologic signs and died. This outbreak was diagnosed retrospectively by the presence of Hendra virus in the brain of the patient.

A survey of wildlife in the outbreak areas was conducted and identified pteropid fruit bats as the most likely source of Hendra virus with a seroprevalence of 47%. All of the other 46 species sampled were negative. Virus isolations from the reproductive tract and urine of wild bats indicated that transmission to horses may have occurred via exposure to bat urine or birthing fluids.

Outbreaks

A total of thirteen outbreaks of Hendra virus have occurred since 1994, all involving infection of horses. Four of these outbreaks have spread to humans as a result of direct contact with infected horses.

- August 1994, Mackay, Queensland: Death of two horses and one person.
- September 1994, Brisbane, Queensland: 14 horses died from a total of 20 infected. Two people infected with one death.
- January 1999, Cairns, Queensland: Death of one horse.
- October 2004, Cairns, Queensland: Death of one horse. A vet involved in autopsy of the horse was infected with Hendra virus and suffered a mild illness.
- December 2004, Townsville, Queensland: Death of one horse.
- June 2006, Sunshine Coast, Queensland: Death of one horse.
- October 2006, Murwillumbah, New South Wales: Death of one horse.
- July 2007, Clifton Beach, Queensland: Infection of one horse (euthanized).
- July 2008, Redlands, Brisbane, Queensland: Death of five horses; four died from the Henda virus, the remaining animal recovered but was euthanized because of the threat to health. Two veterinary workers from the affected property were infected leading to the death of one, veterinary surgeon Dr. Ben Cuneen, on the 20th of August, 2008. The second veterinarian was hospitalized after pricking herself with a needle she had used to euthanize the horse that had recovered. A nurse exposed to the disease while assisting Cuneen in caring for the infected horses was also hospitalized.
- July 2008, Cannonvale, Queensland: Death of two horses.
- August 2009, Cawarral, Queensland: Death of one horse; the death of three other horses is being investigated. Queensland veterinary surgeon Alister Rodgers tested positive after treating the horses. On September 1, 2009 after two weeks in a coma, he became the fourth person to die from exposure to the virus.
- May 2010, Tewantin, Queensland: Death of one horse.

The distribution of black and spectacled flying foxes covers the outbreak sites, and the timing of incidents indicates a seasonal pattern of outbreaks possibly related to the seasonality of fruit bat birthing. As there is no evidence of transmission to humans directly from bats, it is thought that human infection only occurs via an intermediate host.

Pathology

Flying foxes are unaffected by Hendra virus infection. Symptoms of Hendra virus infection of humans may be respiratory, including hemorrhage and edema of the lungs, or encephalitic resulting in meningitis. In horses, infection usually causes pulmonary edema and congestion.

Nipah virus

Emergence

Pteropus vampyrus (Large flying fox), one of the natural reservoirs of Nipah virus

Nipah virus was identified in 1999 when it caused an outbreak of neurological and respiratory disease on pig farms in peninsular Malaysia, resulting in 105 human deaths and the culling of one million pigs. In Singapore, 11 cases including one death occurred in abattoir workers exposed to pigs imported from the affected Malaysian farms. The Nipah virus has been classified by the Centers for Disease Control and Prevention as a Category C agent. The name "Nipah" is taken after the place, *Kampung Nipah* in Negeri Sembilan where the virus was first isolated from humans in that area.

The outbreak was originally mistaken for Japanese encephalitis (JE), however, physicians in the area noted that persons who had been vaccinated against JE were not protected, and the number of cases among adults was unusual Despite the fact that these observations were recorded in the first month of the outbreak, the Ministry of Health failed to react accordingly and instead launched a nationwide campaign to educate people on the dangers of JE and its vector, Culex mosquitoes.

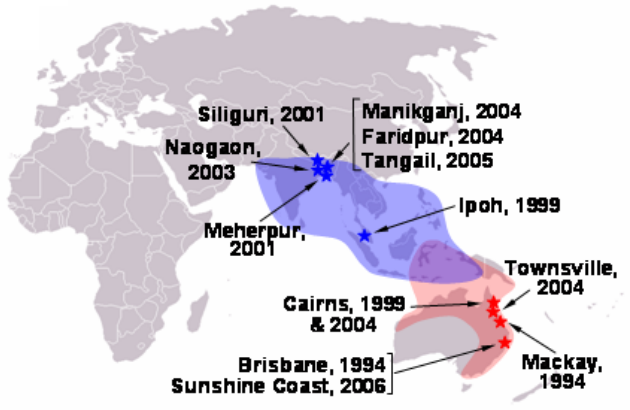
Symptoms of infection from the Malaysian outbreak were primarily encephalitic in humans and respiratory in pigs. Later outbreaks have caused respiratory illness in humans, increasing the likelihood of human-to-human transmission and indicating the existence of more dangerous strains of the virus.

Based on seroprevalence data and virus isolations, the primary reservoir for Nipah virus was identified as Pteropid fruit bats including *Pteropus vampyrus* (Large Flying Fox) and *Pteropus hypomelanus* (Small Flying-fox), both of which occur in Malaysia.

The transmission of Nipah virus from flying foxes to pigs is thought to be due to an increasing overlap between bat habitats and piggeries in peninsular Malaysia. At the index farm, fruit orchards were in close proximity to the piggery, allowing the spillage of urine, faeces and partially eaten fruit onto the pigs. Retrospective studies demonstrate that viral spillover into pigs may have been occurring in Malaysia since 1996 without detection. During 1998, viral spread was aided by the transfer of infected pigs to other farms where new outbreaks occurred.

Outbreaks

Eight more outbreaks of Nipah virus have occurred since 1998, all within Bangladesh and neighbouring parts of India. The outbreak sites lie within the range of Pteropus species (*Pteropus giganteus*). As with Hendra virus, the timing of the outbreaks indicates a seasonal effect.



Locations of henipavirus outbreaks (red stars–Hendra virus; blue stars–Nipah virus) and distribution of henipavirus flying fox reservoirs (red shading–Hendra virus ; blue shading–Nipah virus)

- 2001 January 31 – February 23, Siliguri, India: 66 cases with a 74% mortality rate. 75% of patients were either hospital staff or had visited one of the other patients in hospital, indicating person-to-person transmission.
- 2001 April – May, Meherpur district, Bangladesh: 13 cases with nine fatalities (69% mortality).
- 2003 January, Naogaon district, Bangladesh: 12 cases with eight fatalities (67% mortality).
- 2004 January – February, Manikganj and Rajbari provinces, Bangladesh: 42 cases with 14 fatalities (33% mortality).
- 2004 19 February – 16 April, Faridpur district, Bangladesh: 36 cases with 27 fatalities (75% mortality). Epidemiological evidence strongly suggests that this outbreak involved person-to-person transmission of Nipah virus, which had not previously been confirmed. 92% of cases involved close contact with at least one other person infected with Nipah virus. Two cases involved a single short exposure to an ill patient, including a rickshaw driver who transported a patient to hospital. In addition, at least six cases involved acute respiratory distress syndrome which has not been reported previously for Nipah virus illness in humans. This symptom is likely to have assisted human-to-human transmission through large droplet dispersal.
- 2005 January, Tangail district, Bangladesh: 12 cases with 11 fatalities (92% mortality). The virus was probably contracted from drinking date palm juice contaminated by fruit bat droppings or saliva.
- 2007 February – May, Nadia District, India: up to 50 suspected cases with 3-5 fatalities. The outbreak site borders the Bangladesh district of Kushtia where eight cases of Nipah virus encephalitis with five fatalities occurred during March and April 2007. This was preceded by an outbreak in Thakurgaon during January and February affecting seven people with three deaths. All three outbreaks showed evidence of person-to-person transmission.
- 2008 February - March, Manikganj and Rajbari provinces, Bangladesh: Nine cases with eight fatalities.

- 2011 February- till: The outbreak of Nipah Virus is occurred at Hatibanda, Lalmonirhat, Bangladesh on the onset of 2011. There have a record of 21 school childrens death due to infection of Nipah virus on 4th February, 2011. IEDCR has confirmed the infection is due to this virus . Local schools are declared for one week leave to prevent the spread. However, people are also requested to avoid consumption of fruits or fruit products (e.g. raw date palm juice) contaminated with urine or saliva from infected fruit bats was the most likely source of infection.

Eleven isolated cases of Nipah virus encephalitis have also been documented in Bangladesh since 2001.

Nipah virus has been isolated from Lyle's flying fox (*Pteropus lylei*) in Cambodia and viral RNA found in urine and saliva from *P. lylei* and Horsfield's roundleaf bat (*Hipposideros larvatus*) in Thailand. Infective virus has also been isolated from

environmental samples of bat urine and partially-eaten fruit in Malaysia. Antibodies to henipaviruses have also been found in fruit bats in Madagascar (*Pteropus rufus, Eidolon dupreanum*) and Ghana (*Eidolon helvum*) indicating a wide geographic distribution of the viruses. No infection of humans or other species have been observed in Cambodia, Thailand or Africa.

Pathology

In humans, the infection presents as fever, headache and drowsiness. Cough, abdominal pain, nausea, vomiting, weakness, problems with swallowing and blurred vision are relatively common. About a quarter of the patients have seizures and about 60% become comatose and might need mechanical ventilation. In patients with severe disease, their conscious state may deteriorate and they may develop severe hypertension, fast heart rate, and very high temperature.

Nipah virus is also known to cause relapse encephalitis. In the initial Malaysian outbreak, a patient presented with relapse encephalitis some 53 months after his initial infection. There is no definitive treatment for Nipah encephalitis, apart from supportive measures, such as mechanical ventilation and prevention of secondary infection. Ribavirin, an antiviral drug, was tested in the Malaysian outbreak and the results were encouraging, though further studies are still needed.

In animals, especially in pigs, the virus causes porcine respiratory and neurologic syndrome also known as barking pig syndrome or one mile cough.

Causes of emergence

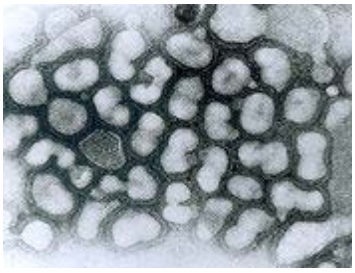
The emergence of henipaviruses parallels the emergence of other zoonotic viruses in recent decades. SARS coronavirus, Australian bat lyssavirus, Menangle virus and probably Ebola virus and Marburg virus are also harbored by bats and are capable of infecting a variety of other species. The emergence of each of these viruses has been linked to an increase in contact between bats and humans, sometimes involving an intermediate domestic animal host. The increased contact is driven both by human encroachment into the bats' territory (in the case of Nipah, specifically pigpens in said territory) and by movement of bats towards human populations due to changes in food distribution and loss of habitat.

There is evidence of habitat loss for flying foxes both in South Asia and Australia (particularly along the east coast) as well as encroachment of human dwellings and agriculture into the remaining habitats, creating greater overlap of human and flying fox distributions.

Chapter 10

Influenza A Virus

Orthomyxoviridae



Electron micrograph of Influenza A viruses

Virus classification

Group: Group V ((-)ssRNA)

Family: *Orthomyxoviridae*

Genera

Influenzavirus A
Influenzavirus B
Influenzavirus C
Isavirus
Thogotovirus

Influenza A virus causes influenza in birds and some mammals and is the only species of Influenzavirus A. Influenzavirus A is a genus of the Orthomyxoviridae family of viruses. Strains of all subtypes of influenza A virus have been isolated from wild birds, although disease is uncommon. Some isolates of influenza A virus cause severe disease both in domestic poultry and, rarely, in humans. Occasionally viruses are transmitted from wild aquatic birds to domestic poultry and this may cause an outbreak or give rise to human influenza pandemics.

Influenza A viruses are negative sense, single-stranded, segmented RNA viruses. There are several subtypes, labeled according to an H number (for the type of hemagglutinin)

and an N number (for the type of neuraminidase). There are 16 different H antigens (H1 to H16) and nine different N antigens (N1 to N9). The newest H type (H16) was isolated from Black-headed Gulls caught in Sweden and the Netherlands in 1999 and reported in the literature in 2005.

Each virus subtype has mutated into a variety of strains with differing pathogenic profiles; some pathogenic to one species but not others, some pathogenic to multiple species.

A filtered and purified Influenza A vaccine for humans was developed and many countries have stockpiled it to allow a quick administration to the population in the event of an Avian influenza pandemic. Avian influenza is sometimes called avian flu, and commonly bird flu.

Variants and subtypes

Some Variants are identified and named according to the isolate that they are like and thus are presumed to share lineage (example Fujian flu virus like); according to their typical host (example Human flu virus); according to their subtype (example H3N2); and according to their deadliness (example LP, Low Pathogenic). So a flu from a virus similar to the isolate A/Fujian/411/2002(H3N2) is called Fujian flu, human flu, and H3N2 flu.

Variants are sometimes named according to the species (host) the strain is endemic in or adapted to. The main variants named using this convention are:

- Bird flu
- Human flu
- Swine influenza
- Equine influenza
- Canine influenza

Variants have also sometimes been named according to their deadliness in poultry, especially chickens:

- Low Pathogenic Avian Influenza (LPAI)
- Highly Pathogenic Avian Influenza (HPAI), also called: deadly flu or death flu

Most known strains are extinct strains. For example, the annual flu subtype H3N2 no longer contains the strain that caused the Hong Kong flu.

Annual flu

The annual flu (also called "seasonal flu" or "human flu") in the U.S. "results in approximately 36,000 deaths and more than 200,000 hospitalizations each year. In addition to this human toll, influenza is annually responsible for a total cost of over $10 billion in the U.S.".

The annually updated trivalent influenza vaccine consists of hemagglutinin (HA) surface glycoprotein components from influenza H3N2, H1N1, and B influenza viruses.

Measured resistance to the standard antiviral drugs amantadine and rimantadine in H3N2 has increased from 1% in 1994 to 12% in 2003 to 91% in 2005.

"[C]ontemporary human H3N2 influenza viruses are now endemic in pigs in southern China and can reassort with avian H5N1 viruses in this intermediate host."

Structure and genetics

"The physical structure of all influenza A viruses is similar. The virions or virus particles are enveloped and can be either spherical or filamentous in form. In clinical isolates that have undergone limited passages in eggs or tissue culture, there are more filamentous than spherical particles, whereas passaged laboratory strains consist mainly of spherical virions."

The Influenza A virus genome is contained on eight single (non-paired) RNA strands that code for eleven proteins (HA, NA, NP, M1, M2, NS1, NEP, PA, PB1, PB1-F2, PB2). The total genome size is 13,588 bases. The segmented nature of the genome allows for the exchange of entire genes between different viral strains during cellular cohabitation. The eight RNA segments are:

- HA encodes hemagglutinin (about 500 molecules of hemagglutinin are needed to make one virion) "The extent of infection into host organism is determined by HA. Influenza viruses bud from the apical surface of polarized epithelial cells (e.g. bronchial epithelial cells) into lumen of lungs and are therefore usually pneumotropic. The reason is that HA is cleaved by tryptase clara which is restricted to lungs. However HAs of H5 and H7 pantropic avian viruses subtypes can be cleaved by furin and subtilisin-type enzymes, allowing the virus to grow in other organs than lungs."
- NA encodes neuraminidase (about 100 molecules of neuraminidase are needed to make one virion).
- NP encodes nucleoprotein.
- M encodes two matrix proteins (the M1 and the M2) by using different reading frames from the same RNA segment (about 3000 matrix protein molecules are needed to make one virion).
- NS encodes two distinct non-structural proteins (NS1 and NEP) by using different reading frames from the same RNA segment.
- PA encodes an RNA polymerase.
- PB1 encodes an RNA polymerase and PB1-F2 protein (induces apoptosis) by using different reading frames from the same RNA segment.
- PB2 encodes an RNA polymerase.

The genome segments have common terminal sequences, and the ends of the RNA strands are partially complementary, allowing them to bond to each other by hydrogen

bonds. After transcription from negative-sense to positive-sense RNA the +RNA strands get the cellular 5' cap added by cap snatching, which involves the viral protein NS1 binding to the cellular pre-mRNAs. The cap is then cleaved from the cellular pre-mRNA using a second viral protein, PB2. The short oligo cap is then added to the influenza +RNA strands, allowing its processing as messenger RNA by ribosomes. The +RNA strands also serve for synthesis of -RNA strands for new virions.

The RNA synthesis and its assembly with the nucleoprotein takes place in the cell nucleus, the synthesis of proteins takes place in the cytoplasm. The assembled virion cores leave the nucleus and migrate towards the cell membrane, with patches of viral transmembrane proteins (hemagglutinin, neuraminidase and M2 proteins) and an underlying layer of the M1 protein, and bud through these patches, releasing finished enveloped viruses into the extracellular fluid.

In nonhumans

Avian influenza

Fowl act as natural asymptomatic carriers of Influenza A viruses. Prior to the current H5N1 epizootic, strains of Influenza A virus had been demonstrated to be transmitted from wild fowl to only birds, pigs, horses, seals, whales and humans; and only between humans and pigs and between humans and domestic fowl; and not other pathways such as domestic fowl to horse.

Wild aquatic birds are the natural hosts for a large variety of influenza A viruses. Occasionally viruses are transmitted from these birds to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics.

H5N1 has been shown to be transmitted to tigers, leopards, and domestic cats that were fed uncooked domestic fowl (chickens) with the virus. H3N8 viruses from horses have crossed over and caused outbreaks in dogs. Laboratory mice have been infected successfully with a variety of avian flu genotypes.

Influenza A viruses spread in the air and in manure and survives longer in cold weather. It can also be transmitted by contaminated feed, water, equipment and clothing; however, there is no evidence that the virus can survive in well-cooked meat. Symptoms in animals vary, but virulent strains can cause death within a few days.

"Highly pathogenic avian influenza virus is on every top ten list available for potential agricultural bioweapon agents".

Avian influenza viruses that the OIE and others test for in order to control poultry disease include: H5N1, H7N2, H1N7, H7N3, H13N6, H5N9, H11N6, H3N8, H9N2, H5N2, H4N8, H10N7, H2N2, H8N4, H14N5, H6N5, H12N5 and others.

Known outbreaks of highly pathogenic flu in poultry 1959-2003

Year	Area	Affected	Subtype
1959	Scotland	chicken	H5N1
1963	England	turkey	H7N3
1966	Ontario (Canada)	turkey	H5N9
1976	Victoria (Australia)	chicken	H7N7
1979	Germany	chicken	H7N7
1979	England	turkey	H7N7
1983	Pennsylvania (USA)*	chicken, turkey	H5N2
1983	Ireland	turkey	H5N8
1985	Victoria (Australia)	chicken	H7N7
1991	England	turkey	H5N1
1992	Victoria (Australia)	chicken	H7N3
1994	Queensland (Australia)	chicken	H7N3
1994	Mexico*	chicken	H5N2
1994	Pakistan*	chicken	H7N3
1997	New South Wales (Australia)	chicken	H7N4
1997	Hong Kong (China)*	chicken	H5N1
1997	Italy	chicken	H5N2
1999	Italy*	turkey	H7N1
2002	Hong Kong (China)	chicken	H5N1
2002	Chile	chicken	H7N3
2003	Netherlands*	chicken	H7N7

**Outbreaks with significant spread to numerous farms, resulting in great economic losses. Most other outbreaks involved little or no spread from the initially infected farms.*

1979: "More than 400 harbor seals, most of them immature, died along the New England coast between December 1979 and October 1980 of acute pneumonia associated with influenza virus, A/Seal/Mass/1/180 (H7N7)."

1995: "[V]accinated birds can develop asymptomatic infections that allow virus to spread, mutate, and recombine (ProMED-mail, 2004j). Intensive surveillance is required to detect these “silent epidemics” in time to curtail them. In Mexico, for example, mass vaccination of chickens against epidemic H5N2 influenza in 1995 has had to continue in order to control a persistent and evolving virus (Lee et al., 2004)."

1997: "Influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species. For example, until 1997, only H1N1 viruses circulated widely in the U.S. pig population. However, in 1997, H3N2 viruses from humans were introduced into the pig population and caused widespread disease among pigs. Most recently, H3N8 viruses from horses have crossed over and caused outbreaks in dogs."

2000: "In California, poultry producers kept their knowledge of a recent H6N2 avian influenza outbreak to themselves due to their fear of public rejection of poultry products; meanwhile, the disease spread across the western United States and has since become endemic."

2003: In Netherlands H7N7 influenza virus infection broke out in poultry on several farms.

2004: In North America, the presence of avian influenza strain H7N3 was confirmed at several poultry farms in British Columbia in February 2004. As of April 2004, 18 farms had been quarantined to halt the spread of the virus.

2005: Tens of millions of birds died of H5N1 influenza and hundreds of millions of birds were culled to protect humans from H5N1. H5N1 is endemic in birds in southeast Asia and represents a long term pandemic threat.

2006: H5N1 spreads across the globe killing hundreds of millions of birds and over 100 people causing a significant H5N1 impact from both actual deaths and predicted possible deaths.

Swine flu
: Swine influenza (or "pig influenza") refers to a subset of Orthomyxoviridae that create influenza in pigs and are endemic in pigs. The species of Orthomyxoviridae that can cause flu in pigs are Influenza A virus and Influenza C virus but not all genotypes of these two species infect pigs. The known subtypes of Influenza A virus that create influenza in pigs and are endemic in pigs are H1N1, H1N2, H3N1 and H3N2.

Horse flu
: Horse flu (or "Equine influenza") refers to varieties of Influenza A virus that affect horses. Horse 'flu viruses were only isolated in 1956. There are two main types of virus called equine-1 (H7N7) which commonly affects horse heart muscle and equine-2 (H3N8) which is usually more severe.

Dog flu
: Dog flu (or "canine influenza") refers to varieties of Influenza A virus that affect dogs. The equine influenza virus H3N8 was found to infect and kill - with respiratory illness - greyhound race dogs at a Florida racetrack in January 2004.

H3N8
: H3N8 is now endemic in birds, horses and dogs.

Human influenza virus

Japanese commuter wearing a face mask.

"Human influenza virus" usually refers to those subtypes that spread widely among humans. H1N1, H1N2, and H3N2 are the only known Influenza A virus subtypes currently circulating among humans.

Genetic factors in distinguishing between "human flu viruses" and "avian influenza viruses" include:

PB2: (RNA polymerase): Amino acid (or residue) position 627 in the PB2 protein encoded by the PB2 RNA gene. Until H5N1, all known avian influenza viruses had a Glu at position 627, while all human influenza viruses had a lysine.
HA: (hemagglutinin): Avian influenza HA bind alpha 2-3 sialic acid receptors while human influenza HA bind alpha 2-6 sialic acid receptors. Swine influenza viruses have the ability to bind both types of sialic acid receptors.

"About 52 key genetic changes distinguish avian influenza strains from those that spread easily among people, according to researchers in Taiwan, who analyzed the genes of more than 400 A type flu viruses." "How many mutations would make an avian virus capable of infecting humans efficiently, or how many mutations would render an influenza virus a pandemic strain, is difficult to predict. We have examined sequences from the 1918 strain, which is the only pandemic influenza virus that could be entirely derived from avian strains. Of the 52 species-associated positions, 16 have residues typical for human strains; the others remained as avian signatures. The result supports the hypothesis that the 1918 pandemic virus is more closely related to the avian influenza A virus than are other human influenza viruses."

Human flu symptoms usually include fever, cough, sore throat, muscle aches, conjunctivitis and, in severe cases, severe breathing problems and pneumonia that may be fatal. The severity of the infection will depend to a large part on the state of the infected person's immune system and if the victim has been exposed to the strain before, and is therefore partially immune.

Highly pathogenic H5N1 avian influenza in a human is far worse, killing 50% of humans that catch it. In one case, a boy with H5N1 experienced diarrhea followed rapidly by a coma without developing respiratory or flu-like symptoms.

The Influenza A virus subtypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are:

- H1N1 caused "Spanish Flu" and the 2009 swine flu outbreak
- H2N2 caused "Asian Flu" in the late 1950s
- H3N2 caused "Hong Kong Flu" in the late 1960s
- H5N1 considered a global influenza pandemic threat through its spread in the mid-2000s
- H7N7 has unusual zoonotic potential
- H1N2 is currently endemic in humans and pigs
- H9N2, H7N2, H7N3, H5N2, H10N7.

H1N1

H1N1 is currently pandemic in both human and pig populations. A variant of H1N1 was responsible for the Spanish flu pandemic that killed some 50 million to 100 million people worldwide over about a year in 1918 and 1919. Another variant was named a pandemic threat in the 2009 flu pandemic. Controversy arose in October, 2005, after the H1N1 genome was published in the journal, *Science*, because of fears that this information could be used for bioterrorism.

H2N2

The Asian Flu was a pandemic outbreak of H2N2 avian influenza that originated in China in 1957, spread worldwide that same year during which a influenza vaccine was developed, lasted until 1958 and caused between one and four million deaths.

H3N2

H3N2 is currently endemic in both human and pig populations. It evolved from H2N2 by antigenic shift and caused the Hong Kong Flu pandemic of 1968 and 1969 that killed up to 750,000. "An early-onset, severe form of influenza A H3N2 made headlines when it claimed the lives of several children in the United States in late 2003."

The dominant strain of annual flu in January 2006 was H3N2. Measured resistance to the standard antiviral drugs amantadine and rimantadine in H3N2 increased from 1% in 1994 to 12% in 2003 to 91% in 2005.

"[C]ontemporary human H3N2 influenza viruses are now endemic in pigs in southern China and can reassort with avian H5N1 viruses in this intermediate host."

H5N1

H5N1 is the world's major influenza pandemic threat.
"When he compared the 1918 virus with today's human flu viruses, Dr. Taubenberger noticed that it had alterations in just 25 to 30 of the virus's 4,400 amino acids. Those few changes turned a bird virus into a killer that could spread from person to person."

H7N7

H7N7 has unusual zoonotic potential. In 2003 in Netherlands 89 people were confirmed to have H7N7 influenza virus infection following an outbreak in poultry on several farms. One death was recorded.

H1N2

H1N2 is currently endemic in both human and pig populations. The new H1N2 strain appears to have resulted from the reassortment of the genes of the currently circulating influenza H1N1 and H3N2 subtypes. The hemagglutinin protein of the H1N2 virus is similar to that of the currently circulating H1N1 viruses and the neuraminidase protein is similar to that of the current H3N2 viruses.

H9N2

Low pathogenic avian influenza A (H9N2) infection was confirmed in 1999, in China and Hong Kong in two children, and in 2003 in Hong Kong in one child. All three fully recovered.

H7N2

One person in New York in 2003 and one person in Virginia in 2002 were found to have serologic evidence of infection with H7N2. Both fully recovered.

H7N3

In North America, the presence of avian influenza strain H7N3 was confirmed at several poultry farms in British Columbia in February 2004. As of April 2004, 18 farms had been quarantined to halt the spread of the virus. Two cases of humans with avian influenza have been confirmed in that region. "Symptoms included conjunctivitis and mild influenza-like illness." Both fully recovered.

H5N2

Japan's Health Ministry said January 2006 that poultry farm workers in Ibaraki prefecture may have been exposed to H5N2 in 2005. The H5N2 antibody titers of paired sera of 13 subjects increased fourfold or more.

H10N7

In 2004 in Egypt H10N7 was reported for the first time in humans. It caused illness in two infants in Egypt. One child's father is a poultry merchant.

Evolution

Taubenberger says:

> "All influenza A pandemics since [the Spanish flu pandemic], and indeed almost all cases of influenza A worldwide (excepting human infections from avian viruses such as H5N1 and H7N7), have been caused by descendants of the 1918 virus, including "drifted" H1N1 viruses and reassorted H2N2 and H3N2 viruses. The latter are composed of key genes from the 1918 virus, updated by

subsequently incorporated avian influenza genes that code for novel surface proteins, making the 1918 virus indeed the "mother" of all pandemics.

Researchers from the National Institutes of Health used data from the Influenza Genome Sequencing Project and concluded that during the ten-year period examined most of the time the hemagglutinin gene in H3N2 showed no significant excess of mutations in the antigenic regions while an increasing variety of strains accumulated. This resulted in one of the variants eventually achieving higher fitness, becoming dominant, and in a brief interval of rapid evolution rapidly sweeping through the population and eliminating most other variants.

Chapter 11

Myxomatosis

European Rabbit in Shropshire, England, infected with the Myxomatosis disease, caused by the Myxoma virus

Myxomatosis (sometimes shortened to "myxi" or "myxo") is a disease which affects rabbits and is caused by the Myxoma virus. It was first observed in Uruguay in laboratory rabbits in the late 19th century. It was introduced into Australia in 1950 in an attempt to control the rabbit population.

Effects of the disease

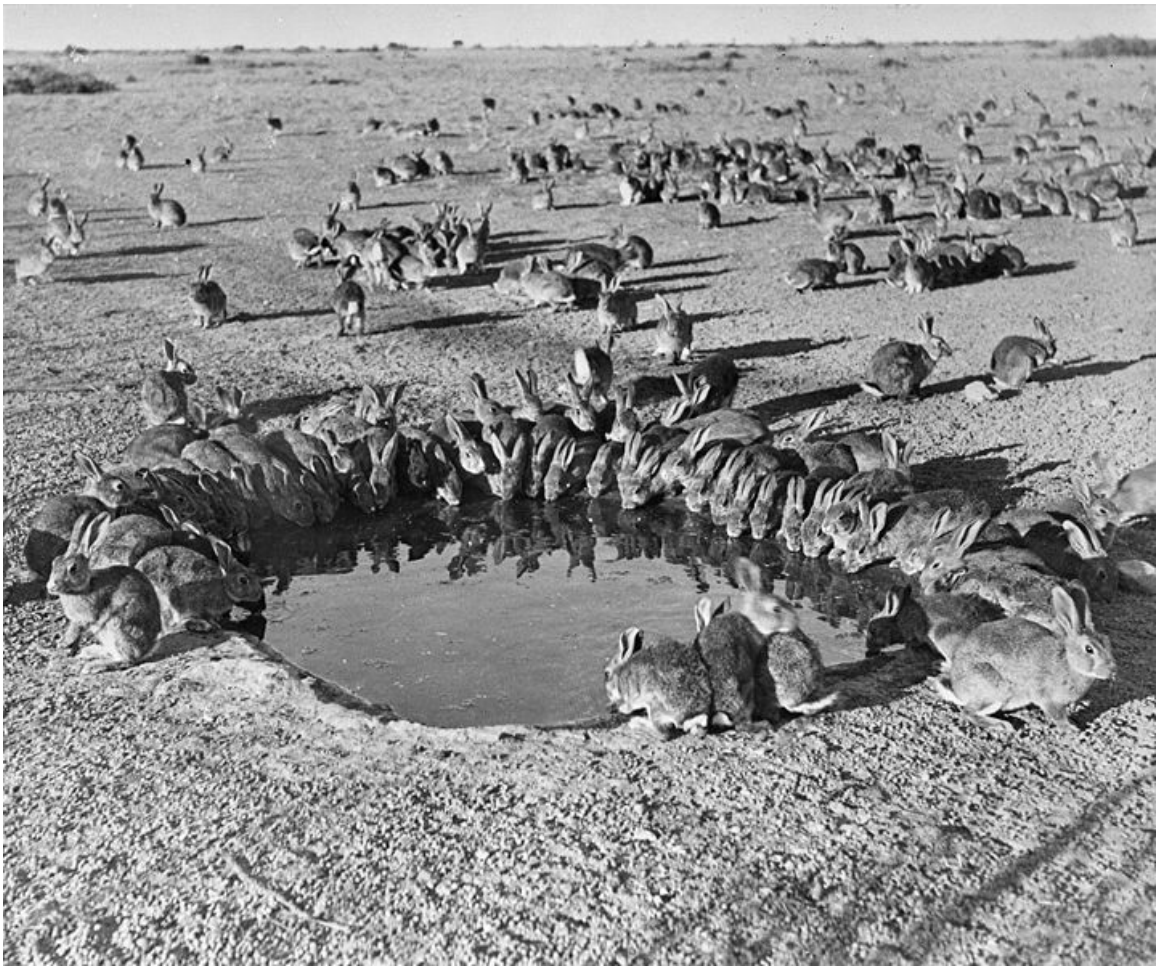
In rabbits of the genus *Sylvilagus* (cottontail rabbits), myxomatosis only causes localized skin tumors, but the European rabbit (*Oryctolagus cuniculus*) is more severely affected. At first, normally the disease is visible by lumps (myxomata) and puffiness around the head and genitals. It then may progress to acute conjunctivitis and possibly blindness; however, this also may be the first indication of the disease. The rabbits become listless, lose appetite, and develop a fever. Secondary bacterial infections occur in most cases which cause pneumonia and purulent inflammation of the lungs. In typical cases where the rabbit has no resistance death may take place with frightening rapidity, often in as little as 48 hours. Death usually occurs within 14 days.

Treatment

It is crucial to prevent the misdiagnosis of myxomatosis with Pasteurellosis. Pasteurellosis is a bacterial infection which can be treated with antibiotics. Rabbits treated for Pasteurellosis must often be treated with antibiotics for several weeks to several months. Some rabbits may require surgical intervention in order to remove purulent tissues and abscess. Once Pasteurellosis has become well entrenched, however, there is no guarantee the animal will survive. By contrast, at this writing, there is no treatment for rabbits suffering Myxomatosis, other than palliative care to ease the suffering of individual animals, and the treatment for secondary and opportunistic infections, in the hopes the treated animal will survive. Though the vectors of communication are similar, either contact with an infected animal, cage, feeding or water dishes, and insects, Pasteurellosis can also be spread through breeding, specifically infecting the sexual organs of the animal. Likewise, it is advisable to arrive at the correct diagnosis for the benefit of both rabbit and owner. Of course, if nobody wants the rabbit to survive, such concerns need not be entertained. In cases of Myxomatosis and rabbit hemorrhagic disease (RHD) the owner is often urged to euthanize the animal to ease its suffering. Often the difference between diagnosing a fatal viral infection and a complex, but treatable bacterial infection, like Pasteurellosis, will likely include medications, X-rays, surgery, convalescent, and follow-up care. While surgery and antibiotics may successfully treat Pasteurellosis, they will not treat Myxomatosis or RHD. The owner and attending veterinarian must quickly discern between untreatable diseases and treatable conditions. Myxomatosis and RHD are highly communicable, and untreatable at any stage, whereas rabbits suffering from diseases/conditions other than Myxomatosis and RHD, such as poisoning, heat exhaustion, E. coli or Clostridium perfringens type E enterotoxemia can benefit from timely veterinary intervention.

Spread of the disease

After its discovery in 1896 in imported rabbits in Uruguay, a relatively harmless strain spread quickly throughout the wild populations in South America.



Rabbits around a waterhole in the myxomatosis trial site on Wardang Island in 1938.

In Australia, the virus was first field-tested for population control in 1938. A full-scale release was performed in 1950. It was devastatingly effective, reducing the estimated rabbit population from 600 million to 100 million in two years. However, the rabbits remaining alive were those least affected by the disease. Genetic resistance to myxomatosis was observed soon after the first release and most rabbits acquired partial immunity in the first two decades. Resistance has been increasing slowly since the 1970s, and the disease now only kills about 50% of infected rabbits. In an attempt to increase that number, a second virus (rabbit calicivirus) was introduced into the rabbit population in 1996.

Myxomatosis was unintentionally introduced to France by the bacteriologist Dr. Paul Armand Delille, following his use of the virus to rid his private estate of rabbits in June 1952 (controversially, he inoculated two of the rabbits on his land). Within four months the virus had spread 50 km; Armand suspected this was due to poachers taking infected rabbits from his estate. By 1954, 90% of the wild rabbits in France were dead. The disease spread throughout Europe. It reached the UK in 1953, being illegally imported onto an estate in West Sussex. Some in the UK deliberately spread the disease, placing

sick rabbits in burrows, while many others deplored the cruelty and suffering. The government refused to legislate to make deliberate spread of the disease illegal. By 1955, about 95% of rabbits in the UK were dead. Rabbits suffering in the last stages of the disease, commonly called "mixy" or "myxie" rabbits, are still a common sight in the UK. Unfortunately, the disease has wider consequences, apart from the death of rabbits: the Iberian Lynx, among others, is now almost extinct because the declining rabbit population which encompasses about 80% of its diet, has caused mass starvation. It is not uncommon for shooters to specifically target infected rabbits, viewing the act as being merciful. However, in 2005 the UK Land Registry conducted a survey of 16,000 hectares of its land and reported that the rabbit population had increased three-fold every two years - likely a product of increasing genetic resistance to the virus.

Myxomatosis is spread by direct contact with an affected animal or by being bitten by fleas or mosquitoes that have fed on an infected rabbit. The myxomatosis virus does not replicate in these insect hosts, but can be physically carried by an insect's mouthparts, i.e. from an infected rabbit to another susceptible animal. Due to the potential of insect vector transmission, pet rabbits may be susceptible in enzootic areas and vaccination is highly recommended.

Use of vaccine

A vaccine is available for pet rabbits (ATCvet code: QI08AD02), but is illegal in Australia due to fears that the immunity conferred by the vaccine could be transmitted through the wild rabbit population, since the vaccine uses a live virus, the Shope fibroma virus.

Natural resistance

The development of resistance to the disease seems to have taken different courses. In Australia, the virus initially killed rabbits very quickly, about 4 days after infection. This gave little time for the infection to spread. However, a less virulent form of the virus has become prevalent there, spreading more effectively by being less lethal. In Europe, many rabbits are genetically resistant to the original virus that was spread. The survival rate of diseased rabbits has now increased to 35% when in the 1950s it was zero. It is conjectured that this is because the main transmission vector in Australia is the mosquito, while in Europe it is the rabbit flea.

Chapter 12

Newcastle Disease

Newcastle disease virus	
Virus classification	
Group:	Group V ((-)ssRNA)
Order:	*Mononegavirales*
Family:	*Paramyxoviridae*
Genus:	*Avulavirus*
Species:	***Newcastle disease virus***

Newcastle disease is a contagious bird disease affecting many domestic and wild avian species. First found in Newcastle, United Kingdom in 1926, then by Burnet in 1943 in Australia in connection with laboratory infection where the virus was isolated from a ocular discharge of a patient to show the specific antibody titre in the patient's blood. Newcastle has a negative sense single stranded genome which codes for a RNA directed RNA polymerase, hemagglutinin- neuraminidase protein, fusion protein, matrix protein, phosphoprotein and nucleoprotein in the 5' to 3' direction. Its effects are most notable in domestic poultry due to their high susceptibility and the potential for severe impacts of an epizootic on the poultry industries. It is endemic to many countries.

Newcastle Disease was discovered in Newcastle upon Tyne, England in 1926 (Doyle), but also at this time slightly different strains were found in other parts of the world.

Exposure of humans to infected birds (for example in poultry processing plants) can cause mild conjunctivitis and influenza-like symptoms, but the Newcastle disease virus (NDV) otherwise poses no hazard to human health. Interest in the use of NDV as an anticancer agent has arisen from the ability of NDV to selectively kill human tumour cells with limited toxicity to normal cells.

No treatment for NDV exists, but the use of prophylactic vaccines and sanitary measures reduces the likelihood of outbreaks.

The causal agent

Description

The causal agent, *Newcastle disease virus* (NDV), is a negative-sense single-stranded RNA virus. Transmission occurs by exposure to faecal and other excretions from infected birds, and through contact with contaminated feed, water, equipment and clothing.

Strains

NDV strains can be categorised as velogenic (highly virulent), mesogenic (intermediate virulence) or lentogenic (nonvirulent). Velogenic strains produce severe nervous and respiratory signs, spread rapidly and cause up to 90% mortality. Mesogenic strains cause coughing, affect egg quality and production and result in up to 10% mortality. Lentogenic strains produce mild signs with negligible mortality.

Use as an anti-cancer agent

In 1999, promising results were reported using an attenuated strain of the Newcastle virus codenamed MTH-68 in cancer patients by researchers who had isolated the strain in 1968. It appears that the virus preferentially targets and replicates in certain types of tumor cells, leaving normal cells almost unaffected. In 2006 researchers from the Hebrew University also succeeded in isolating a variant of the Newcastle Disease Virus codenamed NDV-HUJ which showed promising results in 14 Glioblastoma multiforme patients.

Use as a biological weapon

Newcastle disease was one of more than a dozen agents that the United States researched as potential biological weapons before the nation suspended its biological weapons program.

Transmission

NDV is spread primarily through direct contact between healthy birds and the bodily discharges of infected birds. The disease is transmitted through infected birds' droppings and secretions from the nose, mouth, and eyes. NDV spreads rapidly among birds kept in confinement, such as commercially raised chickens.

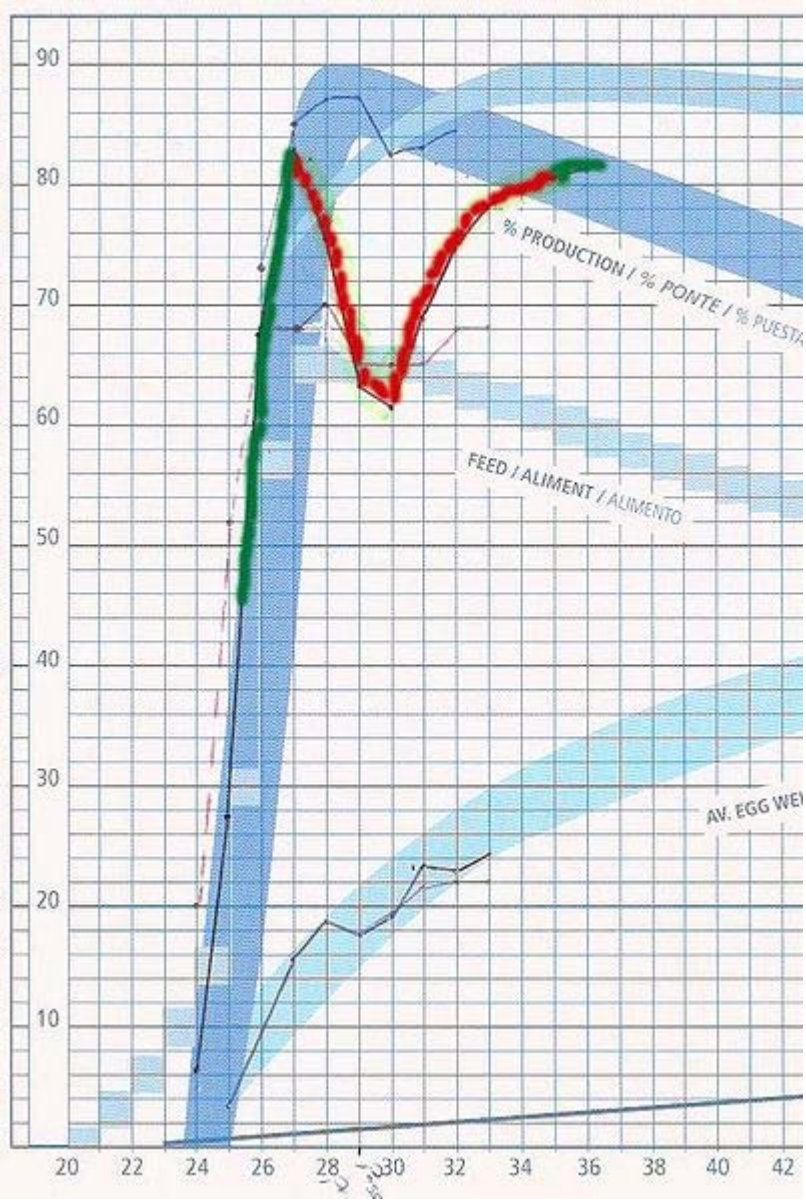
High concentrations of the NDV are found in birds' bodily discharges; therefore, the disease can be spread easily by mechanical means. Virus-bearing material can be picked up on shoes and clothing and carried from an infected flock to a healthy one.

NDV can survive for several weeks in a warm and humid environment on birds' feathers, manure, and other materials. It can survive indefinitely in frozen material. However, the virus is destroyed rapidly by dehydration and by the ultraviolet rays in sunlight.

Smuggled pet birds, especially Amazon parrots from Latin America, pose a great risk of introducing NDV into the US. Amazon parrot**Bold text**s that are carriers of the disease but do not show symptoms are capable of shedding NDV for more than 400 days.

Clinical findings

Symptoms



Egg drop after a (otherwise asymptomatic) Newcastle disease infection in a duly vaccinated broiler parent flock

Signs of infection with NDV vary greatly depending on factors such as the strain of virus and the health, age and species of the host.

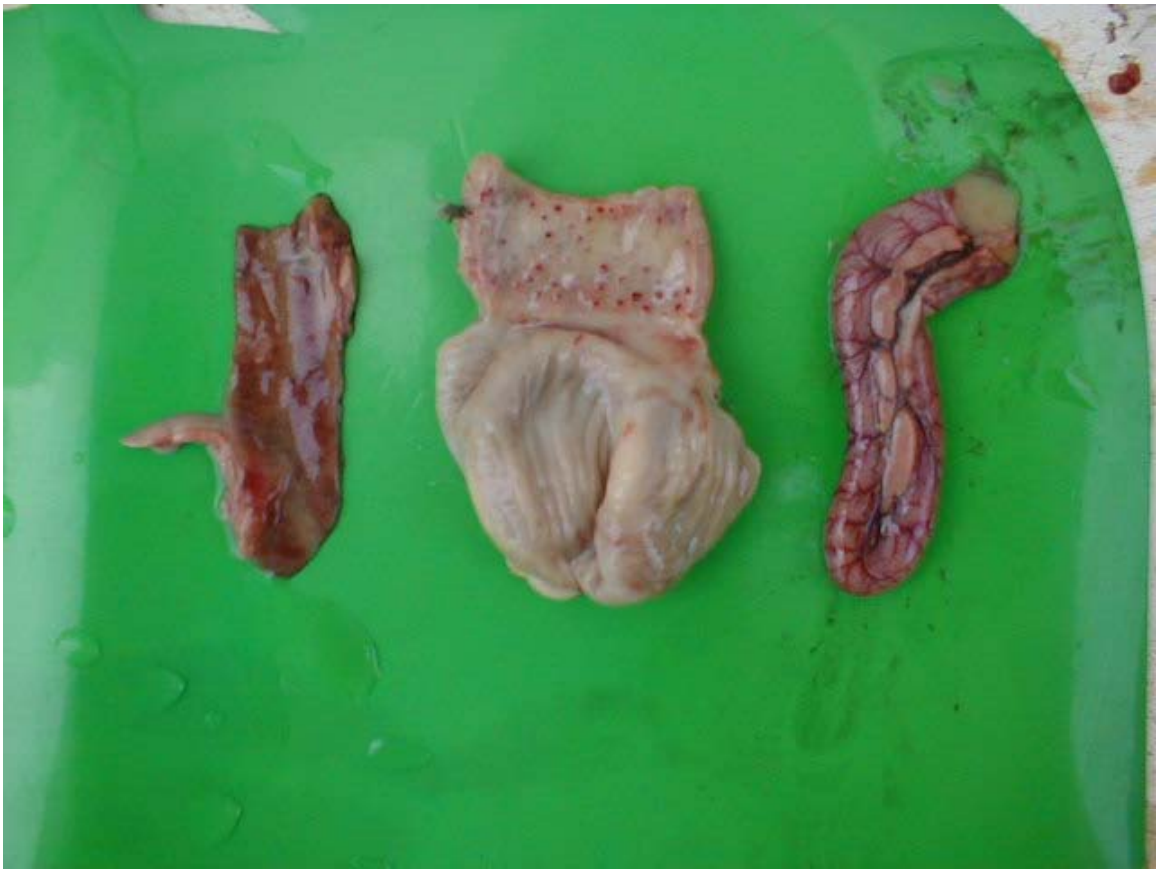
The incubation period for the disease ranges from 2 to 15 days. An infected bird may exhibit the following signs:

They can include respiratory signs (gasping, coughing), nervous signs (depression, inappetence, muscular tremors, drooping wings, twisting of head and neck, circling, complete paralysis), swelling of the tissues around the eyes and neck, greenish, watery diarrhoea, misshapen, rough- or thin-shelled eggs and reduced egg production.

In acute cases, the death is very sudden, and, in the beginning of the outbreak, the remaining birds do not seem to be sick. In flock with good immunity, however, the signs (respiratory an digestive) are mild and progressive, and are followed after 7 days by nervous symptoms, especially twisted heads.

Torticollis in a mallard.

Same symptom in a broiler.



PM lesions on proventriculus, gizzard and duodenum.

Post-mortem lesions

Typical are the petechiae in proventriculus and on submucosae of gizzard; there is also severe enteritis of the duodenum. The lesions are scarce in hyperacute cases (first day of outbreak).

Diagnosis

Immunological tests

Enzyme Linked Immunosorbent Assay (ELISA), PCR, Sequence technology.

Virus isolation

Samples

For routine isolation of NDV from chickens, turkeys, and other birds, samples are obtained by swabbing the trachea and the cloaca. Cotton swabs can be used. The virus can also be isolated from the lungs, brain spleen, liver, and kidneys.

Handling

Prior to shipping samples should be stored at 4 C. (refrigerator). Samples must be shipped in a padded envelope or box. Samples may be sent by regular mail, but overnight is recommended.

Prevention

Any animals that are showing symptoms of Newcastle Disease should be quarantined immediately. New birds should also be vaccinated before being introduced to your flock. There is an inactivated viral vaccine available, as well as various combination vaccines.

Chapter 13

Ovine Rinderpest

Ovine rinderpest	
Virus classification	
Group:	Group V ((-)ssRNA)
Order:	*Mononegavirales*
Family:	*Paramyxoviridae*
Genus:	*Morbillivirus*
Species:	***Peste-des-petits-ruminants virus***

Ovine rinderpest, also commonly known as ***peste des petits ruminants*** (**PPR**), is a contagious disease affecting goats and sheep in Africa (from Tropic of Cancer to Equator), the Middle-East and the Indian subcontinent. But since June 2008, the disease invaded Morocco , which indicates a crossing of the natural barrier of Sahara. It is caused by a species of the Morbillivirus genus of viruses. The disease is highly contagious, and has roughly an 80 percent mortality rate in acute cases.

Disease appellations

Traditionally, the name *kata* was given to stomatitis and pneumoenteritis of the Nigerian dwarf goat. *Peste des Petits Ruminants* was the French name of a similar disease of sheep and goats first described in the Ivory Coast in 1942. These diseases have been shown to be very close to each other.

Many authors prefer the name "Ovine Rinderpest". But official agencies such as the FAO and OIE use the French name "*Peste des Petits Ruminants*", "*Peste Des Petits Ruminants*", "*Peste-des-Petits-Ruminants*" or "*Peste-des-petits-ruminants*", even in English. The French acronym, PPR, is commonly used among veterinary professionals in East Africa.

Epidemiology

Geographical repartition

The disease is present in West Africa, part of Central Africa (Gabon, Central African Republic), East Africa (north of the Equator), Middle East and Indian subcontinent including Nepal and Myanmar.

In North Africa, only Egypt was once hit. But since summer 2008, Morocco is suffering a generalized outbreak with 133 known cases in 129 provinces, mostly affecting sheep. The outbreak has precipitated the vaccination of a large number of the 17 million sheep and five million goats in the country.

Contamination

The disease is spread from a region to another by sick animals. As virus is early inactivated outside the body, indirect contamination is generally limited.

In an affected flock, even in pest-free regions, the disease do not progress very rapidly, although close contact between animals. New clinical cases may be oberved daily for a one-month period.

Symptoms

They are similar to those of rinderpest in cattle. They vary following the previous immunitary status of sheep (enzootic or newly infected country). They also vary following sheep breed.

Incubation period is two to six days.

Hyperacute cases

Hyperacute cases are found dead without previous symptoms. They die with a serous, foamy or haemorrhagic discharge coming out of the nose.

Acute cases at onset

In acute cases, animals are recumbent, sometimes in self-auscultation position.

Body temperature is high (40.5 to 41°C.) in the beginning of the onset in acute cases.

The most typical signs are seen in the digestive tract. When entering an affected flock, one sees many animals with hind limbs stained by sticky faeces. Some sheep have an arched back and show pain to defecate. Tenesmus may be noticed when taking rectal temperature. Fluid faeces are olive green to brown.

Examination of the mouth shows ulceration of the buccal mucosae, especially on the inner face of the lips, and neighboring gum. They can be periodontitis.

There is serous nasal exsudate and conjunctivitis.

Evolution of acute cases

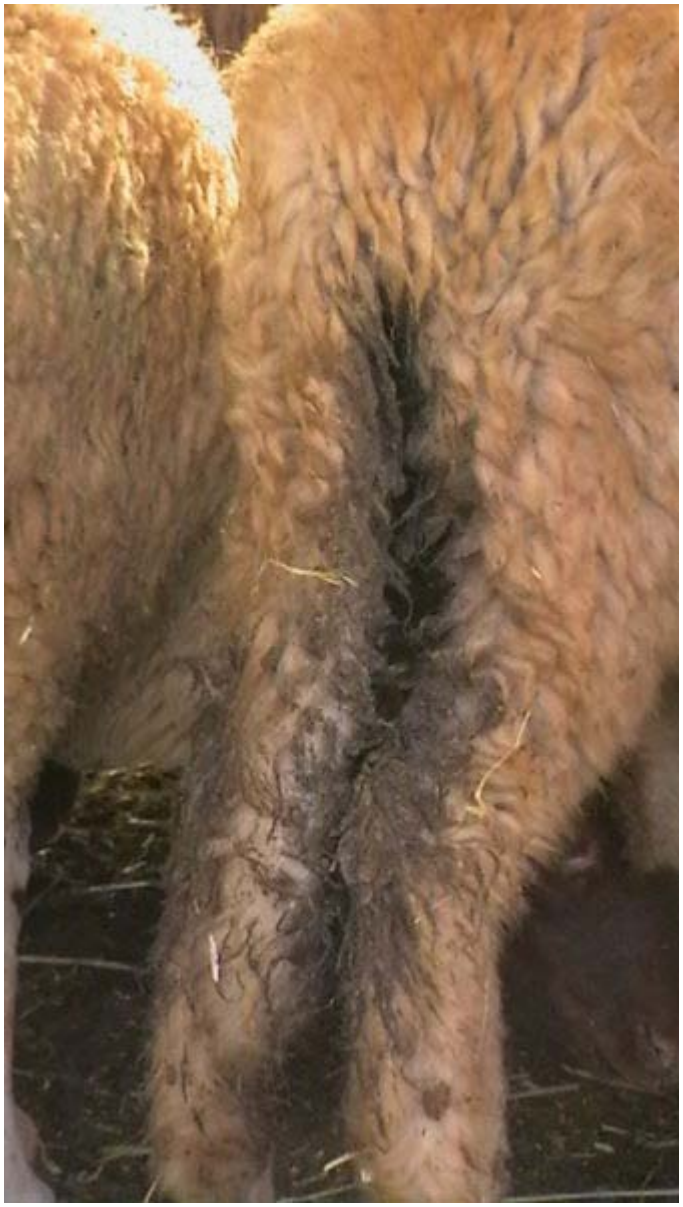
Nasal discharge becomes mucopurulent and may obstruct the nose.

A dry, fitful coughing develops.

Death occurs from 5 to 10 days after the onset of the fever.

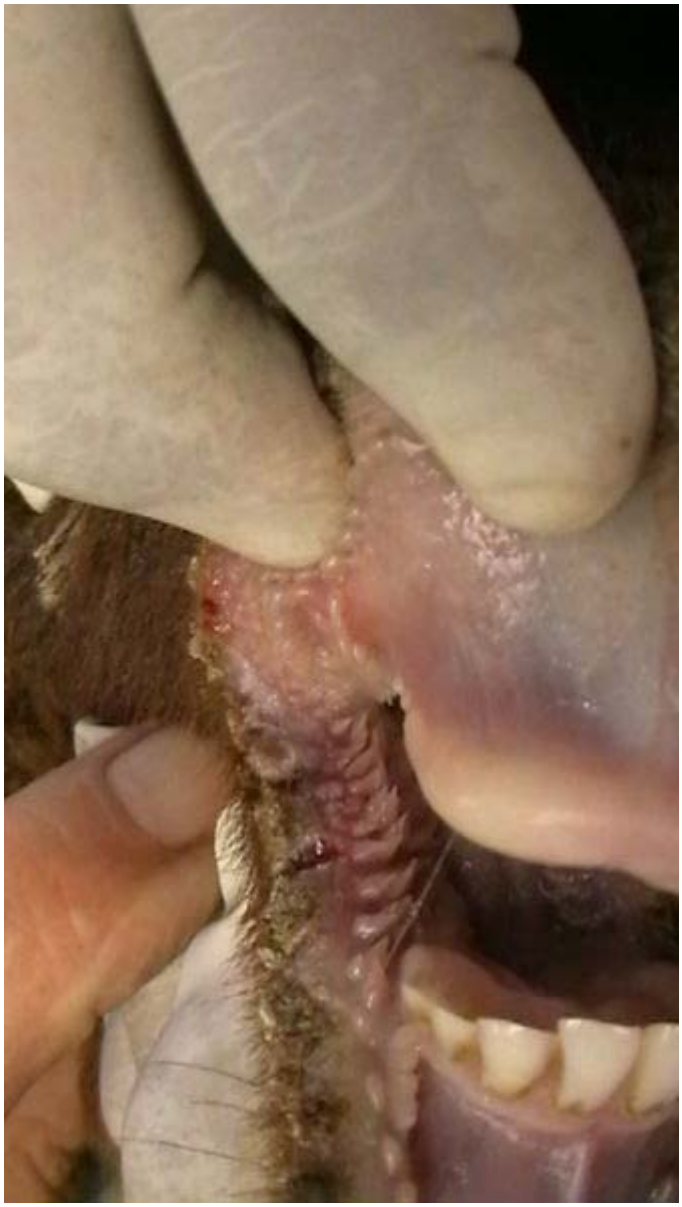
Some animals may recover, but a dry, stertorous coughing often persists for some days. Besides coughing, there is a intensive labial dermatitis with scab formation, resembling orf.

Self-auscultation in an acute case



Hind legs stained with sticky diarrhoea

Arched back (painful defecation)



inflammation and erosion of the mouth

Periodontitis

Mucopurulent nasal exsudate

Orf-like scabs on lips in a recovering case, Day 8

Post-mortem lesions

Field veterinarians should be aware that the pathognomonic lesions are situated in the digestive tract. Quick post-mortem examination will lead to the discovery of many haemorrhagic patches on the serous membranes, and intense pneumonia. They is a risk is to conclude to enzootic pneumonia, and not open the mouth, oesophagus and different parts of intestine.

Erosions and inflammation is widespread on buccal mucosa. The same lesions are also present in pharynx, oesophagus, and on mucus-producing epithelia of the gut, from abomasum to rectum. Zebra-striped lesions on coecum and colon are said to be typical in some cases. Rarely, they are also petechiae on the rumen mucosa.