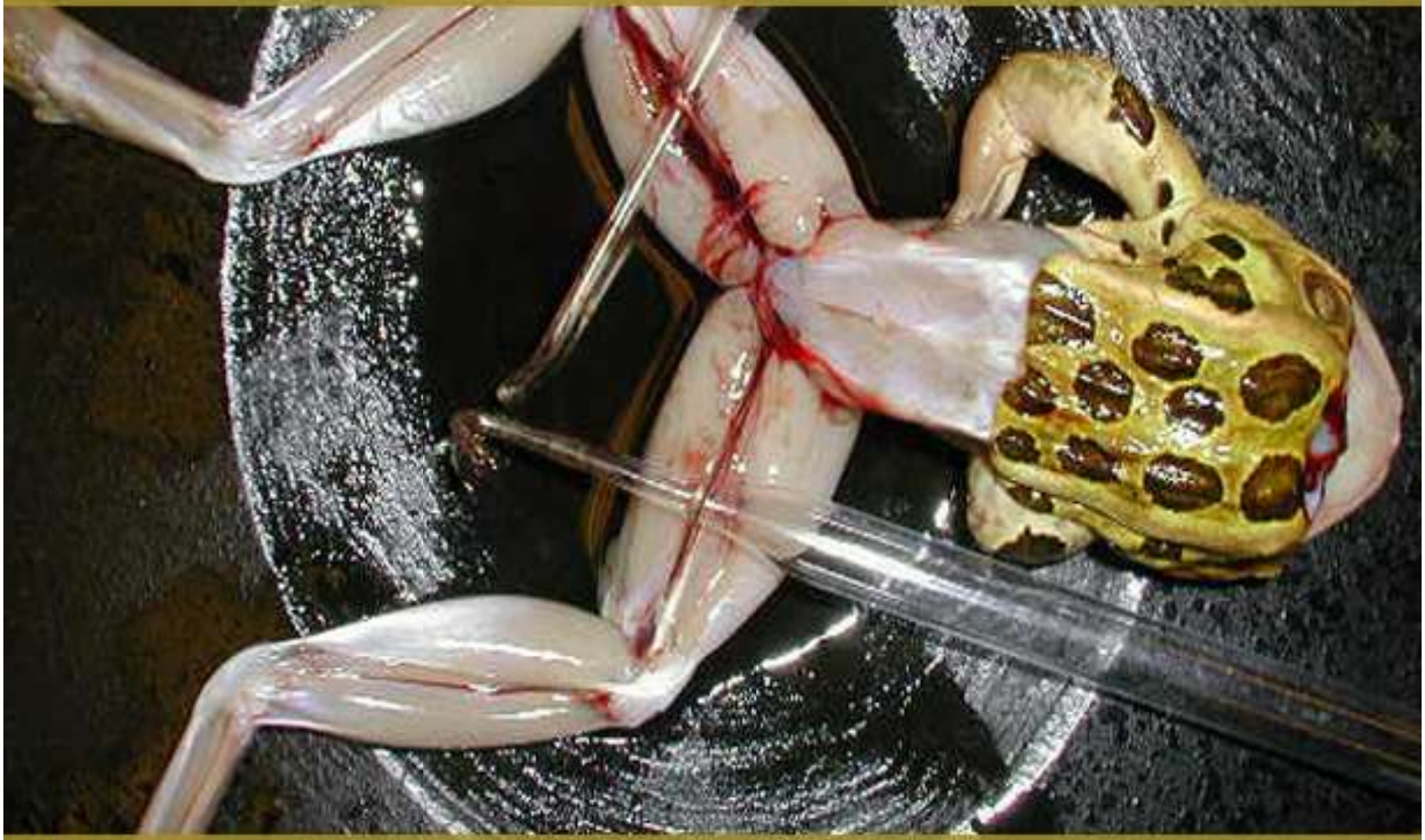
# Animal Virology and Physiology



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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

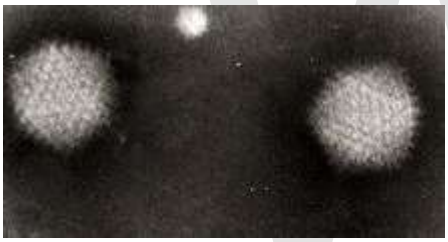
Attempts are made in the United States not to minimize the presence of highly pathogenic avian influenza (HPAI) in poultry in thorough routine surveillance of poultry flocks in commercial poultry operations. Detection of a HPAI virus may result in immediate culling of the flock. Less pathogenic viruses 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**<br>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

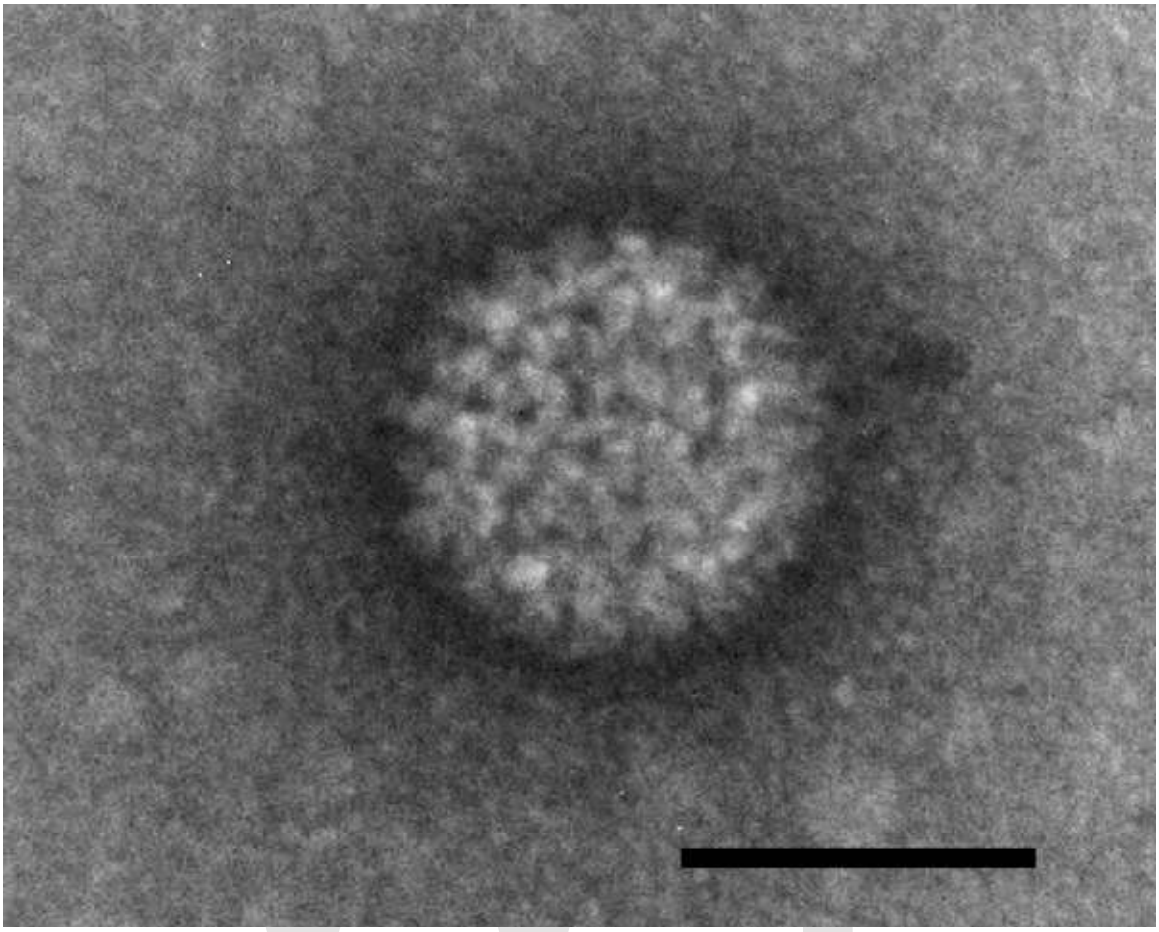
| Bluetongue virus |
| --- |
| **Virus classification** |

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

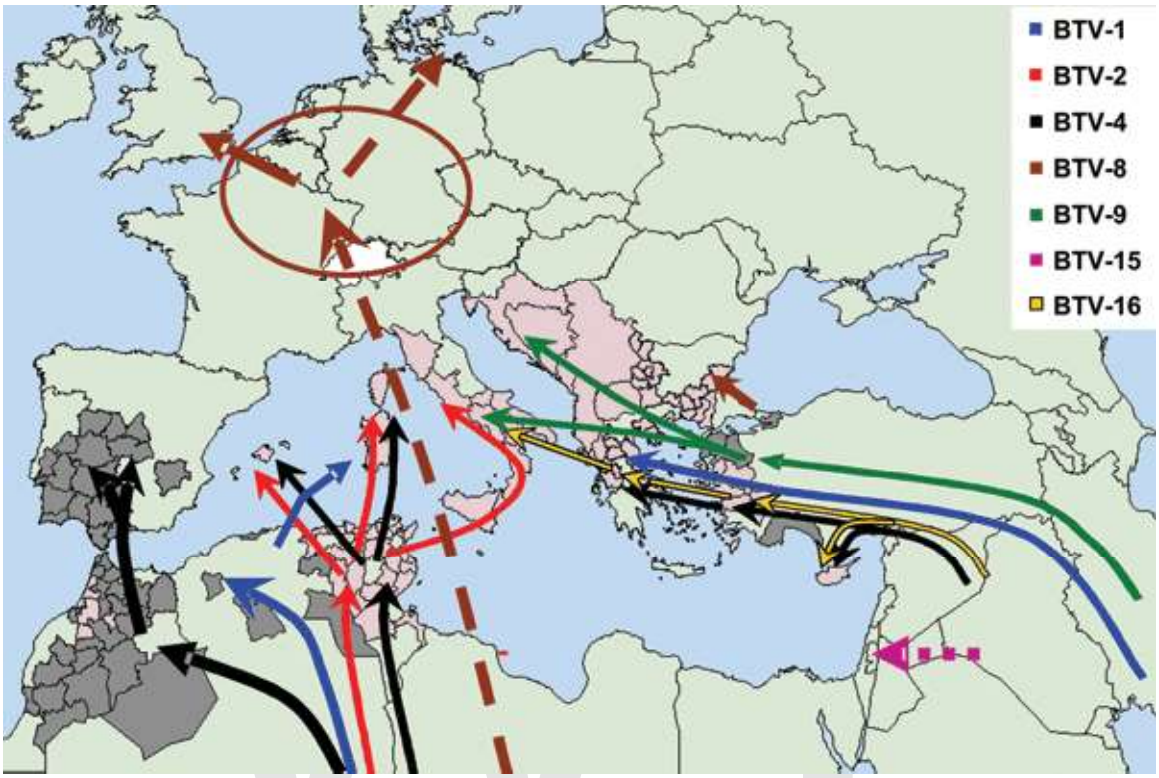
Bluetongue virus (BTV) is well characterized both genetically (the sequence was completed in 1989) and structurally. Understanding of the molecular biology of the virus and mapping the role of each protein in virus life cycle has benefited significantly through the availability of recombinant BTV proteins 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.

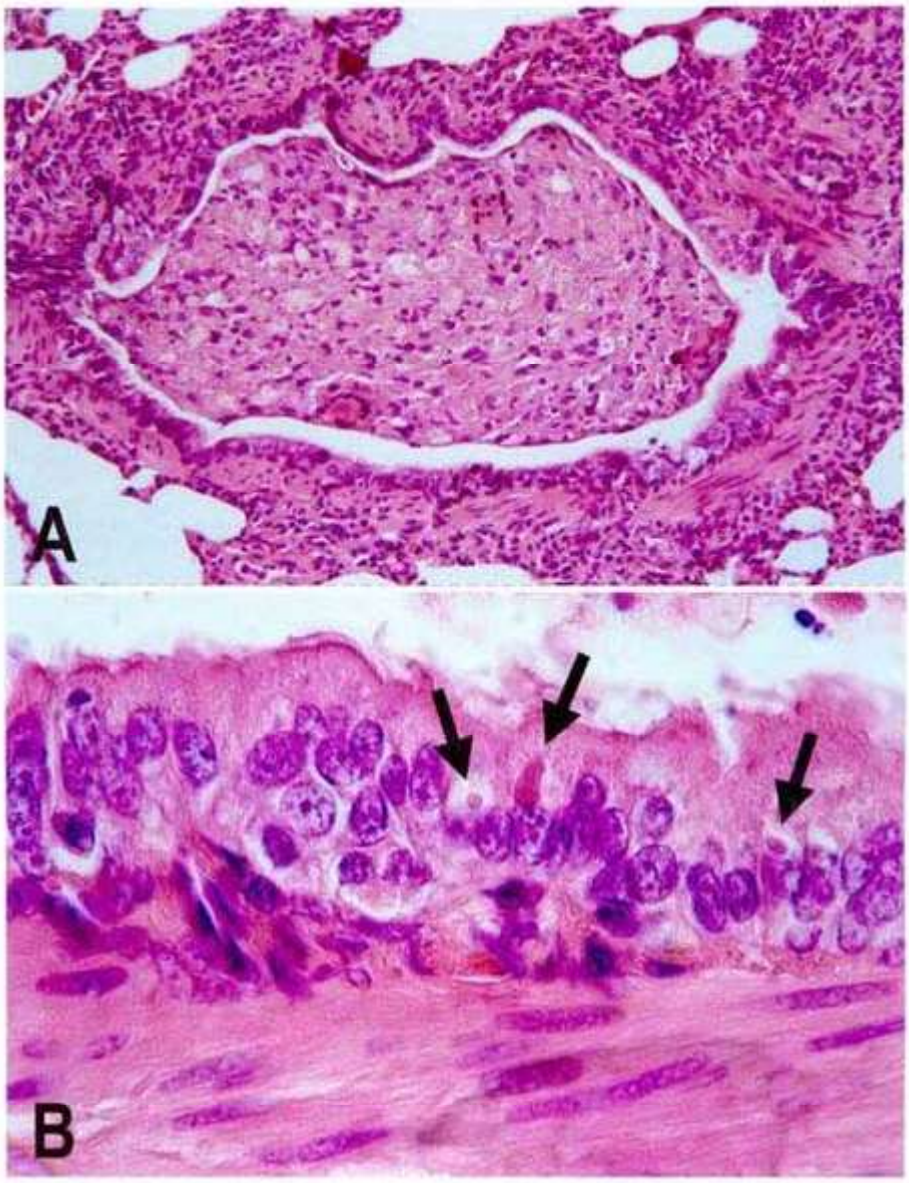
## Etymology

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.

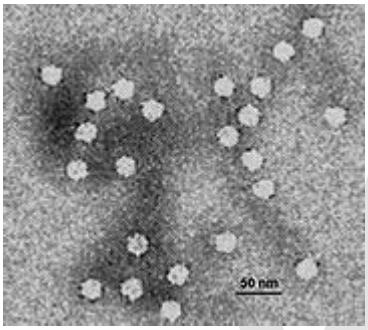
## *Canine distemper virus and Paget's disease*

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**

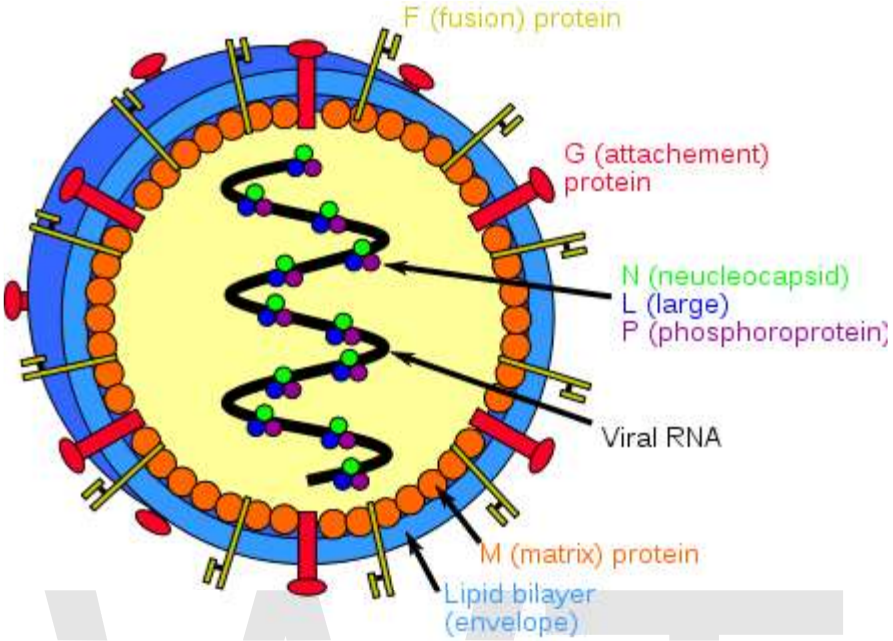
*Hendravirus*

**Species**

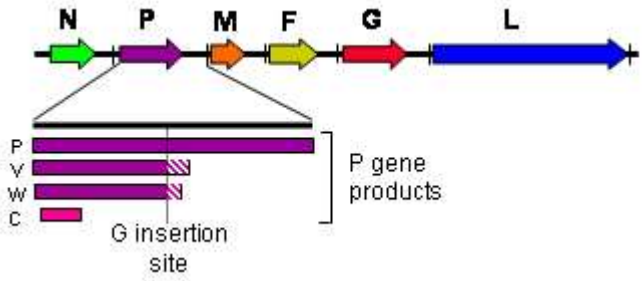
*Nipahvirus*

**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.

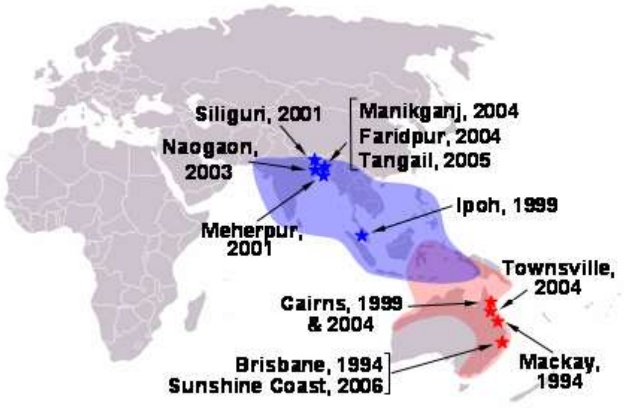
Symptoms of infection from the Malaysian outbreak were primarily encephalitic in humans and respiratory in pigs. Later outbreaks have caused respiratory illness in humans, increasing the likelihood of human-to-human transmission and indicating the existence of more dangerous strains of the virus.

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*

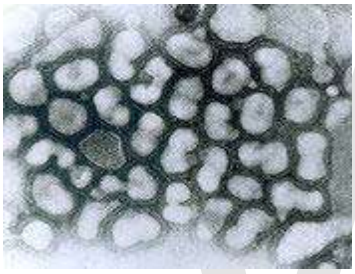
The emergence of henipaviruses parallels the emergence of other zoonotic viruses in recent decades. SARS coronavirus, Australian bat lyssavirus, Menangle virus and probably Ebola virus and Marburg virus are also harbored by bats and are capable of infecting a variety of other species. The emergence of 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.

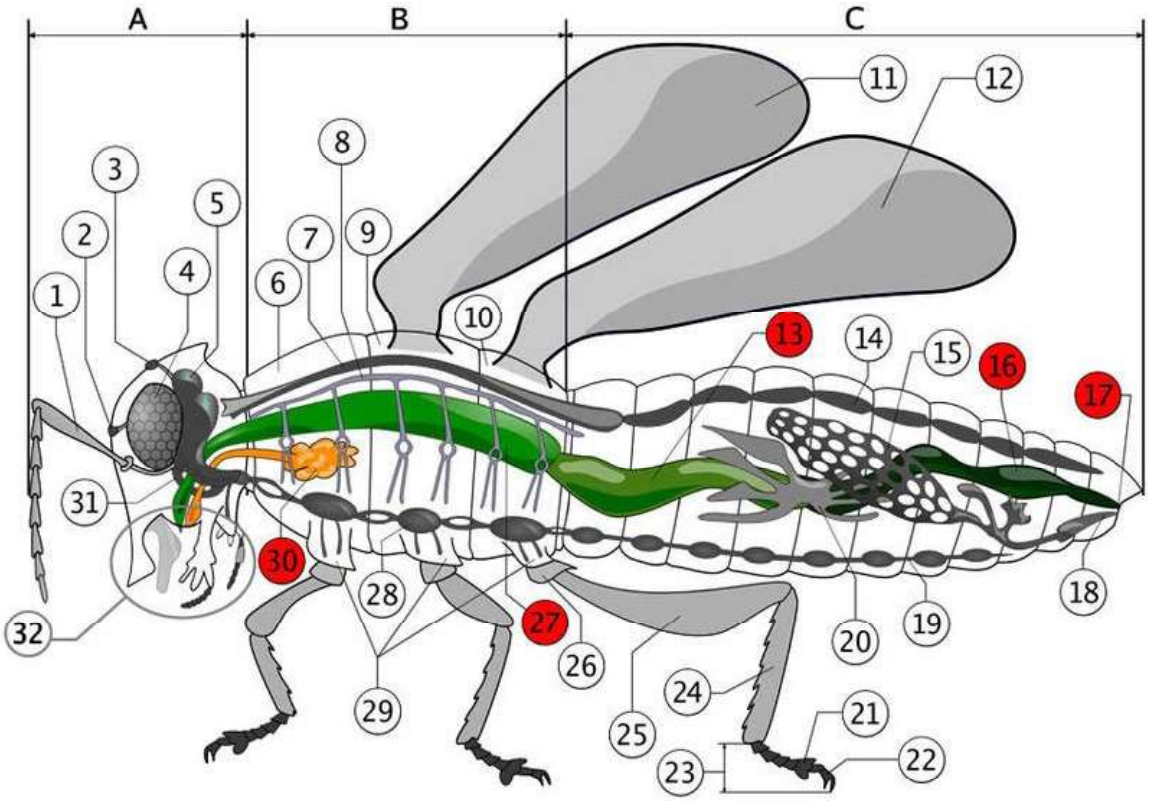
# Chapter 11

# Insect Physiology

**Insect physiology** includes the physiology and biochemistry of insect organ systems.

Although diverse, insects are quite similar in overall design, internally and externally. The insect is made up of three main body regions (tagmata), the head, thorax and abdomen. The head comprises six fused segments with compound eyes, ocelli, antennae and mouthparts, which differ according to the insect's particular diet, e.g. grinding, sucking, lapping and chewing. The thorax is made up of three segments the pro, meso and meta thorax, each supporting a pair of legs which may also differ, depending on function, e.g. jumping, digging, swimming and running. Usually the middle and the last segment of the thorax have paired wings. The abdomen generally comprises eleven segments and contains the digestive and reproductive organs (McGavin, 2001). A general overview of the internal structure and physiology of the insect is presented, including digestive, circulatory, respiratory, muscular, endocrine and nervous systems, as well as sensory organs, temperature control, flight and molting.

## *Digestive System*




Insect digestive system
**A**- Head **B**- Thorax **C**- Abdomen
13. mid-gut (stomach)
15. ovary
16. hind-gut (intestine, rectum & anus)
17. anus
27. fore-gut (crop, gizzard)
30. salivary gland

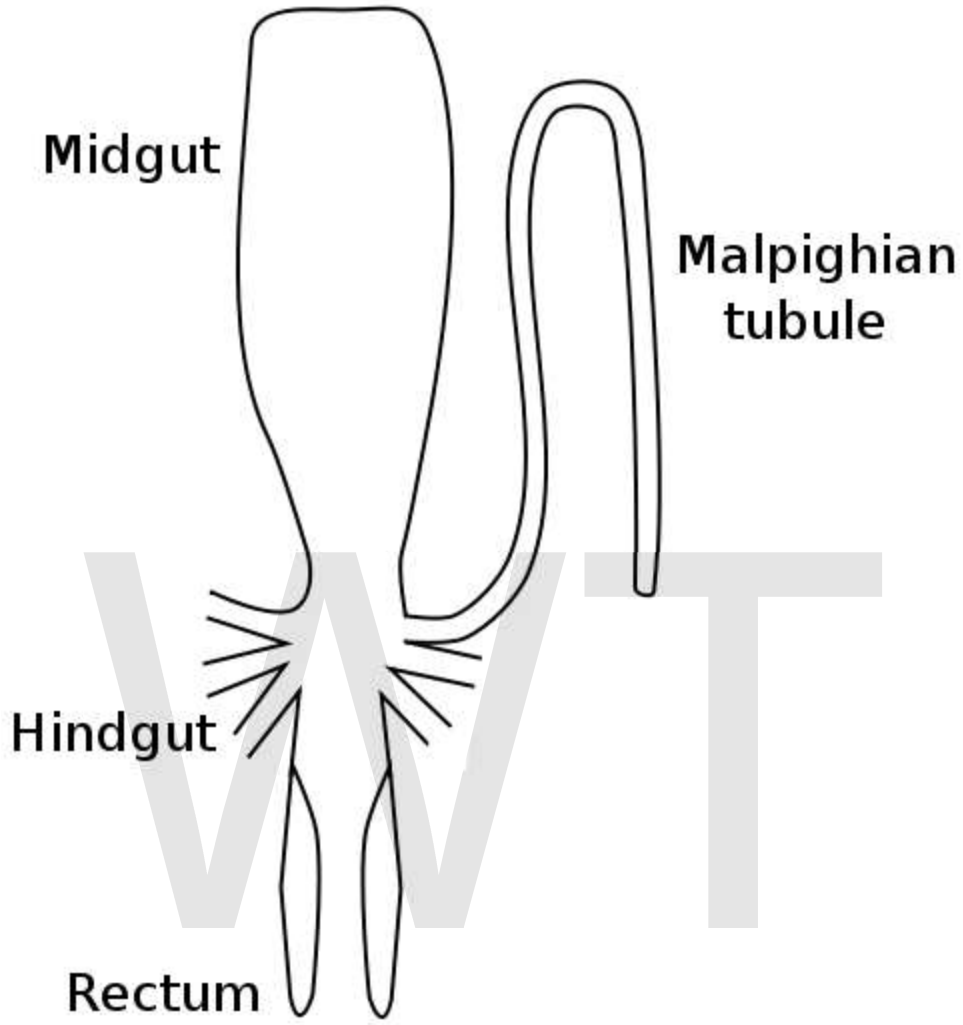
An insect uses its digestive system to extract nutrients and other substances from the food it consumes. Most of this food is ingested in the form of macromolecules and other complex substances (such as proteins, polysaccharides, fats, and nucleic acids) which must be broken down by catabolic reactions into smaller molecules (i.e. amino acids, simple sugars, etc.) before being used by cells of the body for energy, growth, or reproduction. This break-down process is known as digestion.

The insect's digestive system is a closed system, with one long enclosed coiled tube called the alimentary canal which runs lengthwise through the body. The alimentary canal only allows food to enter the mouth, and then gets processed as it travels toward the anus. The insects alimentary canal has specific sections for grinding and food storage, enzyme production and nutrient absorption (McGavin, 2001; Triplehorn & Johnson, 2005). Sphincters control the food and fluid movement between three regions. The three

regions include the foregut (stomatodeum)(27,) the midgut (mesenteron)(13), and the hindgut (proctodeum)(16).

In addition to the alimentary canal, insects also have paired salivary glands and salivary reservoirs. These structures usually reside in the thorax (adjacent to the fore-gut). The salivary glands (30) produce saliva, the salivary ducts lead from the glands to the reservoirs and then forward through the head to an opening called the salivarium behind the hypopharynx; which movements of the mouthparts help mix saliva with food in the buccal cavity. Saliva mixes with food which travels through salivary tubes into the mouth, beginning the process of breaking it down.

The stomatedeum and proctodeum are invaginations of the epidermis and are lined with cuticle (intima). The mesenteron is not lined with cuticle but with rapidly dividing and therefore constantly replaced, epithelial cells (McGavin, 2001; Triplehorn & Johnson, 2005). The cuticle sheds with every moult along with the exoskeleton (Triplehorn & Johnson, 2005). Food is moved down the gut by muscular contractions called peristalsis (Elzinga, 2004).




Stylised diagram of insect digestive tract showing malpighian tubule (Orthopteran type)

1. **Stomatodeum**(foregut): This region stores, grinds and transports food to the next region (Gullan & Cranston, 2005). Included in this are the buccal cavity, the pharynx, the oesophagus, the crop (stores food), and proventriculus or gizzard (grinds food) (Triplehorn & Johnson, 2005). Salivary secretions from the labial glands dilute the ingested food. In mosquitoes (Diptera), which are blood-feeding insects, anticoagulants and blood thinners are also released here.

2. **Mesenteron**(midgut): Digestive enzymes in this region are produced and secreted into the lumen and here nutrients are absorbed into the insect's body. Food is enveloped by this part of the gut as it arrives from the foregut by the peritrophic membrane which is a mucopolysaccharide layer secreted from the midgut's epithelial cells (McGavin, 2001). It

is thought that this membrane prevents food pathogens from contacting the epithelium and attacking the insects' body (McGavin, 2001). It also acts as a filter allowing small molecules through, but preventing large molecules and particles of food from reaching the midgut cells (Gullan & Cranston, 2005). After the large substances are broken down into smaller ones, digestion and consequent nutrient absorption takes place at the surface the epithelium (McGavin, 2001). Microscopic projections from the mid-gut wall, called microvilli, increase surface area and allow for maximum absorption of nutrients.

3. **Proctodeum**(hindgut): This is divided into three sections; the anterior is the ileum, the middle portion, the colon, and the wider, posterior section is the rectum (Gullan & Cranston, 2005). This extends from the pyloric valve which is located between the mid and the hindgut to the anus (Triplehorn & Johnson, 2005). Here absorption of water, salts and other beneficial substances take place before excretion (Gullan & Cranston, 2005). Like other animals, the removal of toxic metabolic waste requires water. However, for very small animals like insects, water conservation is a priority. Because of this, blind-ended ducts called Malpighian tubules come into play (McGavin, 2001). These ducts emerge as evaginations at the anterior end of the hindgut and are the main organs of osmoregulation and excretion (Triplehorn & Johnson, 2005; Gullan & Cranston, 2005). These extract the waste products from the haemolymph, in which all the internal organs are bathed (McGavin, 2001). These tubules continually produce the insect's uric acid, which is transported to the hindgut, where important salts and water are re-absorbed by both the hindgut and rectum. Excrement is then voided as insoluble and non-toxic uric acid granules (McGavin, 2001). Excretion and osmoregulation in insects are not orchestrated by the Malpighian tubules alone, but require a joint function of the ileum and/or rectum (Gullan & Cranston, 2005).

## *Circulatory System*

Insect blood or haemolymph's main function is that of transport and it bathes the insect's body organs. Making up usually less than 25% of an insect's body weight, it transports hormones, nutrients and wastes and has a role in, osmoregulation, temperature control, immunity, storage (water, carbohydrates and fats) and skeletal function. It also plays an essential part in the moulting process (McGavin, 2001; Triplehorn & Johnson, 2005). An additional role of the haemolymph in some orders, can be that of predatory defence. It can contain unpalatable and malodourous chemicals that will act as a deterrent to predators (Gullan & Cranston, 2005).

Haemolymph contains molecules, ions and cells (Gullan & Cranston, 2005). Regulating chemical exchanges between tissues, haemolymph is encased in the insect body cavity or haemocoel (Elzinga, 2004; Gullan & Cranston, 2005). It is transported around the body by combined heart (posterior) and aorta (anterior) pulsations which are located dorsally just under the surface of the body (McGavin, 2001; Gullan & Cranston, 2005; Triplehorn & Johnson, 2005). It differs from vertebrate blood in that it doesn't contain any red blood cells and therefore is without high oxygen carrying capacity, and is more similar to lymph found in vertebrates (Elzinga, 2004; Gullan & Cranston, 2005).

Body fluids enter through one way valved ostia which are openings situated along the length of the combined aorta and heart organ. Pumping of the haemolymph occurs by waves of peristaltic contraction, originating at the body's posterior end, pumping forwards into the dorsal vessel, out via the aorta and then into the head where it flows out into the haemocoel (Elzinga, 2004; Gullan & Cranston, 2005). The haemolymph is circulated to the appendages unidirectionally with the aid of muscular pumps or accessory pulsatile organs which are usually found at the base of the antennae or wings and sometimes in the legs (Gullan & Cranston, 2005). Pumping rate accelerates due to periods of increased activity (Triplehorn & Johnson, 2005). Movement of haemolymph is particularly important for thermoregulation in orders such as Odonata, Lepidoptera, Hymenoptera and Diptera (Gullan & Cranston, 2005).

## *Respiratory system*

Insect respiration is accomplished without lungs using a system of internal tubes and sacs through which gases either diffuse or are actively pumped, delivering oxygen directly to tissues that need oxygen and eliminate carbon dioxide via their cells (Gullan & Cranston, 2005). Since oxygen is delivered directly, the circulatory system is not used to carry oxygen, and is therefore greatly reduced; it has no closed vessels (i.e., no veins or arteries), consisting of little more than a single, perforated dorsal tube which pulses peristaltically, and in doing so helps circulate the hemolymph inside the body cavity.

Air is taken in through spiracles, openings which are positioned laterally in the pleural wall, usually a pair on the anterior margin of the meso and meta thorax, and pairs on each of the eight or less abdominal segments, Numbers of spiracles vary from 1 to 10 pairs (McGavin 2001; Elzinga, 2004; Gullan & Cranston, 2005; Triplehorn & Johnson, 2005). The oxygen passes through the tracheae to the trachioles, and enters the body by the process of diffusion. Carbon dioxide leaves the body by the same process (Triplehorn & Johnson, 2005).

The major tracheae are thickened spirally like a flexible vacuum hose to prevent them from collapsing and often swell into air sacs. Larger insects can augment the flow of air through their tracheal system, with body movement and rhythmic flattening of the tracheal air sacs (Triplehorn & Johnson, 2005). Spiracles are closed and opened by means of valves and can remain partly or completely closed for extended periods in some insects, which minimises water loss (McGavin, 2001; Triplehorn & Johnson, 2005,).

There are many different patterns of gas exchange demonstrated by different groups of insects. Gas exchange patterns in insects can range from continuous, diffusive ventilation, to discontinuous gas exchange.

Terrestrial and a large proportion of aquatic insects perform gaseous exchange as previously mentioned under an open system. Other smaller numbers of aquatic insects have a closed tracheal system, for example, Odonata, Tricoptera, Ephemeroptera, which have tracheal gills and no functional spiracles. Endoparasitic larvae are without spiracles and also operate under a closed system. Here the tracheae separate peripherally, covering

the general body surface which results in a cutaneous form of gaseous exchange. This peripheral tracheal division may also lie within the tracheal gills where gaseous exchange may also take place (Gullan & Cranston, 2005).

## *Muscular system*

Many insects are able to lift twenty times their own body weight and may jump distances that are many times greater than their own length. This is not because they are strong but because they are so small. Muscle power is proportional to its cross-sectional area. Because the mass(the insect's body), that is moved is in proportion to its volume and the fact that they also have a better leverage system than we humans do, they can jump remarkable distances. (Elzinga, 2004; Triplehorn & Johnson, 2005).

The muscular system of insects ranges from a few hundred muscles to a few thousand (Triplehorn & Johnson, 2005). Unlike vertebrates that have both smooth and striated muscles, insects have only striated muscles. Muscle cells are amassed into muscle fibres and then into the functional unit, the muscle (Elzinga, 2004). Muscles are attached to the body wall, with attachment fibres running through the cuticle and to the epicuticle, where they can move different parts of the body including appendages such as wings (Gullan & Cranston, 2005; Triplehorn & Johnson, 2005). The muscle fibre has many cells with a plasma membrane and outer sheath or sarcolemma (Gullan & Cranston, 2005). The sarcolemma is invaginated and can make contact with the tracheole carrying oxygen to the muscle fibre. Arranged in sheets or cylindrically, contractile myofibrils run the length of the muscle fibre. Myofibrils comprising a fine actin filament enclosed between a thick pair of myosin filaments slide past each other instigated by nerve impulses (Gullan & Cranston, 2005).

Muscles can be divided into four categories:

1. **Visceral**: these muscles surround the tubes and ducts and produce peristalsis as demonstrated in the digestive system (Elzinga, 2004).
2. **Segmental**: causing telescoping of muscle segments required for moulting, increase in body pressure and locomotion in legless larvae (Elzinga, 2004).
3. **Appendicular**: originating from either the sternum or the tergum and inserted on the coxae these muscles move appendages as one unit. (Elzinga, 2004) These are arranged segmentally and usually in antagonistic pairs (Triplehorn & Johnson, 2005). Appendage parts of some insects, e.g. the galea and the lacinia of the maxillae, only have flexor muscles. Extension of these structures is by haemolymph pressure and cuticle elasticity (Triplehorn & Johnson, 2005).
4. **Flight**: Flight muscles are the most specialised category of muscle and are capable of rapid contractions. Nerve impulses are required to initiate muscle contractions and therefore flight. These muscles are also known as neurogenic or synchronous muscles. This is because there is a one to one correspondence between action potentials and muscle contractions. In insects with higher wing stroke frequencies the muscles contract more frequently than at the rate that the

nerve impulse reaches them and are known as asynchronous muscles (McGavin, 2001; Gullan & Cranston, 2005).

Flight has allowed the insect to disperse, escape from enemies, environmental harm, and colonise new habitats (McGavin, 2001). One of the insect's key adaptations, the mechanics of flight differ from other flying animals because their wings are not modified appendages (McGavin, 2001; Elzinga, 2004). Fully developed and functional wings occur only in adult insects (Gullan & Cranston, 2005). To fly, gravity and drag (air resistance to movement) has to be overcome (Gullan & Cranston, 2005). Most insects fly by beating their wings and to power their flight they have either direct flight muscles attached to the wings, or an indirect system where there is no muscle to wing connection and instead they are attached to a highly flexible box like thorax (Gullan & Cranston, 2005).

Direct flight muscles generate the upward stroke by the contraction of the muscles attached to the base of the wing inside the pivotal point. Outside the pivotal point the downward stroke is generated through contraction of muscles that extend from the sternum to the wing. Indirect flight muscles are attached to the tergum and sternum. Contraction makes the tergum and base of the wing pull down. In turn this movement lever the outer or main part of the wing in strokes upward. Contraction of the second set of muscles, which run from the back to the front of the thorax, powers the downbeat. This deforms the box and lifts the tergum (Gullan & Cranston, 2005).

## *Endocrine system*

Hormones are the chemical substances that are transported in the insect's body fluids (haemolymph) that carry messages away from their point of synthesis to sites that where physiological processes are influenced. These hormones are produced by glandular, neuroglandular and neuronal centres (Gullan & Cranston, 2005). Insects have several organs that produce hormones, controlling reproduction, metamorphosis and moulting (Triplehorn & Johnson, 2005). It has been suggested that a brain hormone is responsible for caste dermination in termites and diapause interruption in some insects (Triplehorn & Johnson, 2005).

Four endocrine centers have been identified:

1. **Neurosecretory cells** in the brain can produce one or more hormones that affect growth, reproduction, homeostasis and metamorphosis (Gullan & Cranston, 2005; Triplehorn & Johnson, 2005).
2. **Corpora cardiaca** are a pair of neuroglandular bodies that are found behind the brain and on either sides of the aorta. These not only produce their own neurohormones but they store and release other neurohormones including PTTH prothoracicotropic hormone (brain hormone), which stimulates the secretory activity of the prothoracic glands, playing an integral role in moulting.
3. **Prothoracic glands** are diffuse, paired glands located at the back of the head or in the thorax. These glands secrete an ecdysteroid called ecdysone, or the moulting

hormone, which initiates the epidermal moulting process (Gullan & Cranston, 2005). Additionally it plays a role in accessory reproductive glands in the female, differentiation of ovarioles and in the process of egg production.

4. **Corpora allata** are small, paired glandular bodies originating from the epithelium located on either side of the foregut. They secrete the juvenile hormone, which regulate reproduction and metamorphosis (Gullan & Cranston, 2005; Triplehorn & Johnson, 2005).

## *The Nervous System*

Insects have a complex nervous system which incorporates a variety of internal physiological information as well as external sensory information (Gullan & Cranston, 2005) Like invertebrates the basic component is the neuron or nerve cell. This is made up of a dendrite with two projections that receive stimuli and an axon, which transmits information to another neuron or organ, like a muscle. As for vertebrates, chemicals (neurotransmitters such as acetylcholine and dopamine) are released at synapses (Gullan & Cranston, 2005).

**Central nervous system**: An insect's sensory, motor and physiological processes are controlled by the central nervous system along with the endocrine system (Gullan & Cranston, 2005). Being the principal division of the nervous system, it consists of a brain, a ventral nerve cord and a subesophageal ganglion. This is connected to the brain by two nerves, extending around each side of the oesophagus.

The brain has three lobes:

- **Procerebrum,** innervating the compound eyes and the ocelli
- **Deutocerebrum,** innervating the antennae
- **Tritocerebrum,** innervating the foregut and the labrum (Gullan & Cranston, 2005; Triplehorn & Johnson, 2005).

The ventral nerve cord extends from the suboesophageal ganglion posteriorly (Triplehorn & Johnson, 2005). A layer of connective tissue called the neurolemma covers the brain, ganglia, major peripheral nerves and ventral nerve cords.

The head capsule (made up of six fused segments) has six pairs of ganglia. The first three pairs are fused into the brain, while the three following pairs are fused into the subesophageal ganglion. The thoracic segments have one ganglion on each side, which are connected into a pair, one pair per segment. This arrangement is also seen in the abdomen but only in the first eight segments. Many species of insects have reduced numbers of ganglia due to fusion or reduction. Some cockroaches have just six ganglia in the abdomen, whereas the wasp *Vespa crabro* has only two in the thorax and three in the abdomen. And some, like the house fly *Musca domestica*, have all the body ganglia fused into a single large thoracic ganglion. The ganglia of the central nervous system act as the coordinating centres with their own specific autonomy where each may coordinate impulses in specified regions of the insect's body (Triplehorn & Johnson, 2005).

**Peripheral nervous system**: This consists of motor neuron axons that branch out to the muscles from the ganglia of the central nervous system, parts of the sympathetic nervous system and the sensory neurons of the cuticular sense organs that receive chemical, thermal, mechanical or visual stimuli from the insects environment (Gullan & Cranston, 2005). The sympathetic nervous system includes nerves and the ganglia that innervate the gut both posteriorly and anteriorly, some endocrine organs, the spiracles of the tracheal system and the reproductive organs (Gullan & Cranston, 2005).

**Sense Organs**: Chemical senses include the use of chemoreceptors, related to taste and smell, affecting mating, habitat selection, feeding and parasite-host relationships. Taste is usually located on the mouthparts of the insect but in some insects, such as bees, wasps and ants, taste organs can also be found on the antennae. Taste organs can also be found on the tarsi of moths, butterflies and flies. Olfactory sensilla enable insects to smell and are usually found in the antennae (McGavin, 2001). Chemoreceptor sensitivity related to smell in some substances, is very high and some insects can detect particular odours that are at low concentrations miles from their original source (Triplehorn & Johnson, 2005).

Mechanical senses provide the insect with information that may direct orientation, general movement, flight from enemies, reproduction and feeding and are elicited from the sense organs that are sensitive to mechanical stimuli such as pressure, touch and vibration (Triplehorn & Johnson, 2005). Hairs (setae) on the cuticle are responsible for this as they are sensitive to vibration touch and sound (McGavin, 2001).

Hearing structures or tympanal organs are located on different body parts such as, wings, abdomen, legs and antennae. These can respond to various frequencies ranging from 100 to 240 kHz depending on insect species (Triplehorn & Johnson, 2005). Many of the joints of the insect have tactile setae that register movement. Hair beds and groups of small hair like sensilla, determine proprioreception or information about the position of a limb, and are found on the cuticle at the joints of segments and legs. Pressure on the body wall or strain gauges are detected by the campiniform sensilla and internal stretch receptors sense muscle distension and digestive system stretching (McGavin 2001; Triplehorn & Johnson, 2005,).

The compound eye and the ocelli supply insect vision. The compound eye consists of individual light receptive units called ommatidia. Some ants may have only one or two, however dragonflies may have over 10,000. The more ommatidia the greater the visual acuity. These units have a clear lens system and light sensitive retina cells. By day, the image flying insects receive is made up of a mosaic of specks of differing light intensity from all the different ommatidia. At night or dusk, visual acuity is sacrificed for light sensitivity (McGavin, 2001). The ocelli are unable to form focussed images but are sensitive mainly, to differences in light intensity (Triplehorn & Johnson, 2005). Colour vision occurs in all orders of insects. Generally insects see better at the blue end of the spectrum than at the red end. In some orders sensitivity ranges can include ultraviolet (McGavin, 2001).

A number of insects have temperature and humidity sensors (McGavin, 2001) and insects being small, cool more quickly than larger animals. Insects are generally considered cold-blooded or ectothermic, their body temperature rising and falling with the environment. However, flying insects raise their body temperature through the action of flight, above environmental temperatures (Elzinga, 2004; Triplehorn & Johnson, 2005,).

The body temperature of butterflies and grasshoppers in flight may be 5°C or 10°C above environmental temperature, however moths and bumblebees, insulated by scales and hair, during flight, may raise flight muscle temperature 20–30°C above the environment temperature. Most flying insects have to maintain their flight muscles above a certain temperature to gain power enough to fly. Shivering, or vibrating the wing muscles allow larger insects to actively increase the temperature of their flight muscles, enabling flight (Triplehorn & Johnson, 2005).

Until very recently, no one had ever documented the presence of nociceptors (the cells that detect and transmit sensations of pain) in insects, though recent findings of nociception in larval fruit flies challenges this and raises the possibility that some insects may be capable of feeling pain.

## *Reproductive System*

Most insects have a high reproductive rate. With a short generation time, they evolve faster and can adjust to environmental changes more rapidly than other slower breeding animals (McGavin, 2001). Although there are many forms of reproductive organs in insects, there remains a basic design and function for each reproductive part. These individual parts may vary in shape (gonads), position (accessory gland attachment), and number (testicular and ovarian glands), with different insect groups (Gullan & Cranston, 2005).

### Female Reproductive System

The female insect's main reproductive function is to produce eggs, including the egg's protective coating, and to store the male spermatozoa until egg fertilisation is ready. The female reproductive organs include, paired ovaries which empty their eggs (oocytes) via the calyces into lateral oviducts, joining to form the common oviduct. The opening (gonopore) of the common oviduct is concealed in a cavity called the genital chamber and this serves as a copulatory pouch (bursa copulatrix) when mating (Gullan & Cranston, 2005). The external opening to this is the vulva. Often in insects the vulva is narrow and the genital chamber becomes pouch or tube like and is called the vagina. Related to the vagina is a saclike structure, the spermatheca, where spermatozoa are stored ready for egg fertilisation. A secretory gland (Gullan & Cranston, 2005; Triplehorn & Johnson, 2005) nourishes the contained spermatozoa in the vagina.

Egg development is mostly completed by the insect's adult stage and is controlled by hormones that control the initial stages of oogenesis and yolk deposition (Gullan &

Cranston, 2005). Most insects are oviviparous, where the young hatch after the eggs have been laid (Triplehorn & Johnson, 2005).

Insect sexual reproduction starts with sperm entry that stimulates oogenesis, meiosis occurs and the egg moves down the genital tract. Accessory glands of the female secrete an adhesive substance to attach eggs to an object and they also supply material that provides the eggs with a protective coating. Oviposition takes place via the female ovipositor (Elzinga, 2004; Triplehorn & Johnson, 2005).

### Male reproductive system

The male's main reproductive function is to produce and store spermatozoa and provide transport to the reproductive tract of the female (Gullan & Cranston, 2005). Sperm development is usually completed by the time the insect reaches adulthood (Triplehorn & Johnson, 2005). The male has two testes, which contain follicles in which the spermatozoa are produced. These open separately into the sperm duct or vas deferens and this stores the sperm (Gullan & Cranston, 2005). The vas deferentia then unite posteriorally to form a central ejaculatory duct, this opens to the outside on an aedeagus or a penis (Triplehorn & Johnson, 2005). Accessory glands secrete fluids that comprise the spermatophore. This becomes a package that surrounds and carries the spermatozoa, forming a sperm-containing capsule (Gullan & Cranston, 2005; Triplehorn & Johnson, 2005,).

**Sexual and asexual reproduction** Most insects reproduce via sexual reproduction, i.e. the egg is produced by the female, fertilised by the male and oviposited by the female. Eggs are usually deposited in a precise microhabitat on or near the required food (Elzinga, 2004). However, some adult females can reproduce without male input. This is known as parthenogenesis and in the most common type of parthenogenesis the offspring are essentially identical to the mother. This is most often seen in aphids and scale insects (Elzinga, 2004).

## *Metamorphosis and insect's life cycle*

An insect's life-cycle can be divided into three types:

- **Ametabolous,** no metamorphosis, these insects are primitively wingless where the only difference between adult and nymph is size, e.g. Order: Thysanura (Silverfish) (Triplehorn & Johnson, 2005).
- **Hemimetabolous,** or incomplete metamorphosis. The terrestrial young are called nymphs and aquatic young are called naiads. Insect young are usually similar to the adult. Wings appear as buds on the nymphs or early instars. When the last moult is completed the wings expand to the full adult size, e.g. Order: Odonata (Dragonflies).
- **Holometabolus,** or complete metamorphosis. These insects have a different form in their immature and adult stages, have different behaviours and live in different habitats. The immature form is called larvae and remains similar in form but

increases in size. They usually have chewing mouthparts even if the adult form mouth parts suck. At the last larval instar phase the insect forms into a pupa, it doesn't feed and is inactive, and here wing development is initiated, and the adult emerges e.g. Order: Lepidoptera (Butterflies and Moths), (Triplehorn & Johnson, 2005).

## *Molting*

As an insect grows it needs to replace the rigid exoskeleton regularly (McGavin 2001; Triplehorn & Johnson, 2005,). Molting may occur up to three or four times or, in some insects, fifty times or more during its life (McGavin, 2001). A complex process controlled by hormones, it includes the cuticle of the body wall, the cuticular lining of the tracheae, foregut, hindgut and endoskeletal structures (McGavin 2001; Triplehorn & Johnson, 2005).

The stages of molting:

1. **Apolysis**—molting hormones are released into the haemolymph and the old cuticle separates from the underlying epidermal cells. The epidermis increases in size due to mitosis and then the new cuticle is produced. Enzymes secreted by the epidermal cells digest the old endocuticle, not affecting the old sclerotised exocuticle.
2. **Ecdysis**—this begins with the splitting of the old cuticle, usually starting in the midline of the thorax's dorsal side. The rupturing force is mostly from haemolymph pressure that has been forced into thorax by abdominal muscle contractions caused by the insect swallowing air or water. After this the insect wriggles out of the old cuticle.
3. **Sclerotinisation**—after emergence the new cuticle is soft and this a particularly vulnerable time for the insect as its hard protective coating is missing. After an hour or two the exocuticle hardens and darkens. The wings expand by the force of haemolymph into the wing veins (McGavin, 2001; Triplehorn & Johnson, 2005).

## Chapter 12

# Lactation

Kittens nursing

Lactation of pigs

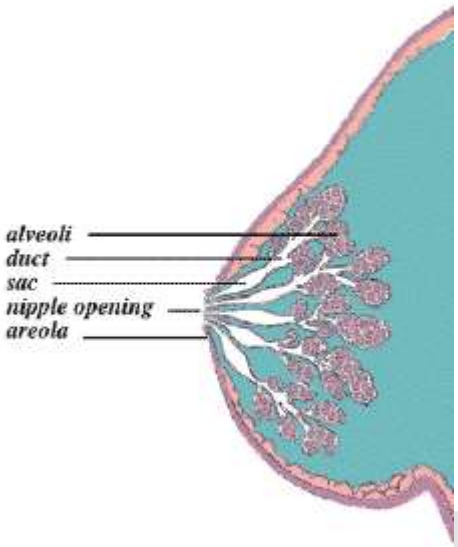
**Lactation** describes the secretion of milk from the mammary glands and the period of time that a mother lactates to feed her young. The process occurs in all female mammals, however it predates mammals. In humans the process of feeding milk is called breastfeeding or nursing. In most species milk comes out of the mother's nipples; however, the platypus (a non-placental mammal) releases milk through ducts in its abdomen. In only one species of mammal, the Dayak fruit bat, is milk production a normal male function. In some other mammals, the male may produce milk as the result of a hormone imbalance. This phenomenon may also be observed in newborn infants as well (for instance witch's milk).

*Galactopoiesis* is the maintenance of milk production. This stage requires prolactin (PRL) and oxytocin.

## *Purpose*

The chief function of lactation is to provide nutrition and immune protection to the young after birth. In almost all mammals, lactation induces a period of infertility, which serves to provide the optimal birth spacing for survival of the offspring.

## *Human lactation*




When the baby sucks its mother's breast, a hormone called oxytocin compels the milk to flow from the alveoli, through the ducts (milk canals) into the sacs (milk pools) behind the areola and then into the baby's mouth

### Hormonal influences

From the twenty-fourth week of pregnancy (the second and third trimesters), a woman's body produces hormones that stimulate the growth of the milk duct system in the breasts:

- Progesterone—influences the growth in size of alveoli and lobes, high levels of progesterone inhibit lactation before birth. Progesterone levels drop after birth, this triggers the onset of copious milk production.
- Oestrogen—stimulates the milk duct system to grow and differentiate. Like progesterone high levels of oestrogen also inhibit lactation. Oestrogen levels also drop at delivery and remain low for the first several months of breastfeeding. It is recommended that breastfeeding mothers avoid oestrogen-based birth control methods, as a spike in estrogen levels may reduce a mother's milk supply.
- Prolactin—contributes to the increased growth and differentiation of the alveoli, also influences differentiation of ductal structures. High levels of prolactin during pregnancy and breastfeeding also increase insulin resistance, increase growth factor levels (IGF-1) and modify lipid metabolism in preparation for breastfeeding. During lactation prolactin is the main factor maintaining tight junctions of the ductal epithelium and regulating milk production through osmotic balance.
- Growth hormone is structurally very similar to prolactin and contributes to its galactopoietic function.

- ATCH and glucocorticoids have an important lactation inducing function in several animal species. ACTH is thought to contribute as it is structurally similar to prolactin. Glucocorticoids play a complex regulating role in the maintenance of tight junctions.
- TSH is a very important galactopoietic hormone, its levels are naturally increased during pregnancy.
- Oxytocin—contracts the smooth muscle of the uterus during and after birth, and during orgasm(s). After birth, oxytocin contracts the smooth muscle layer of band-like cells surrounding the alveoli to squeeze the newly-produced milk into the duct system. Oxytocin is necessary for the *milk ejection reflex*, or *let-down* to occur.
- Human placental lactogen (HPL)—From the second month of pregnancy, the placenta releases large amounts of HPL. This hormone appears to be instrumental in breast, nipple, and areola growth before birth.
- Follicle stimulating hormone (FSH)
- Luteinizing hormone (LH)

By the fifth or sixth month of pregnancy, the breasts are ready to produce milk. It is also possible to induce lactation without pregnancy.

## Lactogenesis I

During the latter part of pregnancy, the woman's breasts enter into the *Lactogenesis I* stage. This is when the breasts make colostrum (see below), a thick, sometimes yellowish fluid. At this stage, high levels of progesterone inhibit most milk production. It is not a medical concern if a pregnant woman leaks any colostrum before her baby's birth, nor is it an indication of future milk production.

## Lactogenesis II

At birth, prolactin levels remain high, while the delivery of the placenta results in a sudden drop in progesterone, estrogen, and HPL levels. This abrupt withdrawal of progesterone in the presence of high prolactin levels stimulates the copious milk production of *Lactogenesis II*.

When the breast is stimulated, prolactin levels in the blood rise, peak in about 45 minutes, and return to the pre-breastfeeding state about three hours later. The release of prolactin triggers the cells in the alveoli to make milk. Prolactin also transfers to the breast milk. Some research indicates that prolactin in milk is greater at times of higher milk production, and lower when breasts are fuller, and that the highest levels tend to occur between 2 a.m. and 6 a.m.

Other hormones—notably insulin, thyroxine, and cortisol—are also involved, but their roles are not yet well understood. Although biochemical markers indicate that Lactogenesis II begins about 30–40 hours after birth, mothers do not typically begin

feeling increased breast fullness (the sensation of milk "coming in the breast") until 50–73 hours (2–3 days) after birth.

Colostrum is the first milk a breastfed baby receives. It contains higher amounts of white blood cells and antibodies than mature milk, and is especially high in immunoglobulin A (IgA), which coats the lining of the baby's immature intestines, and helps to prevent pathogens from invading the baby's system. Secretory IgA also helps prevent food allergies. Over the first two weeks after the birth, colostrum production slowly gives way to mature breast milk.

## Lactogenesis III

The hormonal endocrine control system drives milk production during pregnancy and the first few days after the birth. When the milk supply is more firmly established, autocrine (or local) control system begins. This stage is called *Lactogenesis III*

During this stage, the more that milk is removed from the breasts, the more the breast will produce milk. Research also suggests that draining the breasts more fully also increases the rate of milk production. Thus the milk supply is strongly influenced by how often the baby feeds and how well it is able to transfer milk from the breast. Low supply can often be traced to:

- not feeding or pumping often enough
- inability of the infant to transfer milk effectively caused by, among other things:
  - jaw or mouth structure deficits
  - poor latching technique
- rare maternal endocrine disorders
- hypoplastic breast tissue
- a metabolic or digestive inability in the infant, making it unable to digest the milk it receives
- inadequate calorie intake or malnutrition of the mother

## Milk ejection reflex

The release of the hormone oxytocin leads to the *milk ejection* or *let-down reflex*. Oxytocin stimulates the muscles surrounding the breast to squeeze out the milk. Breastfeeding mothers describe the sensation differently. Some feel a slight tingling, others feel immense amounts of pressure or slight pain/discomfort, and still others do not feel anything different.

The let-down reflex is not always consistent, especially at first. The thought of breastfeeding or the sound of any baby can stimulate this reflex, causing unwanted leakage, or both breasts may give out milk when an infant is feeding from one breast. However, this and other problems often settle after two weeks of feeding. Stress or anxiety can cause difficulties with breastfeeding.

A poor milk ejection reflex can be due to sore or cracked nipples, separation from the infant, a history of breast surgery, or tissue damage from prior breast trauma. If a mother has trouble breastfeeding, different methods of assisting the milk ejection reflex may help. These include feeding in a familiar and comfortable location, massage of the breast or back, or warming the breast with a cloth or shower.

### Afterpains

The surge of oxytocin that may also possibly trigger the milk ejection reflex also causes the uterus to contract. During breastfeeding, mothers may feel these contractions as *afterpains*. These may range from period-like cramps to strong labour-like contractions and can be more severe with second and subsequent babies. Some women's breasts also become dry and chapped and even crack open and bleed while breast feeding. Rubbing lanolin on the nipples and areola can help with those problems.

## *Lactation without pregnancy, induced lactation, relactation*

In humans induced lactation and relactation has been observed frequently in primitive cultures and demonstrated with varying success in adoptive mothers. It appears plausible that the possibility of induction of lactation in women (or females of other species) who are not biological mothers does confer an evolutionary advantage especially in groups with high maternal mortality and tight social bonds. The phenomenon has been also observed in most primates, some lemurs and dwarf mongooses.

In humans lactation can be induced by a combination physical and psychological stimulation, by drugs or a combination of those methods.

Also, some couples may use lactation for sexual purposes.

Rare accounts of male lactation (as distinct from galactorrhea) exist in historical medical and anthropological literature, although the phenomenon has not been confirmed by more recent literature.

## *Evolution*

Darwin correctly recognised that mammary glands developed from cutaneous glands and postulated the hypothesis that they evolved from glands in brood pouches of fish where they provided nourishment for eggs. The later aspect of his hypothesis has not been confirmed, but recently the same mechanism has been postulated for early synapsids. Instead the discus fish (Symphysodon aequifasciata) became known for (biparentally) feeding their offspring by epidermal mucus secretion. A closer look reveals that similar to most mammals the secretion of the nourishing fluid may be controlled by prolactin.

Later therapsids such as cynodonts appear to have secreted complex, nutrient-rich milk. This brought them evolutionary advantage by allowing a decline in egg size.

During early evolution of lactation the secretion was through pilosebaceous glands and *mammary hairs* transported the nourishing fluids to the eggs or youngs. Later the development of the *mammary patch* rendered mammary hairs obsolete.

Other well known example of nourishing young with secretions of glands is the crop milk of pigeons. Like in mammals and disc fish this also appears closely related to prolactin. Other birds such as flamingos and penguins are utilizing similar feeding techniques.

## Chapter 13

# Physiology of Dinosaurs

The **physiology of dinosaurs** has historically been a controversial subject, particularly thermoregulation. Recently, many new lines of evidence have been brought to bear on dinosaur physiology generally, including not only metabolic systems and thermoregulation, but on respiratory and cardiovascular systems as well.

During the early years of dinosaur paleontology, it was widely considered that they were sluggish, cumbersome, and sprawling cold-blooded lizards. However, with the discovery of much more complete skeletons in the western United States, starting in the 1870s, scientists could make more informed interpretations of dinosaur biology and physiology. Edward Drinker Cope, opponent of Othniel Charles Marsh in the Bone Wars, propounded at least some dinosaurs as active and agile, as seen in the painting of two fighting "Laelaps" produced under his direction by Charles R. Knight. In parallel, the development of Darwinian evolution, and the discoveries of *Archaeopteryx* and *Compsognathus*, led Thomas Henry Huxley to propose that dinosaurs were closely related to birds. Despite these considerations, the image of dinosaurs as large reptiles had taken root, and most aspects of their paleobiology were interpreted as being typically reptilian for the first half of the twentieth century. Beginning in the 1960s and with the advent of the Dinosaur Renaissance, views of dinosaurs and the physiology have changed dramatically, including the discovery of feathered dinosaurs in Early Cretaceous age deposits in China, indicating that birds evolved from highly agile maniraptoran dinosaurs.

## *History of study*

### Early interpretations of dinosaurs: 1820s to early 1900s

Reconstruction of *Megalosaurus* from 1854, in Crystal Palace, London

The 1897 painting of "*Laelaps*" (now *Dryptosaurus*) by Charles R. Knight.

The study of dinosaurs began in the 1820s in England. Pioneers in the field, such as William Buckland, Gideon Mantell, and Richard Owen, interpreted the first, very fragmentary remains as belonging to large quadrupedal beasts. Their early work can be seen today in the Crystal Palace Dinosaurs, constructed in the 1850s, which present known dinosaurs as elephantine lizard-like reptiles. Despite these reptilian appearances, Owen speculated that dinosaur heart and respiratory systems were more mammal-like than reptile-like.

### Changing views and the Dinosaur Renaissance

However, in the late 1960s views began to change, beginning with John Ostrom's work on *Deinonychus* and bird evolution. His student, Bob Bakker, popularized the changing thought in a series of papers beginning with *The superiority of dinosaurs* in 1968. In these publications, he argued strenuously that dinosaurs were warm-blooded and active animals, capable of sustained periods of high activity. In most of his writings Bakker framed his arguments as new evidence leading to a revival of ideas popular the late 19th century, frequently referring to an ongoing *dinosaur renaissance*. He used a variety of anatomical and statistical arguments to defend his case, the methodology of which was fiercely debated among scientists.

These debates sparked interest in new methods for ascertaining the palaeobiology of extinct animals, such as bone histology, which have been successfully applied to determining the growth-rates of many dinosaurs.

Today, it is generally thought that many or perhaps all dinosaurs had higher metabolic rates than living reptiles, but also that the situation is more complex and varied than Bakker originally proposed. For example, while smaller dinosaurs may have been true endotherms, the larger forms could have been inertial homeotherms, or many dinosaurs could have had intermediate metabolic rates.

## *Feeding and digestion*

The earliest dinosaurs were almost certainly predators, and shared several predatory features with their nearest non-dinosaur relatives like *Lagosuchus*, including: relatively large, curved, blade-like teeth in large, wide-opening jaws that closed like scissors; relatively small abdomens, as carnivores do not require large digestive systems. Later dinosaurs regarded as predators sometimes grew much larger, but retain the same set of features. Instead of chewing their food, these predators swallowed it whole.

The feeding habits of ornithomimosaurs and oviraptorosaurs are a mystery: although they evolved from a predatory theropod lineage, they have small jaws and lack the blade-like teeth of typical predators, but there is no evidence of their diet or how they ate and digested it.

Features of other groups of dinosaurs indicate they were vegetarians. These features include:

- Jaws that opened only a little and closed so that all the teeth met at the same time
- Large abdomens that could accommodate large amounts of vegetation and store it for the longer time it takes to digest vegetation
- Guts that likely contained Endosymbiotic micro-organisms that digest cellulose, as no known animal can digest this tough material directly

Sauropods, which were vegetarians, did not chew their food, as their teeth and jaws appear suitable only for stripping leaves off plants. Ornithischians, also vegetarians, show a variety of approaches. The armored ankylosaurs and stegosaurs had small heads and weak jaws and teeth, and are thought to have fed in much the same way as sauropods. The pachycephalosaurs had small heads and weak jaws and teeth, but their lack of large digestive systems suggests a different diet, possibly fruits, seeds, or young shoots, which would have been more nutritious than leaves.

On the other hand ornithopods such as *Hypsilophodon*, *Iguanodon* and various hadrosaurs had horny beaks for snipping off vegetation and jaws and teeth that were well-adapted for chewing. The horned ceratopsians had similar mechanisms.

It has often been suggested that at least some dinosaurs used swallowed stones, known as gastroliths, to aid digestion by grinding their food in muscular gizzards, and that this was a feature they shared with birds. In 2007 Oliver Wings reviewed references to gastroliths in scientific literature and found considerable confusion, starting with the lack of an agreed and objective definition of "gastrolith". He found that swallowed hard stones or grit can assist digestion in birds that mainly feed on grain but may not be essential—and that birds that eat insects in summer and grain in winter usually get rid of the stones and grit in summer. Gastroliths have often been described as important for sauropod dinosaurs, whose diet of vegetation required very thorough digestion, but Wings concluded that this idea was incorrect: gastroliths are found with only a small percentage of sauropod fossils; where they have been found, the amounts are too small and in many cases the stones are too soft to have been effective in grinding food; most of these gastroliths are highly polished, but gastroliths used by modern animals to grind food are roughened by wear and corroded by stomach acids; hence the sauropod gastroliths were probably swallowed accidentally. On the other hand he concluded that gastroliths found with fossils of advanced theropod dinosaurs such as *Sinornithomimus* and *Caudipteryx* resemble those of birds, and that the use of gastroliths for grinding food may have appeared early in the group of dinosaurs from which these dinosaurs and birds both evolved.

## *Reproductive biology*

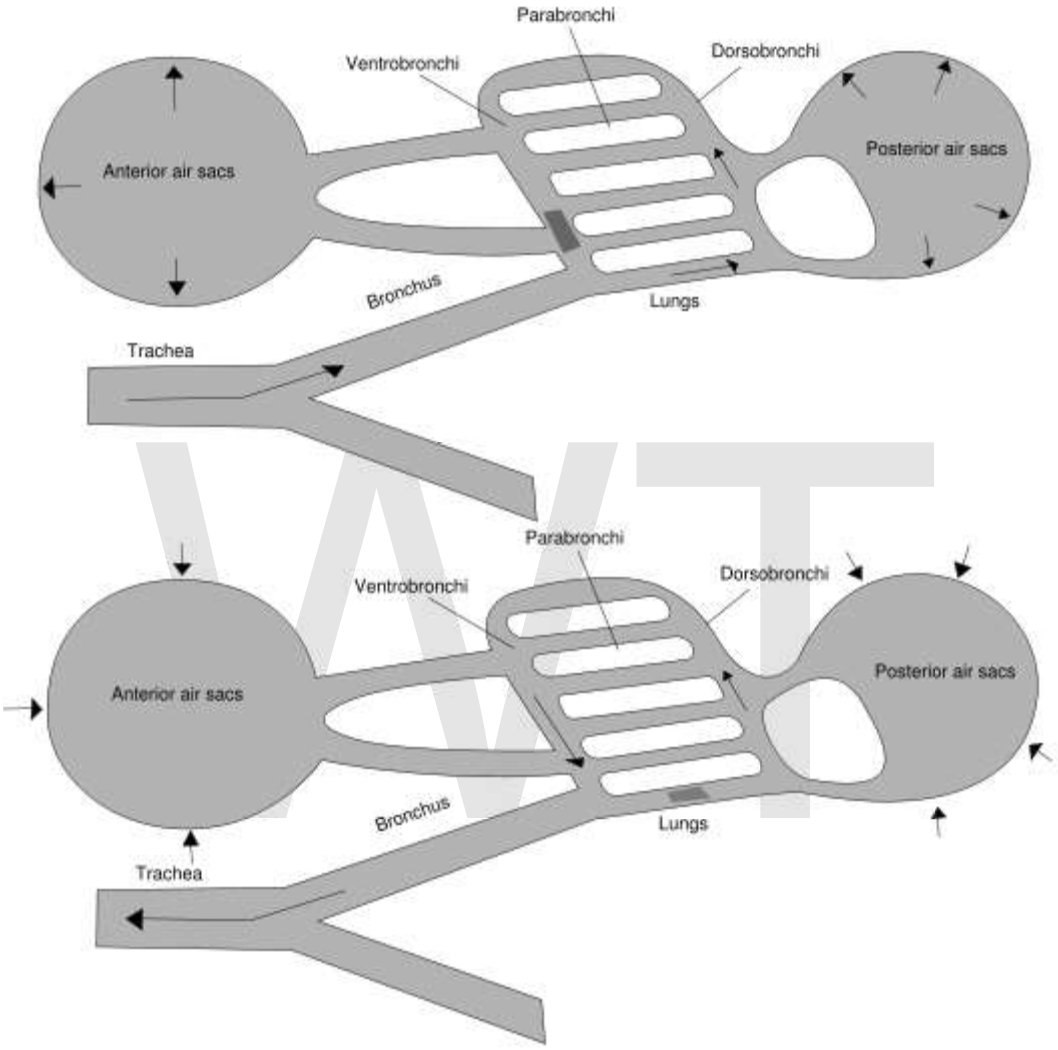
When laying eggs, females of some bird species grow a special type of bone in their limbs between the hard outer bone and the marrow. This medullary bone, which is rich in calcium, is used to make eggshells, and the birds that produced it absorb it when they have finished laying eggs. Medullary bone has been found in fossils of the theropods *Tyrannosaurus* and *Allosaurus* and of the ornithopod *Tenontosaurus*.

Because the line of dinosaurs that includes *Allosaurus* and *Tyrannosaurus* diverged from the line that led to *Tenontosaurus* very early in the evolution of dinosaurs, the presence of medullary bone in both groups suggests that dinosaurs in general produced medullary tissue. On the other hand crocodilians, which are dinosaurs' second closest living relatives after birds, do not produce medullary bone. This tissue may have first appeared in ornithodires, the Triassic archosaur group from which dinosaurs are thought to have evolved.

Medullary bone has been found in specimens of sub-adult size, which suggests that dinosaurs reached sexual maturity before they were fully-grown. Sexual maturity at sub-adult size is also found in reptiles and in medium- to large-sized mammals, but birds and small mammals reach sexual maturity only after they are fully-grown—which happens within their first year. Early sexual maturity is also associated with specific features of animals' life cycles: the young are born relatively well-developed rather than helpless; and the death-rate among adults is high.

## *Respiratory System*

### Air sacs




Birds' lungs obtain fresh air during both exhalation and inhalation, because the air sacs do all the "pumping" and the lungs simply absorb oxygen.

From about 1870 onwards scientists have generally agreed that the post-cranial skeletons of many dinosaurs contained many air-filled cavities (postcranial skeletal pneumaticity, especially in the vertebrae. Pneumatization of the skull (such as paranasal sinuses) is found in both synapsids and archosaurs, but postcranial pneumatization is found only in birds, non-avian saurischian dinosaurs, and pterosaurs.

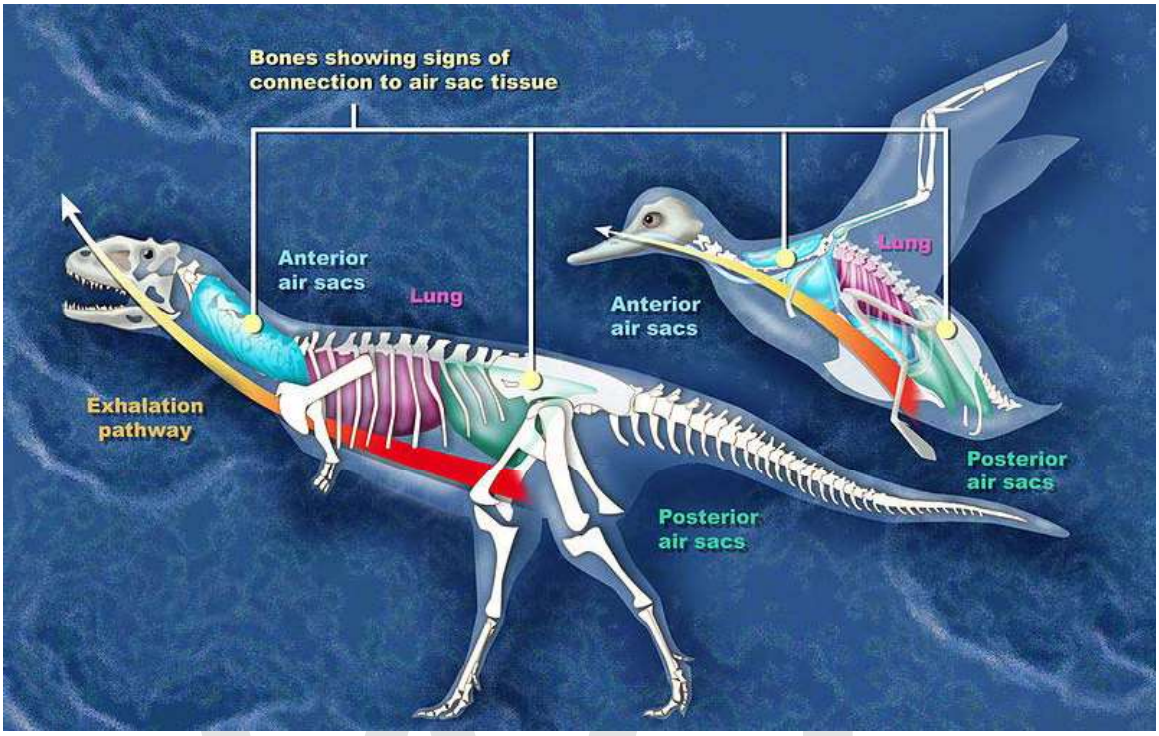
For a long time these cavities were regarded simply as weight-saving devices, but Bakker proposed that they contained air sacs like those that make birds' respiratory systems the most efficient of all animals'.

John Ruben *et al.* (1997, 1999, 2003, 2004) disputed this and suggested that dinosaurs had a "tidal" respiratory system (in and out) powered by a crocodile-like hepatic piston mechanism - muscles attached mainly to the pubis pull the liver backwards, which makes the lungs expand to inhale; when these muscles relax, the lungs return to their previous size and shape, and the animal exhales. They also presented this as a reason for doubting that birds descended from dinosaurs.

Critics have claimed that, without avian air sacs, modest improvements in a few aspects of a modern reptile's circulatory and respiratory systems would enable the reptile to achieve 50% to 70% of the oxygen flow of a mammal of similar size, and that lack of avian air sacs would not prevent the development of endothermy. Very few formal rebuttals have been published in scientific journals of Ruben *et al.*'s claim that dinosaurs could not have had avian-style air sacs; but one points out that the *Sinosauropteryx* fossil on which they based much of their argument was severely flattened and therefore it was impossible to tell whether the liver was the right shape to act as part of a hepatic piston mechanism. Some recent papers simply note without further comment that Ruben *et al.* argued against the presence of air sacs in dinosaurs.

Researchers have presented evidence and arguments for air sacs in sauropods, "prosauropods", coelurosaurs, ceratosaurs, and the theropods *Aerosteon* and *Coelophysis*.

In advanced sauropods ("neosauropods") the vertebrae of the lower back and hip regions show signs of air sacs. In early sauropods only the cervical (neck) vertebrae show these features. If the developmental sequence found in bird embryos is a guide, air sacs actually evolved before the channels in the skeleton that accommodate them in later forms.




Comparison between the air sacs of *Majungasaurus* and a bird

Evidence of air sacs has also been found in theropods. Studies indicate that fossils of coelurosaurs, ceratosaurs, and the theropods *Coelophysis* and *Aerosteon* exhibit evidence of air sacs. *Coelophysis*, from the late Triassic, is one of the earliest dinosaurs whose fossils show evidence of channels for air sacs. *Aerosteon*, a Late Cretaceous allosaur, had the most bird-like air sacs found so far.

Early sauropodomorphs, including the group traditionally called "prosauropods", may also have had air sacs. Although possible pneumatic indentations have been found in *Plateosaurus* and *Thecodontosaurus*, the indentations are very small. One study in 2007 concluded that prosauropods likely had abdominal and cervical air sacs, based on the evidence for them in sister taxa (theropods and sauropods). The study concluded that it was impossible to determine whether prosauropods had a bird-like flow-through lung, but that the air sacs were almost certainly present. A further indication for the presence of air sacs and their use in lung ventilation comes from a reconstruction of the air exchange volume (the volume of air exchanged with each breath) of *Plateosaurus*, which when expressed as a ratio of air volume per body weight at 29 ml/kg is similar to values of geese and other birds, and much higher than typical mammalian values.

So far no evidence of air sacs has been found in ornithischian dinosaurs. But this does not imply that ornithischians could not have had metabolic rates comparable to those of mammals, since mammals also do not have air sacs.

Three explanations have been suggested for the development of air sacs in dinosaurs:

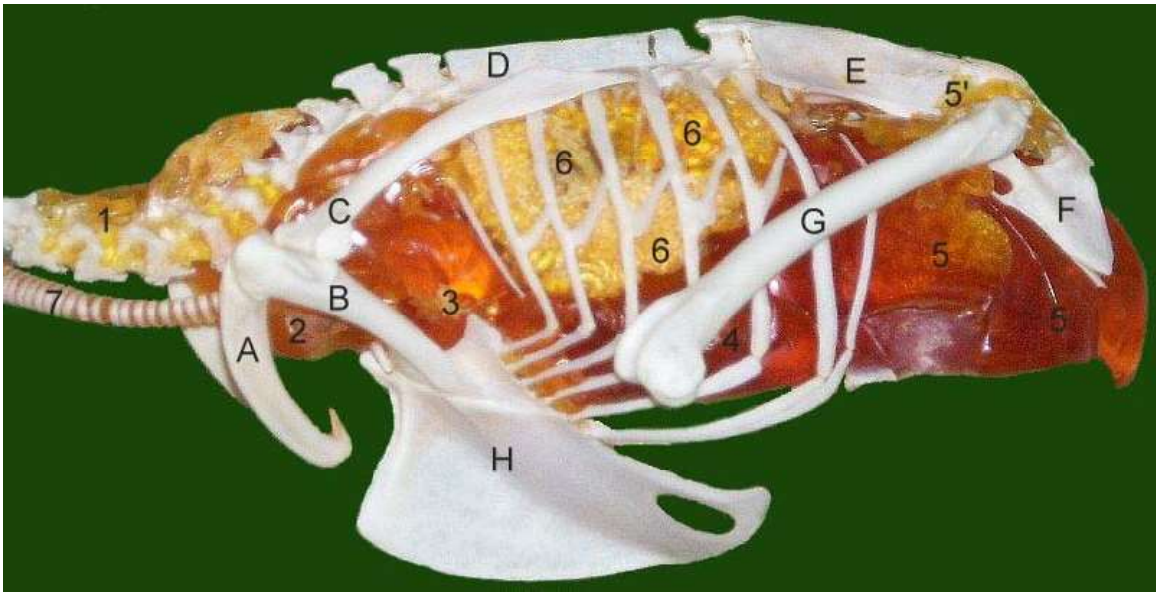
- Increase in respiratory capacity. This is probably the most common hypothesis, and fits well with the idea that many dinosaurs had fairly high metabolic rates.
- Improving balance and maneuvrability by lowering the center of gravity and reducing rotational inertia. However this does not explain the expansion of air sacs in the quadrupedal sauropods.
- As a cooling mechanism. It seems that air sacs and feathers evolved at about the same time in coelurosaurs. If feathers retained heat, their owners would have required a means of dissipating excess heat. This idea is plausible but needs further empirical support.

Calculations of the volumes of various parts of the sauropod *Apatosaurus*' respiratory system support the evidence of bird-like air sacs in sauropods:

- Assuming that *Apatosaurus*, like dinosaurs' nearest surviving relatives crocodilians and birds, did not have a diaphragm, the dead-space volume of a 30-ton specimen would be about 184 liters. This is the total volume of the mouth, trachea and air tubes. If the animal exhales less than this, stale air is not expelled and is sucked back into the lungs on the following inhalation.
- Estimates of its tidal volume – the amount of air moved into or out of the lungs in a single breath – depend on the type of respiratory system the animal had: 904 liters if avian; 225 liters if mammalian; 19 liters if reptilian.

On this basis, *Apatosaurus* could not have had a reptilian respiratory system, as its tidal volume would have been less than its dead-space volume, so that stale air was not expelled but was sucked back into the lungs. Likewise, a mammalian system would only provide to the lungs about $225 - 184 = 41$ liters of fresh, oxygenated air on each breath. *Apatosaurus* must therefore have had either a system unknown in the modern world or one like birds', with multiple air sacs and a flow-through lung. Furthermore, an avian system would only need a lung volume of about 600 liters while a mammalian one would have required about 2,950 liters, which would exceed the estimated 1,700 liters of space available in a 30-ton *Apatosaurus'* chest.

Dinosaur respiratory systems with bird-like air sacs may have been capable of sustaining higher activity levels than mammals of similar size and build can sustain. In addition to providing a very efficient supply of oxygen, the rapid airflow would have been an effective cooling mechanism, which is essential for animals that are active but too large to get rid of all the excess heat through their skins.




The uncinate processes are the small white spurs about half-way along the ribs. The rest of this diagram shows the air sacs and other parts of a bird's respiratory system:1 cervical air sac, 2 clavicular air sac, 3 cranial thoracal air sac, 4 caudal thoracal air sac, 5 abdominal air sac (5' diverticulus into pelvic girdle), 6 lung, 7 trachea

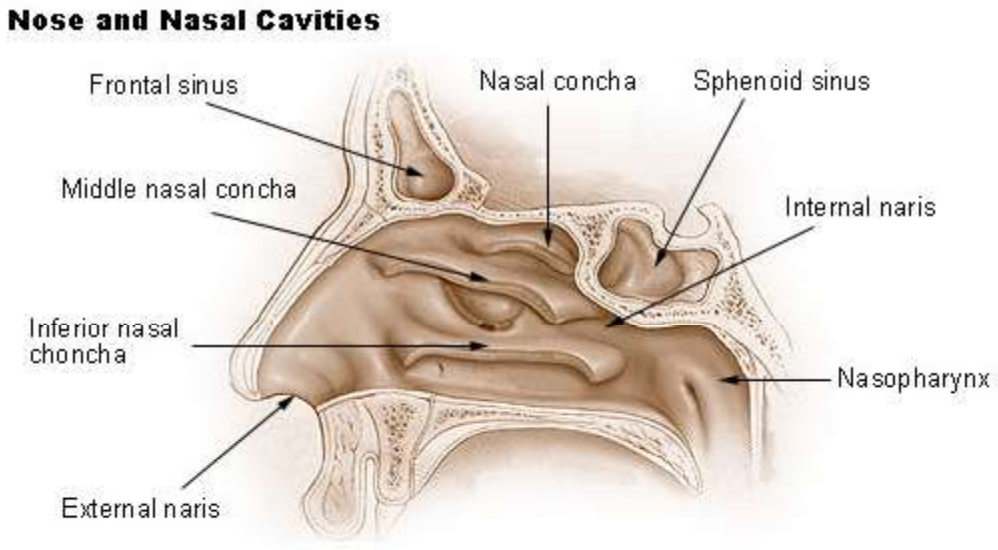
## Uncinate processes on the ribs

Birds have spurs called "uncinate processes" on the rear edges of their ribs, and these give the chest muscles more leverage when pumping the chest to improve oxygen supply. The size of the uncinate processes is related to the bird's lifestyle and oxygen requirements: they are shortest in walking birds and longest in diving birds, which need to replenish their oxygen reserves quickly when they surface. Non-avian maniraptoran dinosaurs also had these uncinate processes, and they were proportionately as long as in modern diving birds, which indicates that maniraptorans needed a high-capacity oxygen supply.

Plates that may have functioned the same way as uncinate processes have been observed in fossils of the ornithischian dinosaur *Thescelosaurus*, and have been interpreted as evidence of high oxygen consumption and therefore high metabolic rate.

## Nasal turbinates

Nasal turbinates (often referred to as "turbinals" or "conchae") are convoluted structures of thin bone in the nasal cavity. In most mammals and birds these are present and lined with mucous membranes that perform two functions. They improve the sense of smell by increasing the area available to absorb airborne chemicals—and they warm and moisten inhaled air, and extract heat and moisture from exhaled air to prevent desiccation of the lungs.




Human nasal turbinates / conchae are rather simple, but similar in position to those of other mammals.

Ruben *et al.* have argued in several papers that:

- No evidence of nasal turbinates has been found in dinosaurs (the papers focussed on coelurosaurs)
- All the dinosaurs they examined had nasal passages that were too narrow and short to accommodate nasal turbinates.
- Hence dinosaurs could not have sustained the breathing rate required for a mammal-like or bird-like metabolic rate while at rest, because their lungs would have dried out.

However, objections have been raised against this argument:

- Nasal turbinates are absent or very small in some birds (e.g. ratites, Procellariiformes and Falconiformes) and mammals (e.g. whales, anteaters, bats, elephants, and most primates), although these animals are fully endothermic and in some cases very active.
- Other studies conclude that nasal passages of these dinosaurs were long enough and wide enough to accommodate nasal turbinates or similar mechanisms to avoid desiccation of the lungs.
- Nasal turbinates are fragile and seldom found in fossils. In particular none have been found in fossil birds.

### *Cardiovascular system*

The possible heart of "Willo" the thescelosaur (center).

In principle one would expect dinosaurs to have had two-part circulations driven by four-chambered hearts, since many would have needed high blood pressure to deliver blood to their heads, which were high off the ground, but vertebrate lungs can only tolerate fairly low blood pressure. In 2000, a skeleton of *Thescelosaurus*, now on display at the North Carolina Museum of Natural Sciences, was described as including the remnants of a four-chambered heart and an aorta. The authors interpreted the structure of the heart as indicating an elevated metabolic rate for *Thescelosaurus*, not reptilian cold-bloodedness. Their conclusions have been disputed; other researchers published a paper where they assert that the heart is really a concretion of entirely mineral "cement". As they note: the anatomy given for the object is incorrect, for example the alleged "aorta" is narrowest where it meets the "heart" and lacks arteries branching from it; the "heart" partially engulfs one of the ribs and has an internal structure of concentric layers in some places; and another concretion is preserved behind the right leg. The original authors defended their position; they agreed that the chest did contain a type of concretion, but one that had formed around and partially preserved the more muscular portions of the heart and aorta.

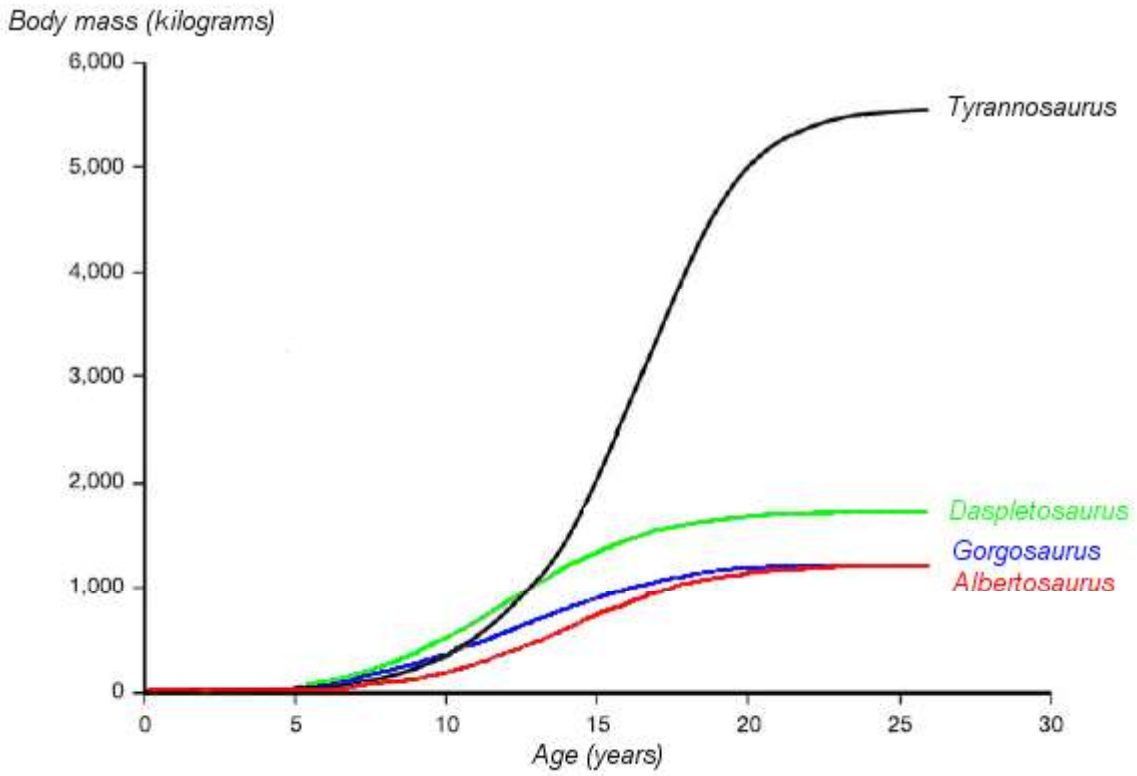
Regardless of the object's identity, it may have little relevance to dinosaurs' internal anatomy and metabolic rate. Both modern crocodilians and birds, the closest living relatives of dinosaurs, have four-chambered hearts, although modified in crocodilians,

and so dinosaurs probably had them as well. However such hearts are not necessarily tied to metabolic rate.

## *Growth and lifecycle*

No dinosaur egg has been found that is larger than a basketball and embryos of large dinosaurs have been found in relatively small eggs, e.g. Maiasaura. Like mammals, dinosaurs stopped growing when they reached the typical adult size of their species, while mature reptiles continue to grow slowly if they have enough food. Dinosaurs of all sizes grew faster than similarly-sized modern reptiles; but the results of comparisons with similarly-sized "warm-blooded" modern animals depend on their sizes:

| Weight (kg) | Comparative growth rate of dinosaurs | Modern animals in this size range |
|---|---|---|
| 0.22 | Slower than marsupials | Rat |
| 1 - 20 | Similar to marsupials, slower than precocial birds (those that are born capable of running) | From guinea pig to Andean Condor |
| 100 - 1000 | Faster than marsupials, similar to precocial birds, slower than placental mammals | From Red Kangaroo to Polar Bear |
| 1500 – 3500 | Similar to most placental mammals | From American Bison to rhinoceros |
| 25000 and over | Very fast, similar to modern whales; but about half that of a scaled-up altricial bird (one that is born helpless) - if one could scale up a bird to 25,000 kilograms (25 LT; 28 ST) | Whales |




A graph showing the hypothesized growth curves (body mass versus age) of four tyrannosaurids. *Tyrannosaurus rex* is drawn in black. Based on Erickson et al. 2004.

*Tyrannosaurus rex* showed a "teenage growth spurt":

- ½ ton at age 10
- very rapid growth to around 2 tons in the mid-teens (about ½ ton per year).
- negligible growth after the second decade.

A 2008 study of one skeleton of the hadrosaur *Hypacrosaurus* concluded that this dinosaur grew even faster, reaching its full size at the age of about 15; the main evidence was the number and spacing of growth rings in its bones. The authors found this consistent with a life-cycle theory that prey species should grow faster than their predators if they lose a lot of juveniles to predators and the local environment provides enough resources for rapid growth.

It appears that individual dinosaurs were rather short-lived, e.g. the oldest (at death) *Tyrannosaurus* found so far was 28 and the oldest sauropod was 38. Predation was probably responsible for the high death rate of very young dinosaurs and sexual competition for the high death rate of sexually mature dinosaurs.

## *Metabolism*

Scientific opinion about the life-style, metabolism and temperature regulation of dinosaurs has varied over time since the discovery of dinosaurs in the mid-19th century. The activity of metabolic enzymes varies with temperature, so temperature control is vital for any organism, whether endothermic or ectothermic. Organisms can be categorized as poikilotherms (poikilo - changing), which are tolerant of internal temperature fluctuations, and homeotherms (homeo - same), which must maintain a constant core temperature. Animals can be further categorized as endotherms, which regulate their temperature internally, and ectotherms, which regulate temperature by the use of external heat sources.

The current consensus view suggests that dinosaur metabolism did not closely match any found in living vertebrates, and, consequently, that they cannot be categorized as either "warm" or "cold-blooded." Rather, they lie somewhere on the spectrum between poikilothermy and homeothermy. Consequently, current research focuses on mechanisms of metabolism and temperature regulation, and the similarities between dinosaurian, avian and mammalian metabolisms.

### What the debate is about

"Warm-bloodedness" is a complex and rather ambiguous term, because it includes some or all of:

- **Homeothermy**, i.e. maintaining a fairly constant body temperature. Modern endotherms maintain a variety of temperatures: 28 °C (82 °F) to 30 °C (86 °F) in monotremes and sloths; 33 °C (91 °F) to 36 °C (97 °F) in marsupials; 36 °C (97 °F) to 38 °C (100 °F) in most placentals; and around 41 °C (106 °F) in birds.
- **Tachymetabolism**, i.e. maintaining a high metabolic rate, particularly when at rest. This requires a fairly high and stable body temperature, since: biochemical processes run about half as fast if an animal's temperature drops by 10C°; most enzymes have an optimum operating temperature and their efficiency drops rapidly outside the preferred range.
- **Endothermy**, i.e. the ability to generate heat internally, for example by "burning" fat, rather than via behaviors such as basking or muscular activity. Although endothermy is in principle the most reliable way to maintain a fairly constant temperature, it is expensive, for example modern mammals need 10 to 13 times as much food as modern reptiles.

Large dinosaurs may also have maintained their temperatures by inertial homeothermy, also known as "bulk homeothermy" or "mass homeothermy". In other words, the thermal capacity of such large animals was so high that that it would take two days or more for their temperatures to change significantly, and this would have smoothed out variations caused by daily temperature cycles. This smoothing effect has been observed in large turtles and crocodilians, but *Plateosaurus*, which weighed about 700 kilograms (1,500 lb), may have been the smallest dinosaur in which it would have been effective.

Inertial homeothermy would not have been possible for small species nor for the young of larger species. Vegetation fermenting in the guts of large herbivores can also produce considerable heat, but this method of maintaining a high and stable temperature would not have been possible for carnivores nor for small herbivores or the young of larger herbivores.

Since the internal mechanisms of extinct creatures are unknowable, most discussion focuses on homeothermy and tachymetabolism.

Assessment of metabolic rates is complicated by the distinction between the rates while resting and while active. In all modern reptiles and most mammals and birds the maximum rates during all-out activity are 10 to 20 times higher than minimum rates while at rest. However in a few mammals these rates differ by a factor of 70. Theoretically it would be possible for a land vertebrate to have a reptilian metabolic rate at rest and a bird-like rate while working flat out. However an animal with such a low resting rate would be unable to grow quickly. The huge herbivorous sauropods may have been on the move so constantly in search of food that their energy expenditure would have been much the same irrespective of whether their resting metabolic rates were high or low.

## Metabolic options

The main possibilities are that:

- Dinosaurs were cold-blooded, like modern reptiles, except that the large size of many would have stabilized their body temperatures.
- They were warm-blooded, more like modern mammals or birds than modern reptiles.
- They were neither cold-blooded nor warm-blooded in modern terms, but had metabolisms that were different from and some ways intermediate between those of modern cold-blooded and warm-blooded animals.
- They included animals with two or three of these types of metabolism.

Dinosaurs were around for about 150 million years, so it is very likely that different groups evolved different metabolisms and thermoregulatory regimes, and that some developed different physiologies from the first dinosaurs.

If all or some dinosaurs had intermediate metabolisms, they may have had the following features:

- Low resting metabolic rates—which would reduce the amount of food they needed and allow them to use more of that food for growth than do animals with high resting metabolic rates.
- Inertial homeothermy
- The ability to control heat loss by expanding and contracting blood vessels just under the skin, as many modern reptiles do.

- Two-part circulations driven by four-chambered hearts.
- High aerobic capacity, allowing sustained activity.

Robert Reid has suggested that such animals could be regarded as "failed endotherms". He envisaged both dinosaurs and the Triassic ancestors of mammals passing through a stage with these features. Mammals were forced to become smaller as archosaurs came to dominate ecological niches for medium to large animals. Their decreasing size made them more vulnerable to heat loss because it increased their ratios of surface area to mass, and thus forced them to increase internal heat generation and thus become full endotherms. On the other hand dinosaurs became medium to very large animals and thus were able to retain the "intermediate" type of metabolism.

## Bone structure

Armand de Ricqlès discovered Haversian canals in dinosaur bones, and argued that they were evidence of endothermy in dinosaurs. These canals are common in "warm-blooded" animals and are associated with fast growth and an active life style because they help to recycle bone to facilitate rapid growth and repair damage caused by stress or injuries. Dense secondary Haversian bone, which is formed during remodeling, is found in many living endotherms as well as dinosaurs, pterosaurs and therapsids. Secondary Haversian canals are correlated with size and age, mechanical stress and nutrient turnover. The presence of secondary Haversian canals suggests comparable bone growth and lifespans in mammals and dinosaurs. Bakker argued that the presence of fibrolamellar bone (produced quickly and having a fibrous, woven appearance) in dinosaur fossils was evidence of endothermy.

However as a result of other, mainly later research, bone structure is not considered a reliable indicator of metabolism in dinosaurs, mammals or reptiles:

- Dinosaur bones often contain lines of arrested growth (LAGs), formed by alternating periods of slow and fast growth; in fact many studies count growth rings to estimate the ages of dinosaurs. The formation of growth rings is usually driven by seasonal changes in temperature, and this seasonal influence has sometimes been regarded as a sign of slow metabolism and ectothermy. But growth rings are found in polar bears and in mammals that hibernate. The relationship between LAGs and seasonal growth dependency remains unresolved.

- Fibrolamellar bone is fairly common in young crocodilians and sometimes found in adults.
- Haversian bone has been found in turtles, crocodilians and tortoises, but is often absent in small birds, bats, shrews and rodents.

Nevertheless de Ricqlès persevered with studies of the bone structure of dinosaurs and archosaurs. In mid-2008 he co-authored a paper that examined bone samples from a wide range of archosaurs, including early dinosaurs, and concluded that:

- Even the earliest archosauriformes may have been capable of very fast growth, which suggests they had fairly high metabolic rates. Although drawing conclusions about the earliest archosauriformes from later forms is tricky, because species-specific variations in bone structure and growth rate are very likely, there are research strategies than can minimize the risk that such factors will cause errors in the analysis.
- Archosaurs split into three main groups in the Triassic: ornithodirans, from which dinosaurs evolved, remained committed to rapid growth; crocodilians' ancestors adopted more typical "reptilian" slow growth rates; and most other Triassic archosaurs had intermediate growth rates.

## Growth rates

Dinosaurs grew from small eggs to several tons in weight relatively quickly. A natural interpretation of this is that that dinosaurs converted food into body weight very quickly, which requires a fairly fast metabolism both to forage actively and to assimilate the food quickly.Developing bone found in juveniles is distinctly porous, which has been linked to vascularization and bone deposition rate, all suggesting growth rates close to those observed in modern birds.

But a preliminary study of the relationship between adult size, growth rate, and body temperature concluded that larger dinosaurs had higher body temperatures than smaller ones had; *Apatosaurus*, the largest dinosaur in the sample, was estimated to have a body temperature exceeding 41 °C (106 °F), whereas smaller dinosaurs were estimated to have body temperatures around 25 °C (77 °F) – for comparison, normal human body temperature is about 37 °C (99 °F). Based on these estimations, the study concluded that large dinosaurs were inertial homeotherms (their temperatures were stabilized by their sheer bulk) and that dinosaurs were ectothermic (in colloquial terms, "cold-blooded", because they did not generate as much heat as mammals when not moving or digesting food). These results are consistent with the relationship between dinosaurs' sizes and growth rates (described above). Studies of the sauropodomorph *Massospondylus* and early theropod *Syntarsus* (*Megapnosaurus*) reveal growth rates of 3 kg/year and 17 kg/year, respectively, much slower than those estimated of Maiasaura and observed in modern birds.

## Oxygen isotope ratios in bone

The ratio of the isotopes $^{16}O$ and $^{18}O$ in bone depends on the temperature the bone formed at: the higher the temperature, the more $^{16}O$. Barrick and Showers (1999) analyzed the isotope ratios in two theropods that lived in temperate regions with seasonal variation in temperature, *Tyrannosaurus* (USA) and *Giganotosaurus* (Argentina):

- dorsal vertebrae from both dinosaurs showed no sign of seasonal variation, indicating that both maintained a constant core temperature despite seasonal variations in air temperature.

- ribs and leg bones from both dinosaurs showed greater variability in temperature and a lower average temperature as the distance from the vertebrae increased.

Barrick and Showers concluded that both dinosaurs were endothermic but at lower metabolic levels than modern mammals, and that inertial homeothermy was an important part of their temperature regulation as adults. Their similar analysis of some Late Cretaceous ornithischians in 1996 concluded that these animals showed a similar pattern.

However this view has been challenged. The evidence indicates homeothermy, but by itself cannot prove endothermy. Secondly, the production of bone may not have been continuous in areas near the extremities of limbs – in allosaur skeketons lines of arrested growth ("LAGs"; rather like growth rings) are sparse or absent in large limb bones but common in the fingers and toes. While there is no absolute proof that LAGs are temperature-related, they could mark times when the extremities were so cool that the bones ceased to grow. If so, the data about oxygen isotope ratios would be incomplete, especially for times when the extremities were coolest. Oxygen isotope ratios may be an unreliable method of estimating temperatures if it cannot be shown that bone growth was equally continuous in all parts of the animal.

## Predator-prey ratios

Bakker argued that:

- cold-blooded predators need much less food than warm-blooded ones, so a given mass of prey can support far more cold-blooded predators than warm-blooded ones.
- the ratio of the total mass of predators to prey in dinosaur communities was much more like that of modern and recent warm-blooded communities than that of recent or fossil cold-blooded communities.
- hence predatory dinosaurs were warm-blooded. And since the earliest dinosaurs (e.g. *Staurikosaurus*, *Herrerasaurus*) were predators, all dinosaurs must have been warm-blooded.

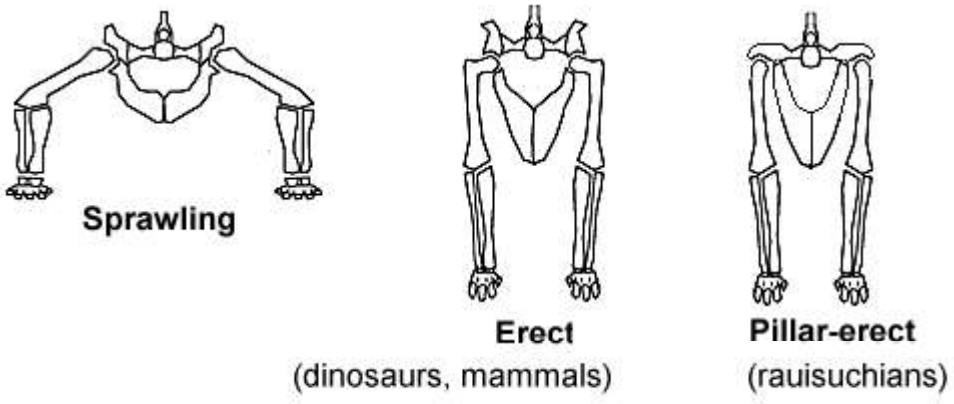
This argument was criticized on several grounds and is no longer taken seriously (the following list of criticisms is far from exhaustive):

- Estimates of dinosaur weights vary widely, and even a small variation can make a large difference to the calculated predator-prey ratio.
- His sample may not have been representative. Bakker obtained his numbers by counting museum specimens, but these have a bias towards rare or especially well-preserved specimens, and do not represent what exists in fossil beds. Even fossil beds may not accurately represent the actual populations, for example smaller and younger animals have less robust bones and are therefore less likely to be preserved.
- There are no published predator-prey ratios for large ectothermic predators, because such predators are very rare and mostly occur only on fairly small

islands. Large ectothermic herbivores are equally rare. So Bakker was forced to compare mammalian predator-prey ratios with those of fish and invertebrate communities, where life expectancies are much shorter and other differences also distort the comparison.

- The concept assumes that predator populations are limited only by the availability of prey. However other factors such as shortage of nesting sites, cannibalism or predation of one predator on another can hold predator populations below the limit imposed by prey biomass, and this would misleadingly reduce the predator-prey ratio. .
- Ecological factors can misleadingly reduce the predator-prey ratio, for example: a predator might prey on only some of the "prey" species present; disease, parasites and starvation might kill some of the prey animals before the predators get a chance to hunt them.
- It is very difficult to state precisely what preys on what. For example the young of herbivores may preyed upon by lizards and snakes while the adults are preyed on by mammals. Conversely the young of many predators live largely on invertrebrates and switch to vertrebrate as they grow.

## Posture and gait




Hip joints and limb postures.

Dinosaurs' limbs were erect and held under their bodies, rather than sprawling out to the sides like those of lizards and newts. The evidence for this is the angles of the joint surfaces and the locations of muscle and tendon attachments on the bones. Attempts to represent dinosaurs with sprawling limbs result in creatures with dislocated hips, knees, shoulders and elbows.

Carrier's constraint states that air-breathing vertebrates with two lungs that flex their bodies sideways during locomotion find it difficult to move and breathe at the same time. This severely limits stamina, and forces them to spend more time resting than moving.

Sprawling limbs require sideways flexing during locomotion (except for tortoises and turtles, which are very slow and whose armor keeps their bodies fairly rigid). However,

despite Carrier's constraint, sprawling limbs are efficient for creatures that spend most of their time resting on their bellies and only move for a few seconds at a time—because this arrangement minimizes the energy costs of getting up and lying down.

Erect limbs increase the costs of getting up and lying down, but avoid Carrier's constraint. This indicates that dinosaurs were active animals because natural selection would have favored the retention of sprawling limbs if dinosaurs had been sluggish and spent most of their waking time resting. An active lifestyle requires a metabolism that quickly regenerates energy supplies and breaks down waste products which cause fatigue, i.e., it requires a fairly fast metabolism and a considerable degree of homeothermy.

Additionally, an erect posture demands precise balance, the result of a rapidly functioning neuromuscular system. This suggests endothermic metabolism, because an ectothermic animal would be unable to walk or run, and thus to evade predators, when its core temperature was lowered. Other evidence for endothermy includes limb length (many dinosaurs possessed comparatively long limbs) and bipedalism, both found today only in endotherms. Many bipedal dinosaurs possessed gracile leg bones with a short thigh relative to calf length. This is generally an adaptation to frequent sustained running, characteristic of endotherms which, unlike ectotherms, are capable of producing sufficient energy to stave off the onset of anaerobic metabolism in the muscle.

Bakker and Ostrom both pointed out that all dinosaurs had erect hindlimbs and that all quadrupedal dinosaurs except the ceratopsians and ankylosaurs had erect forelimbs; and that among living animals only the endothermic ("warm-blooded") mammals and birds have erect limbs (Ostrom acknowledged that crocodilians' occasional "high walk" was a partial exception). Bakker claimed this was clear evidence of endothermy in dinosaurs, while Ostrom regarded it as persuasive but not conclusive.

A 2009 study supported the hypothesis that endothermy was widespread in at least larger non-avian dinosaurs, and that It was plausibly ancestral for all dinosauriforms, based on the biomechanics of running.

## Feathers

Skin impression of the hadrosaur *Edmontosaurus*

There is now no doubt that many theropod dinosaur species had feathers, including *Shuvuuia*, *Sinosauropteryx* and *Dilong* (an early tyrannosaur). These have been interpreted as insulation and therefore evidence of warm-bloodedness.

But impressions of feathers have only been found in coelurosaurs (which includes the ancestors of both birds and tyrannosaurs), so at present feathers give us no information about the metabolisms of the other major dinosaur groups, e.g. coelophysids, ceratosaurs, carnosaurs, sauropods or ornithischians.

In fact the fossilised skin of *Carnotaurus* (an abelisaurid and therefore not a coelurosaur) shows an unfeathered, reptile-like skin with rows of bumps. But an adult *Carnotaurus* weighed about 1 ton, and mammals of this size and larger have either very short hair or naked skins, so perhaps the skin of *Carnotaurus* tells us nothing about whether smaller non-coelurosaurid theropods had feathers.

Skin-impressions of *Pelorosaurus* and other sauropods (dinosaurs with elephantine bodies and long necks) reveal large hexagonal scales, and some sauropods, such as *Saltasaurus*, had bony plates in their skin. The skin of ceratopsians consisted of large polygonal scales, sometimes with scattered circular plates. "Mummified" remains and skin impressions of hadrosaurids reveal pebbly scales. It is unlikely that the ankylosaurids, such as *Euoplocephalus*, had insulation, as most of their surface area was covered in bony knobs and plates. Likewise there is no evidence of insulation in the stegosaurs.

## Polar dinosaurs

Dinosaur fossils have been found in regions that were close to the poles at the relevant times, notably in southeastern Australia, Antarctica and the North Slope of Alaska. There is no evidence of major changes in the angle of the Earth's axis, so polar dinosaurs and the rest of these ecosystems would have had to cope with the same extreme variation of day length through the year that occurs at similar latitudes today (up to a full day with no darkness in summer, and a full day with no sunlight in winter).

Studies of fossilized vegetation suggest that the Alaska North Slope had a maximum temperature of 13 °C (55 °F) and a minimum temperature of 2 °C (36 °F) to 8 °C (46 °F) in the last 35 million years of the Cretaceous (slightly cooler than Portland, Oregon but slightly warmer than Calgary, Alberta). Even so, the Alaska North Slope has no fossils of large cold-blooded animals such as lizards and crocodilians, which were common at the same time in Alberta, Montana, and Wyoming. This suggests that at least some non-avian dinosaurs were warm-blooded. It has been proposed that North American polar dinosaurs may have migrated to warmer regions as winter approached, which would allow them to inhabit Alaska during the summers even if they were cold-blooded. But a round trip between there and Montana would probably have used more energy than a cold-blooded land vertebrate produces in a year; in other words the Alaskan dinosaurs would have to be warm-blooded, irrespective of whether they migrated or stayed for the winter. A 2008 paper on dinosaur migration by Phil R. Bell and Eric Snively proposed that most polar dinosaurs, including theropods, sauropods, ankylosaurians, and hypsilophodonts, probably overwintered, although hadrosaurids like *Edmontosaurus* were probably capable of annual 2,600 km (1,600 mile) round trips.

It is more difficult to determine the climate of southeastern Australia when the dinosaur fossil beds were laid down 115 to 105 million years ago, towards the end of the Early Cretaceous: these deposits contain evidence of permafrost, ice wedges, and hummocky ground formed by the movement of subterranean ice, which suggests mean annual

temperatures ranged between −6 °C (21.2 °F) and 5 °C (41 °F); oxygen isotope studies of these deposits give a mean annual temperature of 1.5 °C (34.7 °F) to 2.5 °C (36.5 °F). However the diversity of fossil vegetation and the large size of some of fossil trees exceed what is found in such cold environments today, and no-one has explained how such vegetation could have survived in the cold temperatures suggested by the physical indicators – for comparison Fairbanks, Alaska presently has a mean annual temperature of 2.9 °C (37.2 °F). An annual migration from and to southeastern Australia would have been very difficult for fairly small dinosaurs in such as *Leaellynasaura*, a vegetarian about 60 centimetres (2.0 ft) to 90 centimetres (3.0 ft) long, because seaways to the north blocked the passage to warmer latitudes. Bone samples from *Leaellynasaura* and *Timimus*, an ornithomimid about 3.5 metres (11 ft) long and 1.5 metres (4.9 ft) high at the hip, suggested these two dinosaurs had different ways of surviving the cold, dark winters: the *Timimus* sample had lines of arrested growth (LAGs for short; similar to growth rings), and it may have hibernated; but the *Leaellynasaura* sample showed no signs of LAGs, so it may have remained active throughout the winter.

## Evidence for behavioral thermoregulation

Some dinosaurs, e.g. *Spinosaurus* and *Ouranosaurus*, had on their backs "sails" supported by spines growing up from the vertebrae. (This was also true, incidentally, for the synapsid *Dimetrodon*.) Such dinosaurs could have used these sails to:

- take in heat by basking with the "sails" at right angles to the sun's rays.
- to lose heat by using the "sails" as radiators while standing in the shade or while facing directly towards or away from the sun.

But these were a very small minority of known dinosaur species. One common interpretation of the plates on stegosaurs' backs is as heat exchangers for thermoregulation, as the plates are filled with blood vessels, which, theoretically, could absorb and dissipate heat.
This might have worked for a stegosaur with large plates, such as *Stegosaurus*, but other stegosaurs, such as *Wuerhosaurus*, *Tuojiangosaurus* and *Kentrosaurus* possessed much smaller plates with a surface area of doubtful value for thermo-regulation. However, the idea of stegosaurian plates as heat exchangers has recently been questioned.

## Other evidence

## Respiration

Endothermy demands frequent respiration, which can result in water loss. In living birds and mammals, water loss is limited by pulling moisture out of exhaled air with mucous-covered respiratory turbinates, tissue-covered bony sheets in the nasal cavity. Several dinosaurs have olfactory turbinates, used for smell, but none have yet been identified with respiratory turbinates.

## Brain size

Because endothermy allows refined neuromuscular control, and because brain matter requires large amounts of energy to sustain, some speculate that increased brain size indicates increased activity and, thus, endothermy. The encephalization quotient (EQ) of dinosaurs, a measure of brain size calculated using brain endocasts, varies on a spectrum from bird-like to reptile-like. Using EQ alone, coelosaurs appear to have been as active as living mammals, while theropods and ornithopods fall somewhere between mammals and reptiles, and other dinosaurs resemble reptiles.

## The crocodilian puzzle and early archosaur metabolism

It appears that the earliest dinosaurs had the features that form the basis for arguments for warm-blooded dinosaurs—especially erect limbs. This raises the question "How did dinosaurs become warm-blooded?" The most obvious possible answers are:

- "Their immediate ancestors (archosaurs) were cold-blooded, and dinosaurs began developing warm-bloodedness very early in their evolution." This implies that dinosaurs developed a significant degree of warm-bloodedness in a very short time, possibly less than 20M years. But in mammals' ancestors the evolution of warm-bloodedness seems to have taken much longer, starting with the beginnings of a secondary palate around the beginning of the mid-Permian and going on possibly until the appearance of hair about 164M years ago in the mid Jurassic).
- "Dinosaurs' immediate ancestors (archosaurs) were at least fairly warm-blooded, and dinosaurs evolved further in that direction." This answer raises 2 problems: (**A**) The early evolution of archosaurs is still very poorly understood - large numbers of individuals and species are found from the start of the Triassic but only 2 species are known from the very late Permian (*Archosaurus rossicus* and *Protorosaurus speneri*); (**B**) Crocodilians evolved shortly before dinosaurs and are closely related to them, but are cold-blooded (see below).

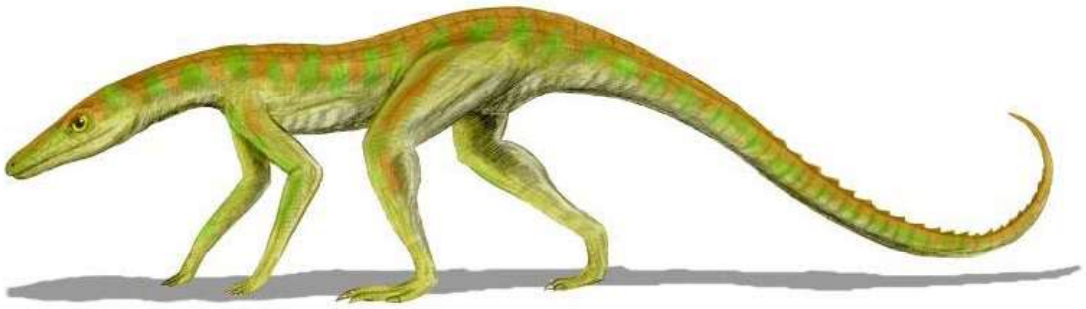
Crocodilians present some puzzles if one regards dinosaurs as active animals with fairly constant body temperatures. Crocodilians evolved shortly before dinosaurs and, second to birds, are dinosaurs' closest living relatives - but modern crocodilians are cold-blooded. This raises some questions:

- If dinosaurs were to a large extent "warm-blooded", when and how fast did warm-bloodedness evolve in their lineage?
- Modern crocodilians are cold-blooded but have several features associated with warm-bloodedness. How did they acquire these features?

Modern crocodilians are cold-blooded but can move with their limbs erect, and have several features normally associated with warm-bloodedness because they improve the animal's oxygen supply:

- 4-chambered hearts. Mammals and birds have four-chambered hearts. Non-crocodilian reptiles have three-chambered hearts, which are less efficient because they allow oxygenated and de-oxygenated blood to mix and therefore send some de-oxygenated blood out to the body instead of to the lungs. Modern crocodilians' hearts are four-chambered, but are smaller relative to body size and run at lower pressure than those of modern mammals and birds. They also have a bypass that makes then functionally three-chambered when under water, conserving oxygen.
- a diaphragm, which aids breathing.
- a secondary palate, which allows the animal to eat and breathe at the same time.
- a hepatic piston mechanism for pumping the lungs. This is different from the lung-pumping mechanisms of mammals and birds but similar to what some researchers claim to have found in some dinosaurs.

So why did natural selection favor these features, which are important for active warm-blooded creatures but of little apparent use to cold-blooded aquatic ambush predators that spend most of their time floating in water or lying on river banks?



Reconstruction of *Terrestrisuchus*, a very slim, leggy Triassic crocodylomorph.

It was suggested in the late 1980s that that crocodilians were originally active, warm-blooded predators and that their archosaur ancestors were warm-blooded. More recently, developmental studies indicate that crocodilian embryos develop fully four-chambered hearts first—then develop the modifications that make their hearts function as three-chambered under water. Using the principle that ontogeny recapitulates phylogeny, the researchers concluded that the original crocodilians had fully 4-chambered hearts and were therefore warm-blooded and that later crocodilians developed the bypass as they reverted to being cold-blooded aquatic ambush predators.

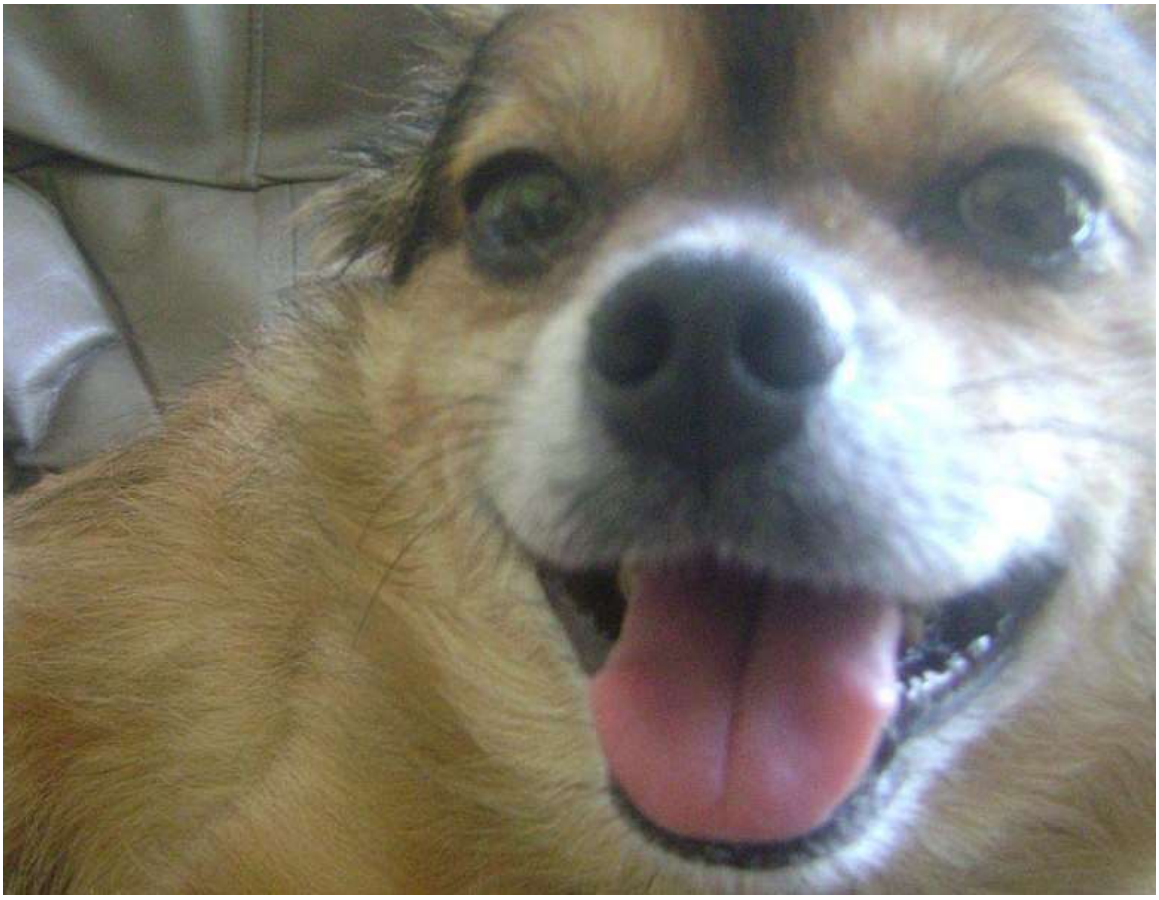
More recent research on archosaur bone structures and their implications for growth rates also suggests that early archosaurs had fairly high metabolic rates and that the Triassic ancestors of crocodilians dropped back to more typically "reptilian" metabolic rates.

If this view is correct, the development of warm-bloodedness in archosaurs (reaching its peak in dinosaurs) and in mammals would have taken more similar amounts of time. It would also be consistent with the fossil evidence:

- The earliest crocodilians, e.g. *Terrestrisuchus*, were slim, leggy terrestrial predators.
- Erect limbs appeared quite early in archosaurs' evolution, and those of rauisuchians are very poorly adapted for any other posture.

# Chapter 14

# Thermoregulation



A dog panting is an example of thermoregulation.

**Thermoregulation** is the ability of an organism to keep its body temperature within certain boundaries, even when the surrounding temperature is very different. This process is one aspect of homeostasis: a dynamic state of stability between an animal's internal environment and its *external* environment (the study of such processes in zoology has been called ecophysiology or physiological ecology). If the body is unable to maintain a normal temperature and it increases significantly above normal, a condition known as

hyperthermia occurs. This occurs when the body is exposed to constant temperatures of approximately 55° C, any prolonged exposure (longer than a few hours) at this temperature and up to around 70° C death is almost inevitable. The opposite condition, when body temperature decreases below normal levels, is known as hypothermia.

Whereas an organism that *thermoregulates* is one that keeps its core body temperature within certain limits, a **thermoconformer** is subject to changes in body temperature according to changes in the temperature outside of its body. It was not until the introduction of thermometers that any exact data on the temperature of animals could be obtained. It was then found that local differences were present, since heat production and heat loss vary considerably in different parts of the body, although the circulation of the blood tends to bring about a mean temperature of the internal parts. Hence it is important to identify the parts of the body that most closely reflect the temperature of the internal organs. Also, for such results to be comparable, the measurements must be conducted under comparable conditions. The rectum has traditionally been considered to reflect most accurately the temperature of internal parts, or in some cases of sex or species, the vagina, uterus or bladder.

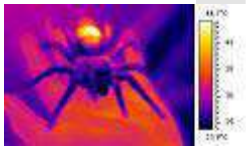
Occasionally the temperature of the urine as it leaves the urethra may be of use. More often the temperature is taken in the mouth, axilla, ear or groin.

## *Classification of animals by thermal characteristics*

**Thermoregulation in animals**

Ectothermy · Endothermy

Poikilothermy · Heterothermy · Homeothermy (Gigantothermy)



Kleptothermy · Bradymetabolism · Tachymetabolism

## Endothermy versus Ectothermy

Organisms can generally be divided into two types of thermoregulators, endotherms and ectotherms. Endotherms create most of their heat via metabolic processes, and are colloquially referred to as warm-blooded. Ectotherms temperature comes mostly from the environment.

## Ectotherms

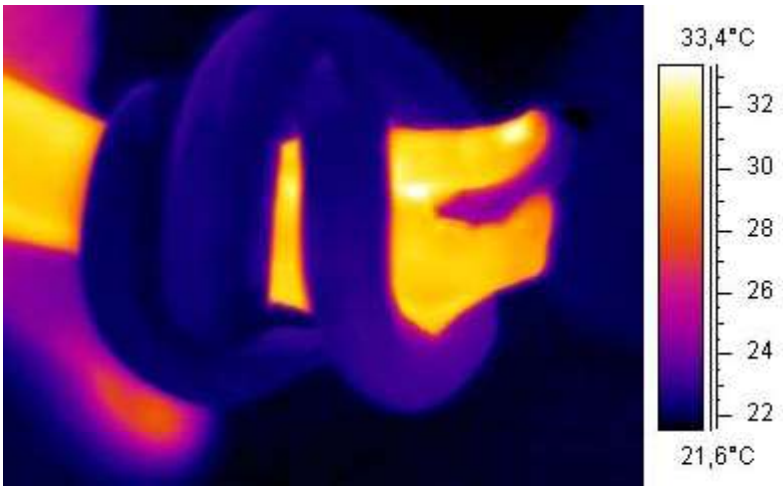
### *Ectothermic cooling*

Seeking shade is one method of cooling. Here Sooty Tern chicks are using a Black-footed Albatross chick for shade.

- Vaporization:
  - Getting wet in a river, lake or sea.
- Convection:
  - Climbing to lower ground from trees, into valleys, burrows, etc.
  - Entering a cold water or air current.
  - Building a nest that allows natural or generated air/water flow for cooling.
- Conduction:
  - Lying on cold ground.
  - Staying wet in a river, lake or sea.
  - Covering in cool mud.
- Radiation:
  - Finding shade.
  - Entering a burrow shaped for radiating heat (Black-box effect).
  - Expanding folds of skin.
  - Exposing wing surfaces.

***Ectothermic heating (or minimizing heat loss)***

- Convection:
  - Climbing to higher ground up trees, ridges, rocks.
  - Entering a warm water or air current.
  - Building an insulated nest or burrow.
- Conduction:
  - Lie on hot rock.
- Radiation:
  - Lie in sun.
  - Fold skin to reduce exposure.
  - Conceal wing surfaces.
- Insulation
  - Change shape to alter surface/volume ratio
  - Inflate the body




Thermographic image of a snake around an arm

To cope with low temperatures, some fish have developed the ability to remain functional even when the water temperature is below freezing; some use natural antifreeze or antifreeze proteins to resist ice crystal formation in their tissues. Amphibians and reptiles cope with heat loss by evaporative cooling and behavioral adaptations.

## Endothermy

An endotherm is an animal that regulates its own body temperature, typically by keeping it a constant level. To regulate body temperature, an organism may need to prevent heat gains in arid environments. Evaporation of water, either across respiratory surfaces or across the skin in those animals possessing sweat glands, helps in cooling body temperature to within the organism's tolerance range. Animals with a body covered by fur have limited ability to sweat, relying heavily on **panting** to increase evaporation of water across the moist surfaces of the lungs and the tongue and mouth. Birds also avoid

overheating by **gular fluttering**, flapping the wings near the gular (throat) skin, similar to panting in mammals, since their thin skin has no sweat glands. Down feathers trap warm air acting as excellent insulators just as hair in mammals acts as a good insulator. Mammalian skin is much thicker than that of birds and often has a continuous layer of insulating fat beneath the dermis — in marine mammals such as whales this is called blubber. Dense coats found in desert endotherms also aid in preventing heat gain.

A cold weather strategy is to temporarily decrease metabolic rate, decreasing the temperature difference between the animal and the air and thereby minimizing heat loss. Furthermore, having a lower metabolic rate is less energetically expensive. Many animals survive cold frosty nights through torpor, a short-term temporary drop in body temperature. Organisms when presented with the problem of regulating body temperature have not only behavioural, physiological and structural adaptations, but also a feedback system to trigger these adaptations to regulate temperature accordingly. The main features of this system are *stimulus, receptor, modulator, effector* and then the feedback of the newly adjusted temperature to the *stimulus.* This cyclical process aids in homeostasis.

### Homeothermy versus Poikilothermy

Homeothermy and poikilothermy refer to how stable an organism's temperature is. Most endothermic organisms are homeothermic, like mammals. However, animals with facultative endothermy are often pokilothermic, meaning their temperature can vary considerably. Similarly, most fish are ectotherms, as all of their heat comes from the surrounding water. However, most are homeotherms because their temperature is very stable.

## *Thermoregulation in vertebrates*

By numerous observations upon humans and other animals, John Hunter showed that the essential difference between the so-called warm-blooded and cold-blooded animals lies in observed constancy of the temperature of the former, and the observed variability of the temperature of the latter. Almost all birds and mammals have a high temperature almost constant and independent of that of the surrounding air (homeothermy). Almost all other animals display a variation of body temperature, dependent on their surroundings (poikilothermy).

Certain mammals are exceptions to this rule, being warm-blooded during the summer or daytime, but cold-blooded during the winter when they hibernate or at night during sleep. J. O. Wakelin Barratt has demonstrated that under certain pathological conditions, a warm-blooded (homeothermic) animal may become temporarily cold-blooded (poikilothermic). He has shown conclusively that this condition exists in rabbits suffering from rabies during the last period of their life, the rectal temperature being then within a few degrees of the room temperature and varying with it. He explains this condition by the assumption that the nervous mechanism of heat regulation has become paralysed. The respiration and heart-rate being also retarded during this period, the resemblance to the

condition of hibernation is considerable. Again, Sutherland Simpson has shown that during deep anaesthesia a warm-blooded animal tends to take the same temperature as that of its environment. He demonstrated that when a monkey is kept deeply anaesthetized with ether and is placed in a cold chamber, its temperature gradually falls, and that when it has reached a sufficiently low point (about 25 °C in the monkey), the employment of an anaesthetic is no longer necessary, the animal then being insensible to pain and incapable of being roused by any form of stimulus; it is, in fact, narcotised by cold, and is in a state of what may be called "artificial hibernation." Once again this is explained by the fact that the heat-regulating mechanism has been interfered with. Similar results have been obtained from experiments on cats.

## Brain control

Thermoregulation in both ectotherms and endotherms is controlled mainly by the preoptic area of the anterior hypothalamus. Such homeostatic control is separate from the sensation of temperature.

## Thermoregulation in birds and mammals

Kangaroo licking its arms to cool down on a very hot day

In cold environments, birds and mammals employ the following adaptations and strategies to minimize heat loss:

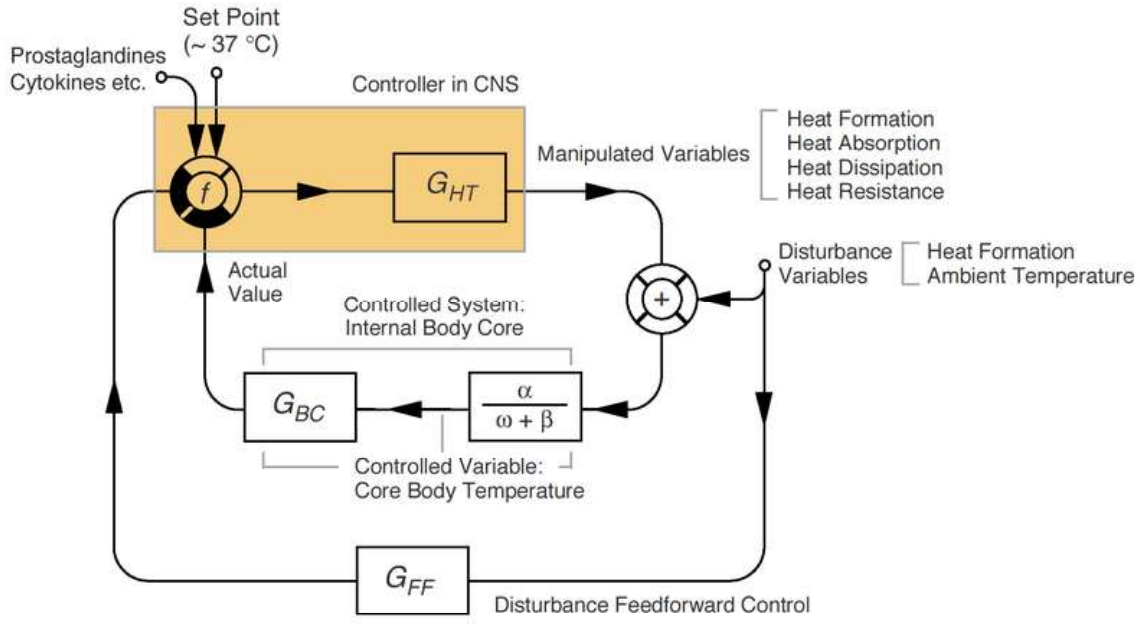
1. using small smooth muscles (erector pili in mammals) which are attached to feather or hair shafts; this non-shivering thermogenesis distorts the surface of the skin as the feather/hair shaft is made more erect (called goose bumps or pimples)
2. increasing body size to more easily maintain core body temperature (warm-blooded animals in cold climates tend to be larger than similar species in warmer climates
3. having the ability to store energy as fat for metabolism

4. have shortened extremities
5. have countercurrent blood flow in extremities - this is where the warm arterial blood travelling to the limb passes the cooler venous blood from the limb and heat is exchanged warming the venous blood and cooling the arterial (e.g. Arctic Wolf or penguins)

In warm environments, birds and mammals employ the following adaptations and strategies to maximize heat loss:

1. behavioural adaptations like living in burrows during the day and being nocturnal
2. evaporative cooling by perspiration and panting
3. storing fat reserves in one place (e.g. camel's hump) to avoid its insulating effect
4. elongated, often vascularized extremities to conduct body heat to the air

## Thermoregulation in humans




Simplified information processing structure of human thermoregulation.

As in other mammals, thermoregulation is an important aspect of human homeostasis. Most body heat is generated in the deep organs, especially the liver, brain, and heart, and in contraction of skeletal muscles. Humans have been able to adapt to a great diversity of climates, including hot humid and hot arid. High temperatures pose serious stresses for the human body, placing it in great danger of injury or even death. For humans, adaptation to varying climatic conditions includes both physiological mechanisms as a byproduct of evolution, and the conscious development of cultural adaptations.

There are four avenues of heat loss: convection, conduction, radiation, and evaporation. If skin temperature is greater than that of the surroundings, the body can lose heat by

radiation and conduction. But if the temperature of the surroundings is greater than that of the skin, the body actually *gains* heat by radiation and conduction. In such conditions, the only means by which the body can rid itself of heat is by evaporation. So when the surrounding temperature is higher than the skin temperature, anything that prevents adequate evaporation will cause the internal body temperature to rise. During sports activities, evaporation becomes the main avenue of heat loss. Humidity affects thermoregulation by limiting sweat evaporation and thus heat loss.

The skin assists in homeostasis (keeping different aspects of the body constant e.g. temperature). It does this by reacting differently to hot and cold conditions so that the inner body temperature remains more or less constant. Vasodilation and sweating are the primary modes by which humans attempt to lose excess body heat. The brain creates much heat through the countless reactions which occur. Even the process of thought creates heat. The head has a complex system of blood vessels, which keeps the brain from overheating by bringing blood to the thin skin on the head, allowing heat to escape. The effectiveness of these methods is influenced by the character of the climate and the degree to which the individual is acclimatized.

## In hot conditions

1. Eccrine sweat glands under the skin secrete sweat (a fluid containing mostly water with some dissolved ions) which travels up the sweat duct, through the sweat pore and onto the surface of the skin. This causes heat loss via evaporative cooling; however, a lot of essential water is lost.
2. The hairs on the skin lie flat, preventing heat from being trapped by the layer of still air between the hairs. This is caused by tiny muscles under the surface of the skin called Arrector pili muscles relaxing so that their attached hair follicles are not erect. These flat hairs increase the flow of air next to the skin increasing heat loss by convection. When environmental temperature is above core body temperature, sweating is the only physiological way for humans to lose heat.
3. Arterioles Vasodilation occurs, this is the process of relaxation of smooth muscle in arteriole walls allowing increased blood flow through the artery. This redirects blood into the superficial capillaries in the skin increasing heat loss by convection and conduction.

*Note:* Most animals can't sweat efficiently. Cats and dogs have sweat glands only on the pads of their feet. Horses and humans are two of the few animals capable of sweating. Many animals pant rather than sweat because the lungs have a large surface area and are highly vascularised. Air is inhaled, cooling the surface of the lungs and is then exhaled losing heat and some water vapour.

## Thermoregulation in hot and humid conditions

In general, humans appear physiologically well adapted to hot dry conditions. However, effective thermoregulation is reduced in hot, humid environments such as the Red Sea and Persian Gulf (where moderately hot summer temperatures are accompanied by

unusually high vapor pressures), tropical environments, and deep mines where the atmosphere can be water-saturated. In hot-humid conditions, clothing can impede efficient evaporation. In such environments, it helps to wear light clothing such as cotton, that is pervious to sweat but impervious to radiant heat from the sun. This minimizes the gaining of radiant heat, while allowing as much evaporation to occur as the environment will allow. Clothing such as plastic fabrics that are impermeable to sweat and thus do not facilitate heat loss through evaporation, can actually contribute to heat stress.

## In cold conditions

1. Sweat stops being produced.
2. The minute muscles under the surface of the skin called erector pili muscles (attached to an individual hair follicle) contract (piloerection), lifting the hair follicle upright. This makes the hairs stand on end which acts as an insulating layer, trapping heat. This is what also causes goose bumps since humans don't have very much hair and the contracted muscles can easily be seen.
3. Arterioles carrying blood to superficial capillaries under the surface of the skin can shrink (constrict), thereby rerouting blood away from the skin and towards the warmer core of the body. This prevents blood from losing heat to the surroundings and also prevents the core temperature dropping further. This process is called vasoconstriction. It is impossible to prevent all heat loss from the blood, only to reduce it. In extremely cold conditions excessive vasoconstriction leads to numbness and pale skin. Frostbite only occurs when water within the cells begins to freeze, this destroys the cell causing damage.
4. Muscles can also receive messages from the thermo-regulatory center of the brain (the hypothalamus) to cause shivering. This increases heat production as respiration is an exothermic reaction in muscle cells. Shivering is more effective than exercise at producing heat because the animal remains still. This means that less heat is lost to the environment via convection. There are two types of shivering: low intensity and high intensity. During low intensity shivering animals shiver constantly at a low level for months during cold conditions. During high intensity shivering animals shiver violently for a relatively short time. Both processes consume energy although high intensity shivering uses glucose as a fuel source and low intensity tends to use fats. This is a primary reason why animals store up food in the winter.
5. Mitochondria can convert fat directly into heat energy, increasing the temperature of all cells in the body. Brown fat is specialized for this purpose, and is abundant in newborns and animals that hibernate.

The process explained above, in which the skin regulates body temperature is a part of thermoregulation. This is one aspect of homeostasis-the process by which the body regulates itself to keep internal conditions constant.

## Diseases and syndromes relating to thermoregulation

- Hypothermia
- Hyperthermia
- Heat stroke
- Raynaud's phenomenon (Raynaud's disease)
- Induced hypothermia
- Erythromelalgia (hyperthermia)

## *Thermoregulation in plants*

Thermogenesis occurs in the flowers of many plants in the Araceae family as well as in cycad cones. In addition, some plants in the Alum family - such as the Eastern Skunk Cabbage, the Philodendron (*Philodendron selloum*), and the Sacred lotus (*Nelumbo nucifera*) are able to thermoregulate themselves, remaining on average 20 °C (36 °F) above air temperature while flowering. Heat is produced by breaking down the starch that was stored in their roots, which requires the consumption of oxygen at a rate approaching that of a flying hummingbird.

One possible explanation for plant thermoregulation is to provide protection against cold temperature. For example, the skunk cabbage is not frost-resistant, yet it begins to grow and flower when there is still snow on the ground. Another theory is that thermogenicity helps attract pollinators, which is borne out by observations that heat production is accompanied by the arrival of beetles or flies.

## *Behavioural temperature regulation*

Animals other than humans regulate and maintain their body temperature with physiological adjustments and behavior. Desert lizards are ectotherms and so unable to metabolically control their temperature but can do this by altering their location. They may do this by in the morning only raising their head from its burrow and then exposing their entire body. By basking in the sun, the lizard absorbs solar heat. It may also absorb heat by conduction from heated rocks that have stored radiant solar energy. To lower their temperature, lizards may seek cooler objects with which to contact, find shade or return to their burrow. They also go to their burrows to avoid cooling when the sun goes down or the temperature falls.

Animals also engage in kleptothermy in which they share or even steal each other's body warmth. In endotherms such as bats and birds (such as the mousebird and emperor penguin) it allows the sharing of body heat (particularly amongst juveniles). This allows the individuals to increase their thermal inertia (as with gigantothermy) and so reduce heat loss. Some ectotherms share burrows of ectotherms. Other animals exploit termite mounds.

Some animals living in cold environments maintain their body temperature by preventing heat loss. Their fur grows more densely to increase the amount of insulation. Some

animals are regionally heterothermic and are able to allow their less insulated extremities to cool to temperatures much lower than their core temperature—nearly to 0 °C. This minimizes heat loss through less insulated body parts, like the legs, feet (or hooves), and nose.


An ostrich can keep its body temperature very constant, even though it can be very hot during the day and cold at night.

## Hibernation, estivation, and daily torpor

To cope with limited food resources and low temperatures, some mammals hibernate in underground burrows. In order to remain in "stasis" for long periods, these animals must build up brown fat reserves and be capable of slowing all body functions. True hibernators (e.g. groundhogs) keep their body temperature down throughout their hibernation while the core temperature of false hibernators (e.g. bears) varies with them sometimes emerging from their dens for brief periods. Some bats are true hibernators which rely upon a rapid, non-shivering thermogenesis of their brown fat deposit to bring them out of hibernation.

Estivation occurs in summer (like siestas) and allows some mammals to survive periods of high temperature and little water (e.g. turtles burrow in pond mud).

Daily torpor occurs in small endotherms like bats and humming birds which temporarily reduce their high metabolic rates to conserve energy.

## *Variations in the temperature of human beings and some animals*

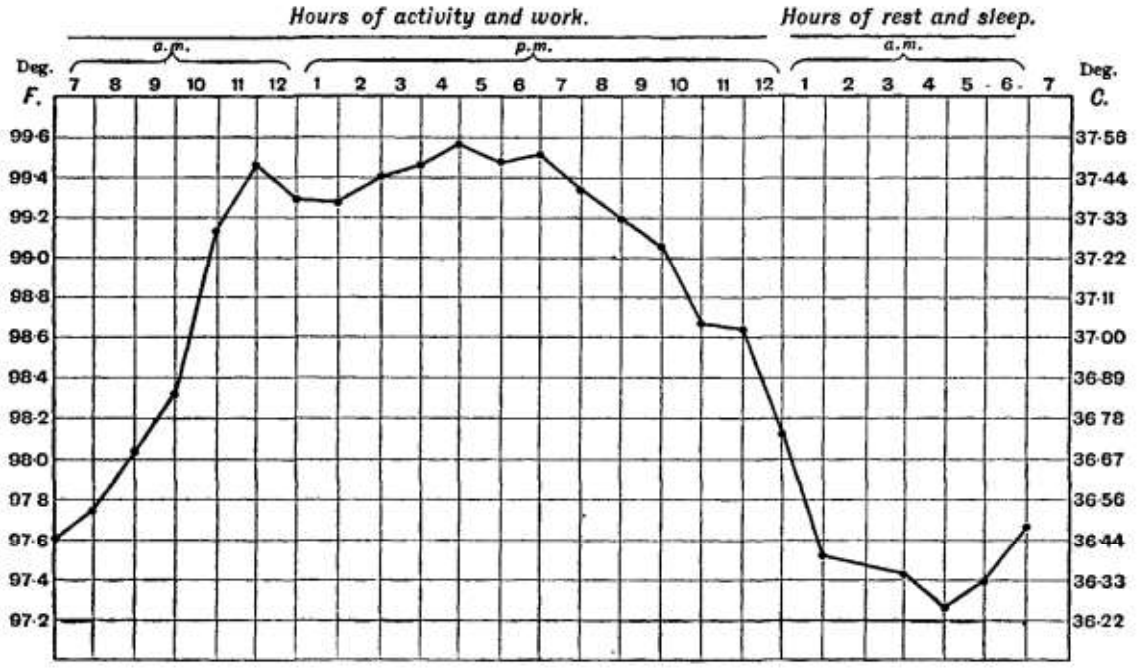



Chart showing diurnal variation in body temperature, ranging from about 37.5 °C from 10 a.m. to 6 p.m., and falling to about 36.3 °C from 2 a.m. to 6 a.m.

### Normal human temperature

Previously, average oral temperature for healthy adults had been considered 37.0 °C (98.6 °F), while normal ranges are 36.1 °C (97.0 °F) to 37.8 °C (100.0 °F). In Poland and Russia, the temperature had been measured axillary. 36.6 °C was considered "ideal" temperature in these countries, while normal ranges are 36 °C to 36.9 °C.

Recent studies suggest that the average temperature for healthy adults is 98.2 °F or 36.8 °C (same result in three different studies). Variations (one standard deviation) from three other studies are:

- 36.4 - 37.1 °C (97.5 - 98.8 °F)
- 36.3 - 37.1 °C (97.3 - 98.8 °F) for males, 36.5 - 37.3 °C (97.7 - 99.1 °F) for females
- 36.6 - 37.3 °C (97.9 - 99.1 °F)

### Variations from thermometer placement

Temperature varies according to thermometer placement, with rectal temperature being 0.3-0.6 °C (0.5-1 °F) higher than oral temperature, while axillary temperature is 0.3-0.6 °C (0.5-1 °F) lower than oral temperature. The average difference between oral and

axillary temperatures of Indian children aged 6–12 was found to be only 0.1 °C (standard deviation 0.2 °C), and the mean difference in Maltese children aged 4–14 between oral and axillary temperature was 0.56 °C, while the mean difference between rectal and axillary temperature for children under 4 years old was 0.38 °C.

## Variations associated with development

Of the lower warm-blooded animals, there are some that appear to be cold-blooded at birth. Kittens, rabbits and puppies, if removed from their surroundings shortly after birth, lose their body heat until their temperature has fallen to within a few degrees of that of the surrounding air. But such animals are at birth blind, helpless and in some cases furless. Animals who are born when in a condition of greater development can maintain a fairly constant body temperature. In strong, healthy human infants a day or two old the temperature rises slightly when removed, but in that of weakly, ill-developed children it either remains stationary or falls. The cause of the variable temperature in infants and young immature animals is the imperfect development of the nervous regulating mechanism.

The average temperature falls slightly from infancy to puberty and again from puberty to middle age, but after that stage is passed the temperature begins to rise again, and by about the eightieth year is as high as in infancy.

## Variations due to circadian rhythms

In humans, a diurnal variation has been observed dependent on the periods of rest and activity, lowest at 11 p.m. to 3 a.m. and peaking at 10 a.m. to 6 p.m. Monkeys also have a well-marked and regular diurnal variation of body temperature which follows periods of rest and activity, and is not dependent on the incidence of day and night; nocturnal monkeys reach their highest body temperature at night and lowest during the day. Sutherland Simpson and J.J. Galbraith observed that all nocturnal animals and birds - whose periods of rest and activity are naturally reversed through habit and not from outside interference - experience their highest temperature during the natural period of activity (night) and lowest during the period of rest (day). Those diurnal temperatures can be reversed by reversing their daily routine.

The temperature curve of diurnal birds is essentially similar to that of man and other homoeothermal animals, except that the maximum occurs earlier in the afternoon and the minimum earlier in the morning. Also that the curves obtained from rabbits, guinea pigs and dogs were quite similar to those from man. These observations indicate that body temperature is partially regulated by circadian rhythms.

## Variations due to women's menstrual cycles

During the follicular phase (which lasts from the first day of menstruation until the day of ovulation), the average basal body temperature in women ranges from 36.45 to 36.7 °C (97.6 to 98.1 °F). Within 24 hours of ovulation, women experience an elevation of 0.15 -

0.45 °C (0.2 - 0.9 °F) due to the increased metabolic rate caused by sharply elevated levels of progesterone. The basal body temperature ranges between 36.7 - 37.3°C (98.1 - 99.2°F) throughout the luteal phase, and drops down to pre-ovulatory levels within a few days of menstruation. Women can chart this phenomenon to determine whether and when they are ovulating, so as to aid conception or contraception.

### Variations due to fever

Fever is a regulated elevation of the set point of core temperature in the hypothalamus, caused by circulating pyrogens produced by the immune system. To the subject, a rise in core temperature due to fever may result in feeling cold in an environment where people without fever do not.

### Variations due to biofeedback

A group of monks known as the Tummo are known to practice biofeedback meditation techniques that allow them to raise their body temperatures substantially.

### Variations due to other factors

In Simpson's & Galbraith's work, the mean temperature of the female was higher than that of the male in all the species examined whose sex had been determined.

Meals sometimes cause a slight elevation, sometimes a slight depression—alcohol seems always to produce a fall. Exercise and variations of external temperature within ordinary limits cause very slight change, as there are many compensating influences at work, which are discussed later. The core temperature of those living in the tropics is within a similar range to those dwelling in the Arctic regions.

## *Low body temperature increases lifespan*

It was long theorised that low body temperature may prolong life. On November 2006, a team of scientists from the Scripps Research Institute reported that transgenic mice which had body temperature 0.3-0.5 C lower than normal mice (due to overexpressing the uncoupling protein 2 in hypocretin neurons (Hcrt-UCP2), which elevated hypothalamic temperature, thus forcing the hypothalamus to lower body temperature) indeed lived longer than normal mice. The lifespan was 12% longer for males and 20% longer for females. Mice were allowed to eat as much as they wanted. The effects of such a genetic change in body temperature on longevity is harder to study in humans. The UCP2 genetic alleles seen in humans so far are associated with obesity

## *Limits compatible with life*

There are limits both of heat and cold that a warm-blooded animal can bear, and other far wider limits that a cold-blooded animal may endure and yet live. The effect of too extreme a cold is to decrease metabolism, and hence to lessen the production of heat.

Both catabolic and anabolic pathways share in this metabolic depression, and, though less energy is used up, still less energy is generated. The effects of this diminished metabolism become telling on the central nervous system first, especially the brain and those parts concerning consciousness; both heart rate and respiration rate decrease; judgment becomes impaired as drowsiness supervenes, becoming steadily deeper until the individual loses consciousness; without medical intervention, death by hypothermia quickly follows. Occasionally, however, convulsions may set in towards the end, and death is caused by asphyxia.

In experiments on cats performed by Sutherland Simpson and Percy T. Herring, the animals were unable to survive when rectal temperature fell below 16°C. At this low temperature respiration became increasingly feeble; heart-impulse usually continued after respiration had ceased, the beats becoming very irregular, apparently ceasing, then beginning again. Death appeared to be mainly due to asphyxia, and the only certain sign that it had taken place was the loss of knee jerks.

Conversely, too high a temperature speeds up the metabolism of different tissues to such a rate that their metabolic capital is soon exhausted. Blood that is too warm produces dyspnea by exhausting the metabolic capital of the respiratory centre; heart rate is increased; the beats then become arrhythmic and eventually cease. The central nervous system is also profoundly affected by hyperthermia and delirium and convulsions may set in. Consciousness may also be lost, propelling the person into a comatose condition. These changes can sometimes also be observed in patients suffering from an acute fever. The lower limit of temperature that humans can endure depends on many factors, but no one can survive a temperature of 45 °C (113 °F) or above for very long. Mammalian muscle becomes rigid with heat rigor at about 50°C, with the sudden rigidity of the whole body rendering life impossible.

H.M. Vernon has done work on the death temperature and paralysis temperature (temperature of heat rigor) of various animals. He found that species of the same class showed very similar temperature values, those from the Amphibia examined being 38.5°C, Fish 39°C, Reptilia 45°C, and various Molluscs 46°C. Also, in the case of Pelagic animals, he showed a relation between death temperature and the quantity of solid constituents of the body. In higher animals, however, his experiments tend to show that there is greater variation in both the chemical and physical characteristics of the protoplasm, and hence greater variation in the extreme temperature compatible with life.

## Chapter 15

# Poikilotherm and Torpor

# Poikilotherm

Common frog is a poikilotherm and needs to be able to function over a wide range of body core temperatures.

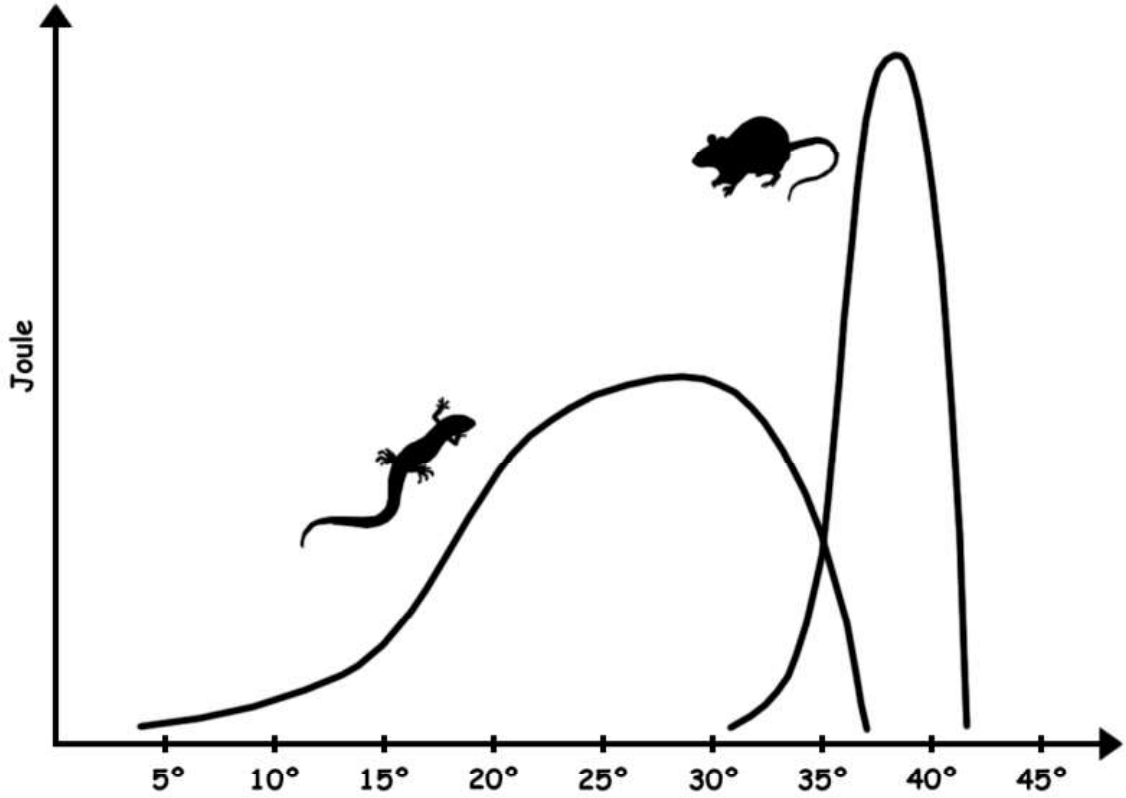
A **poikilotherm** is a organism whose internal temperature varies considerably. It is the opposite of a homeotherm, organisms which maintain thermal homeostasis. Usually the variation is a consequence of the ambient environmental temperature. Many terrestrial ectotherms are poikilothermic. The term is used as a more exact description of the vernacular "cold-blooded", which can also refer to organisms which are ectothermic (primarily obtain heat from their environment). Poikilothermic animals include types of

vertebrate animals, specifically fish, amphibians, and reptiles, as well as a host of invertebrate animals.

## Etymology

The term derives from Ancient Greek, *poikilos* (*ποικίλος*), meaning "varied"; and *thermos* (*θερμός*), meaning "heat".

## Physiology




Sustained energy output (Joule) of a poikilotherme (a lizard) and a homeotherm (a mouse) as a function of core body temperature. The homeotherm has a much higher output, but can only function over a very narrow range of body temperatures.

For an important chemical reaction, poikilotherms may have four to ten enzyme systems that operate at different temperatures. As a result, poikilotherms often have larger, more complex genomes than homeotherms in the same ecological niche. Frogs are a notable example of this effect.

Because their metabolism is variable and generally below that of homeothermic animals, sustained high-energy activities like powered flight in large animals or maintaining a large brain is generally beyond poikilotherm animals. This favoures e.g. sit-and-wait hunting strategy over chasing prey for larger animals with high movement cost. As they do not use their metabolisms to heat or cool themselves, total energy requirement over

time is low. For the same body weight, poikilotherms need half to 1/10 of the energy of homeotherms.

## *Adaptations in poikilotherms*

- Some adaptations are behavioral. Lizards and snakes bask in the sun in the early morning and late evening, and seek shelter around noon.
- Termite mounds are usually oriented in a north-south direction so that they absorb as much heat as possible around dawn and dusk and minimise heat absorption around noon.
- Tuna are able to warm their entire bodies through a heat exchange mechanism called the rete mirabile, which helps keep heat inside the body, and minimises the loss of heat through the gills. They also have their swimming muscles near the center of their bodies instead of near the surface, which minimises heat loss.
- Gigantothermy low ratio of surface area to volume is using a low ratio of surface area to volume minimises heat loss, such as in sea turtles.

## *Ecological niches*

It is comparatively easy for a poikilotherm to accumulate enough energy to reproduce. Poikilotherms in the same ecological niche often have much shorter generations than homeotherms: weeks rather than years.

This difference in energy requirement also means that a given niche of a given ecology can support a greater density of poikilothermic animals as homeothermic animals. This is reflected in e.g. the predator-prey ratio which is usually higher in poikilothermic fauna compared to homeothermic ones. However, in a given niche, homeotherms often drive poikilothermic competitors to extinction because homeotherms can gather food for a greater fraction of each day.

Poikilotherms succeed in some niches, such as islands, or distinct bioregions (such as the small bioregions of the Amazon basin). These often do not have enough food to support a viable breeding population of homeothermic animals. In these niches, poikilotherms such as large lizards, crabs and frogs subplant homeotherms such as birds and mammals.

## *In medicine*

In medicine, loss of normal thermoregulation in humans is referred to as "poikilothermia". This is usually seen with sedative and hypnotic drugs. For example, barbiturates, ethanol, and chloral hydrate may precipitate this effect.

# Torpor

**Daily torpor**, sometimes called **temporary hibernation** is a (usually short-term) state of decreased physiological activity in an animal, usually characterized by a reduced body temperature and rate of metabolism. Animals that go through torpor include birds (notably Cypselomorphae, even tiny hummingbirds) and some mammals such as mice and bats. During the active part of their day, animals that undergo daily torpor maintain normal body temperature and activity levels, but their temperature drops during a portion of the day (usually night) to conserve energy. Torpor is often used to help animals survive during periods of colder temperatures, as it allows the organism to save the amount of energy that would normally be used to maintain a high body temperature.

Torpor may extend for a longer period of time. Some animals such as groundhogs, ground squirrels and jumping mice enter this intensely deep state of hibernation for the duration of the winter. Lungfish switch to the torpor state if their pool dries out; tenrecs switch to the torpor state if food is scarce during the summer in Madagascar. This prolonged and deep torpor during summer months is known as aestivation. Black bears, although often thought of as hibernators, do not truly enter a state of torpor: while their body temperatures lower along with respiration and heartbeat, they do not decrease as significantly as most animals in a state of torpor, and bears are still responsive. Still, there is much debate about this within the scientific community: some feel that black bears are true hibernators that employ a more advanced form of hibernation.

Bats, especially species in temperate regions suffering harsh winters, rely upon torpor to survive. Lowering the body temperature to the ambient temperature allows them to enter torpor for prolonged periods at a lower metabolic cost. Oxygen consumption, heart rate and breathing rates are all lowered significantly meaning less energy is required to survive. Torpor is important in daily cycles to conserve energy as well as prolonged torpor, or hibernation. Pre-hibernation feeding builds up layers of fat which are used as the energy source during torpor. Arousal from torpor in bats is facultative, not obligate, but comes at a high energy cost, meaning awakening must be for a good reason.

## *Other uses of the word*

Torpor is alternatively used as a reference to any non-physiological state of inactivity. As an example, recently naturalists have learned that female crocodiles enter a deep torpor without aggression during their short egg laying period.

## Chapter 16

# Cow Dung

Fresh cow dung

Cow dung being dried for fuel in India.

Water buffalo dung drying on the wall of a house, Yuanyang County, Yunnan

**Cow dung** is the waste of bovine animal species. These species include domestic cattle ("cows"), bison ("buffalo"), yak and water buffalo. Cow dung is the undigested residue of plant matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. Colour ranges from greenish to blackish, often darkening in colour soon after exposure to air.

## *Uses*

Cow dung (usually combined with soiled bedding and urine) is often used as manure (agricultural fertilizer). If not recycled into the soil by species such as earthworms and dung beetles, cow dung can dry out and remain on the pasture, creating an area of grazing land which is unpalatable to livestock.

In many parts of the developing world, caked and dried cow dung is used as fuel.

Dung may also be collected and used to produce biogas to generate electricity and heat. The gas is rich in methane and is used in rural areas of India/Pakistan and elsewhere to provide a renewable and stable source of electricity.

Cow dung is also used to line the floor and walls of buildings owing to its insect repellent properties for some types of insects (not flies or dung beetles). In central Africa, Maasai villages have burned cow dung inside to repel mosquitos. In cold places, cow dung is used to line the walls of rustic houses as a cheap thermal insulator.

Cow dung is also an optional ingredient in the manufacture of adobe mud brick housing depending on the availability of materials at hand.

A deposit of cow dung is referred to in American English as a "cow pie", and in British English as a cowpat. Also known as "cow chips" when dry, it is used in the practice of "cow chip throwing" popularized in Beaver, Oklahoma in 1970 . Another game is Cow Chip Bingo.

## *Ecology*

Cow dung provides food for a wide range of animal and fungus species, which break it down and recycle it into the food chain and into the soil.

In areas where cattle (or other mammals with similar dung) are not native, there are often also no native species which can break down their dung, and this can lead to infestations of pests such as flies and parasitic worms. In Australia, dung beetles from elsewhere have been introduced to help recycle the cattle dung back into the soil.

## *Variants*

A *buffalo chip*, also called a *meadow muffin*, is the name for a large, flat, dried piece of dung deposited by the American Bison from the large amount of grass that it eats. Well

dried buffalo chips were among the few things that could be collected and burned on the prairie and were used by the Plains Indians, settlers and pioneers, and homesteaders as a source of cooking heat and warmth.

Bison dung is sometimes referred to by the name *nik-nik*. This word is a borrowing from the Sioux language (which probably originally borrowed it from a northern source). In modern Sioux, *nik-nik* can refer to the feces of any bovine, including domestic cattle. It has also come to be used, especially in Lakota, to refer to lies or broken promises (especially by the U.S. government). It probably attained this sense by association with the English term "bullshit".

# Chapter 17

# Feces

Horse feces

**Feces**, **faeces**, or **fæces** is a waste product from an animal's digestive tract expelled through the anus (or cloaca) during *defecation*.

## *Etymology*

The word *faeces* is the plural of the Latin word *fæx* meaning "dregs". There is no singular form in the English language, making it a plurale tantum. There are many colloquial terms for feces, of which some are considered profanity (such as *shit*, *crap* and *turd*) while others (such as *poo*, *poop*, *number two*, *dookie* and *doody*) are not. Terms such as *dung*, *scat*, *spoor* and *droppings* are normally used to refer to animal feces.

*Stool* is a common term normally used in reference to human feces. For example, in medicine to diagnose the presence or absence of a medical condition, a stool sample is sometimes requested for testing purposes. The term "stool" can also be used for that of non-human species.

## *Ecology*

The Cassowary disperses plant seeds via its feces.

Earthworm feces aid in provision of minerals and plant nutrients in an accessible form

After an animal has digested eaten material, the remains of that material are expelled from its body as waste. Though it is lower in energy than the food it came from, feces may still contain a large amount of energy, often 50% of that of the original food. This means that of all food eaten, a significant amount of energy remains for the decomposers of ecosystems. Many organisms feed on feces, from bacteria to fungi to insects such as dung beetles, which can sense odors from long distances. Some may specialize in feces, while others may eat other foods as well. Feces serve not only as a basic food, but also a supplement to the usual diet of some animals. This is known as coprophagia, and occurs in various animal species such as young elephants eating their mother's feces to gain essential gut flora, or by other animals such as dogs, rabbits, and monkeys.

Feces are also important as a signal. Kestrels, for instance, are able to detect the feces of their prey (which reflect ultraviolet), allowing them to identify areas where there are large numbers of voles.

Seeds may also be found in feces. Animals which eat fruit are known as frugivores. The advantage for a plant in having fruit is that animals will eat the fruit and unknowingly disperse the seed in doing so. This mode of seed dispersal is highly successful, as seeds dispersed around the base of a plant are unlikely to succeed and are often subject to heavy predation. Provided the seed can withstand the pathway through the digestive system, it is not only likely to be far away from the parent plant, but is even provided with its own fertilizer.

Organisms which subsist on dead organic matter or *detritus* are known as detritivores, and play an important role in ecosystems by recycling organic matter back into a simpler form which plants and other autotrophs may once again absorb. This cycling of matter is known as the biogeochemical cycle. To maintain nutrients in soil it is therefore important that feces return to the area from which they came, which is not always the case in human society where food may be transported from rural areas to urban populations and then feces disposed of into a river or sea.

## *Human feces*

In humans, defecation may occur (depending on the individual and the circumstances) from once every two or three days to several times a day. Extensive hardening of the feces may cause prolonged interruption in the routine and is called constipation.

Human fecal matter varies significantly in appearance, depending on diet and health. Normally it is semisolid, with a mucus coating. Its brown coloration comes from a combination of bile and bilirubin, which comes from dead red blood cells.

In newborn babies, fecal matter is initially yellow/green after the meconium. This coloration comes from the presence of bile alone. In time, as the body starts expelling bilirubin from dead red blood cells, it acquires its familiar brown appearance, unless the baby is breast feeding, in which case it remains soft, pale yellowish, and not completely malodorous until the baby begins to eat significant amounts of other food.

Throughout the life of an ordinary human, one may experience many types of feces. A "green" stool is from rapid transit of feces through the intestines (or the consumption of certain blue or green food dyes in quantity), and "clay-like" appearance to the feces is the result of a lack of bilirubin.

Bile overload is very rare, and not a health threat. Problems as simple as serious diarrhea can cause blood in one's stool. Black stools caused by blood usually indicate a problem in the intestines (the black is digested blood), whereas red streaks of blood in stool are usually caused by bleeding in the rectum or anus.

Food may sometimes make an appearance in the feces. Common undigested foods found in human feces are seeds, nuts, corn and beans, mainly because of their high dietary fiber content. Beets may turn feces different hues of red. Artificial food coloring in some processed foods such as highly colorful packaged breakfast cereals can also cause unusual feces coloring if eaten in sufficient quantities.

Laboratory examination of feces, usually termed as stool examination or stool test, is done for the sake of diagnosis, for example, to detect presence of parasites such as pinworms and/or their eggs (ova) or to detect disease spreading bacteria.

## Personal hygiene

All cultures practice some form of personal cleansing after expelling feces.

- In Western and East Asian society, the use of toilet paper is widespread.
  - Other paper products were also historically used (before the advent of flush toilets).
  - Several companies market toilet tissue or wipes for babies and campers.
  - In some European countries, the use of a bidet for additional cleaning is common.
- In South Asia and Southeast Asia, showers are provided for use in toilets.
- In Islam, washing is prescribed by ritual cleansing with water, of which washing of the anus is part of the ablutions.
- In India, the anus is also washed with water using the left hand. As with all such practices, hand washing after use of the toilet has become a very important public health issue.
- In the United Kingdom, the Indian toilet was adapted as the WC or water closet and widely deployed in England during the reign of Queen Victoria. London was the stage for several instances of food poisoning resulting from workers handling food after using the toilet. Cleansing of the anus was an arbitrary practice left to personal choice and facility available.
- In Ancient Rome, a communal sponge was used, which was then rinsed in a bucket of salt water.
- In Japan, flat sticks were used in ancient times, being replaced by toilet paper as the country became more Westernized. Toilets that include built-in bidets have now become widely popular in private homes; these can be very sophisticated appliances, allowing users to adjust the temperature, direction and force of water jets, and offering warm air to dry the anus and surrounding regions. The toilet automatically flushes when the buttocks leave the seat.

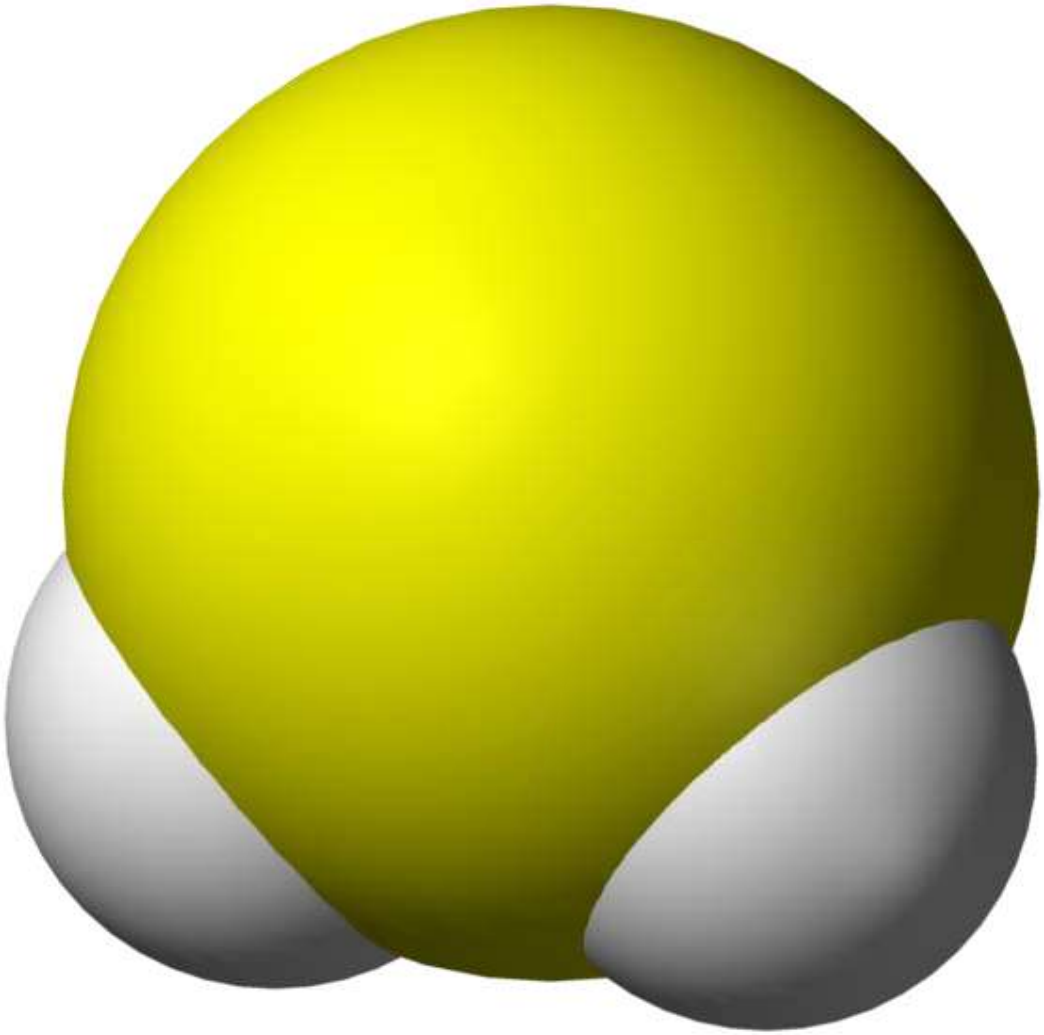
## Bristol Stool Scale

Consistency and shape of stools may be classified medically according to the Bristol Stool Scale.

Pica, a disorder where non-food items are eaten, can cause unusual stool.

Intestinal parasites and their ova (eggs) can sometimes be visible to the naked eye.

## *Odor*



The molecule hydrogen sulfide contributes to the smell of feces.

The distinctive odor of feces is due to bacterial action. Gut flora produce compounds such as indole, skatole, and thiols (sulfur-containing compounds), as well as the inorganic gas hydrogen sulfide. These are the same compounds that are responsible for the odor of flatulence. Consumption of foods with spices may result in the spices being undigested and adding to the odor of feces. The perceived bad odor of feces has been hypothesized to be a deterrent for humans, as consumption or touching it may result in sickness or infection. Of course, human perception of the odor is a subjective matter; an animal that eats feces may be attracted to its odor.

## Pets

Pets can be trained to use litter boxes or wait to be allowed outside and defecate there. Training can be done in several ways, especially dependent on species. An example is crate training for dogs. Several companies market carpet cleaning products aimed at pet owners.

## Uses

Human feces may be used as fertilizer in the form of biosolids (treated sewage sludge). The feces of animals are often used as fertilizer. Some animal feces, especially those of camel, bison and cattle, is used as fuel when dried out. Animal dung, besides being used as fuel, is occasionally used as a cement to make adobe mudbrick huts or even in throwing sports such as cow pat throwing or camel dung throwing contests. Kopi Luwak or Civet coffee, is coffee made from coffee berries which have been eaten by and passed through the digestive tract of the Asian Palm Civet (Paradoxurus hermaphroditus).

Dog feces were used in the tanning process of leather during the Victorian era. Collected dog feces were mixed with water to form a substance known as "bate". Enzymes in the dog feces helped to relax the fibrous structure of the hide prior to the final stages of tanning.

## Animal feces

Fresh bear scat showing a diet of apples

The feces of animals often have special names. For example:

- **Non-human animals** generally –
  - As bulk material – dung
  - Individually – droppings
- **Cattle** –
  - Bulk material – cow dung
  - Individual droppings – cow pats, meadow muffins etc.
- **Deer** (and formerly other quarry animals) – fewmets.
- **Wild carnivores** – scat.
- **Otter** – spraint.
- **Birds** (individual) – droppings (also include urine as white crystals of uric acid).
- **Seabirds** or **bats** (large accumulations) – guano.
- **Herbivorous insects**, such as caterpillars and leaf beetles – frass.
- **Earthworms**, **lugworms** etc. – worm casts (feces extruded at ground surface).
- **Feces when used as fertilizer** (usually mixed with animal bedding and urine) – manure.

Chapter 18

# Kleptothermy, Heterothermy and Ectotherm

## Kleptothermy

Neonatal lab rats huddling for warmth.

**Kleptothermy** is any form of thermoregulation by which an animal shares in the metabolic thermogenesis of another animal. It may or may not be reciprocal, and occurs in both endotherms and ectotherms. Its most common form is huddling.

### *Huddling*

Male Canadian garter snakes huddle around a female after hibernation when mating.

Some species of ectotherms including lizards and snakes, such as boa constrictors and Tiger snakes, increase their effective mass by clustering tightly together. This allows the individuals to increase their thermal inertia (as with gigantothermy) and so reduce heat loss. It is also widespread amongst gregarious endotherms such as bats and birds (such as the mousebird and emperor penguin) where it allows the sharing of body heat (particularly amongst juveniles).

In at least one case this is not reciprocal, and might be accurately described as *heat-stealing*. Some male Canadian red sided garter snakes engage in female mimicry by producing fake pheromones after emerging from hibernation. This causes rival males to cover them in a mistaken attempt to mate, and so transfer heat to them. This allows those males that mimic females to become more quickly revitalized after hibernation (which depends upon raising their body temperature), giving them an advantage in their own attempts to mate.

### *Habitat sharing*

Many ectotherms exploit the heat produced by endotherms by sharing their nests and burrows. For example, mammal burrows are used by geckos and seabird burrows by Australian tiger snakes and New Zealand tuatara Termites create in their mounds high and regulated temperatures and this is exploited by some species of lizards, snakes and crocodiles.

Research has shown such kleptothermy can be advantageous: the Blue-lipped sea krait, when it occupies a burrow of a pair of Wedge-tailed Shearwater incubating their chick, raises its body temperature to 37.5 °C (99.5 °F) compared to 31.7 °C (89.1 °F) when in other habitats. Its body temperature is also more stable. Burrows without birds did not provide this heat being only 28 °C (82 °F).

# Heterothermy

**Heterothermy** (from Greek: *heteros* = "other" *thermē* = "heat.") is a physiological term for animals that exhibit characteristics of both poikilothermy and homeothermy.

### *Temporal heterothermy*

Temporal heterothermy refers to animals that are poikilothermic or homeothermic for a portion of the day, or year. More often than not, it is used as a way to dissociate the fluctuating metabolic rates seen in some small mammals and birds (e.g. bats and hummingbirds), from those of traditional cold blooded animals. In many bat species, body temperature and metabolic rate are elevated only during activity. When at rest, these animals reduce their metabolisms drastically, which results in their body temperature dropping to that of the surrounding environment. This makes them homeothermic when active, and poikilothermic when at rest.

### *Regional heterothermy*

Regional heterothermy describes organisms that are able to maintain different temperature "zones" in different regions of the body. This usually occurs in the limbs, and is made possible through the use of counter-current heat exchangers, such as the rete mirabile found in tuna and certain birds. These exchangers equalise the temperature between hot arterial blood going out to the extremities and cold venous blood coming back, thus reducing heat loss. Penguins and many arctic birds use these exchangers to keep their feet at roughly the same temperature as the surrounding ice. This keeps the birds from getting stuck on an ice sheet. Other animals, like the Leatherback Sea Turtle, use the heat exchangers to gather, and retain heat generated by their muscular flippers. There are even some insects which possess this mechanism, the best-known example being bumblebees, which exhibit counter-current heat exchange at the point of constriction between the mesosoma ("thorax") and metasoma ("abdomen"); heat is

retained in the thorax and lost from the abdomen. Using a very similar mechanism, the internal temperature of a honeybee's thorax can exceed 45°C while in flight.

# Ectotherm

*Pseudemys* turtles (shown here basking for warmth) are ectothermic.

An **ectotherm**, from the Greek *εκτός* (*ektós*) "outside" and *θερμός* (*thermós*) "hot", refers to organisms that control body temperature through external means. As a result, organisms are dependent on environmental heat sources and have relatively low metabolic rates. For example, many reptiles regulate their body temperature by basking in the sun. The opposite of ectothermy is endothermy, where heat is primarily generated as a result of internal metabolic processes. Many ectotherms are also poikilotherms, meaning their temperature varies over a wider range than homeotherms.

Ectotherms are animals that warm their bodies by absorbing heat from their surroundings. In most ectotherms, the body temperature fluctuates with changes in the surrounding temperature; these ectotherms are called poikilotherms. The body temperature of snakes, for example, cools in cold weather and warms up in hot weather. Most marine fish and invertebrates, however, live in water that stays the same temperature. Their body

temperature, therefore, does not change, and these ectotherms are therefore considered homeotherms.

## *Adaptations*

Certain ectotherm behaviors help regulate body temperature. To warm up, reptiles find sunny places, and stretch out for maximum exposure. If it gets too warm, lizards alternate between sun and shade. Amphibians warm up by moving into the sun or diving into warm water. They cool off by entering the shade. In cold weather, honey bees huddle together to retain heat. Butterflies and moths may orient their wings to maximize exposure to solar radiation in order to build up heat before takeoff. Many flying insects, such as honey bees and bumble bees, also raise their internal temperatures endothermically prior to flight, by contracting their flight muscles without moving their wings.

In addition to behaviors, physiological adaptations help ectotherms regulate temperature. Diving reptiles conserve heat because their blood circulates inward toward the body core during a dive. The skin of bullfrogs secretes more mucus when it is hot, allowing more cooling by evaporation. Many ectotherms exist at a lower temperature during torpor, a state of slowed metabolism. This helps them survive a food shortage. If the food supply increases, they come out of torpor in a few hours.

## *Advantages and Disadvantages*

Tropical ectotherms may be particularly vulnerable to climate warming and are experiencing large increases in metabolic rate and will have an increased need for food.