Diagnostic pathology of selected diseases in wildlife

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Introduction

In its broadest sense ‘pathology’ means the ‘study of disease’. In this chapter, the term is equated with gross post-mortem examination and supporting laboratory tests, e.g. histopathology, haematology, cytology and serology. These investigations can help to provide a diagnosis and also to throw light on pathogenesis. It is important to note that some laboratory investigations can best or most easily be conducted on live animals (e.g. serology), others on dead animals (e.g. histology) and a few on either live or dead (e.g. parasitological examination of faeces or skin scrapings).

Pathological investigation is very important in the diagnosis and investigation of wild animal diseases. This investigation may, on occasion, be coupled with clinical observations/examinations and often has to be correlated with epizootiological data, requiring careful analysis and cautious interpretation.

Although, ideally, post-mortem examination and other investigations should be conducted by a specialist wildlife veterinary pathologist, especially if there is a ‘forensic’ component (13), often this is not possible, especially in remote locations. For that reason, field veterinarians and biologists must be prepared to conduct at least a basic necropsy of the dead animal, they should also know which samples to take and how to pack and transport these for laboratory investigation.

Examples will be given in this chapter of the pathology of infectious diseases of wildlife that are of major animal health importance (bovine tuberculosis, Rift Valley fever and rabies), are currently topical (West Nile virus infection), or are recently emerging (transmissible spongiform encephalopathies, morbilliviruses). Particular emphasis is placed on diseases of wild mammals, but examples are also given of conditions that affect birds and amphibians. Fish are not covered, but it is important not to omit them and other taxa. The principles of pathological investigation are usually the same, regardless of

Summary

The prompt detection and effective management of infectious disease in wildlife rely greatly on field diagnosis. Although clinical work is sometimes of value, the cornerstone of diagnosis is pathological examination (gross necropsy with supporting laboratory investigations).

The approach and rationale to gross post-mortem examination are common to all species, despite possible significant differences in technique. Likewise, the principles of sampling are usually comparable, with emphasis on standardisation, the correct use of equipment, and consistency in methods of storage and transportation of specimens. However, the type of sample taken and the laboratory tests required differ, depending upon the circumstances and possible diagnosis. Retention of material is always important.

The principles of diagnostic pathology are discussed, with reference to selected diseases, namely: mycobacteriosis, Rift Valley fever, rabies, spongiform encephalopathies, morbillivirus and poxvirus infections, viral encephalitides, West Nile virus infection and chytridiomycosis. The importance of being able to perform certain investigations in the field, efficiently and safely, is emphasised.

Keywords

whether the affected species are endothermic mammals/birds or ectothermic reptiles/amphibians/fish/invertebrates.

**Post-mortem examination (necropsy)**

Post-mortem examination is important because it can lead to the detection of gross lesions (permitting a diagnosis), or of abnormalities that may point to the presence of clinical or subclinical disease. A standard post-mortem technique is always desirable, with a full range of supporting tests (31). Consistency of method is vital.

During a post-mortem examination or handling of samples, whether in the field or in the laboratory, the risk of spread of pathogens must be considered. It is important to protect staff and local people from zoonoses and to ensure that other infectious agents are not disseminated where they may be a cause of further infection in wildlife or domestic animals (Fig. 1). Hygiene and standard operating procedures are vital: a code of practice for field necropsies is recommended. Routine disinfection is usually adequate but the resistance to chemical and physical agents of certain organisms, such as mycobacteria, or of the prions that appear to be the cause of spongiform encephalopathies, means that special precautions may need to be followed where such diseases are suspected.

Post-mortem techniques will not be discussed in detail here. The reader is advised to consult the above publications and other literature on this topic.

**Other diagnostic tests**

Many and varied diagnostic tests exist. The value of these tests will depend upon the disease that is suspected and the circumstances of examination; for example, when carrying out a post-mortem examination in the field, some tests are impracticable because of lack of facilities or likely delay in getting samples to the laboratory.

The use of field kits has much to commend it and the construction and use of these have been discussed by a number of authors (12, 17). These kits can be used to transport reagents and equipment to the field so that tests can be performed *in situ*. In addition, the development in recent years of many micro-techniques for the detection of antigens, antibodies or specific substances (e.g. enzymes) means that more and more can be
done outside the laboratory (Fig. 3). Nevertheless, the results obtained in such cases still depend upon the quality of the sample and therefore protocols need to be followed.

Important laboratory tests in the diagnosis of wildlife disease include the following:

- histopathology
- microbiology: bacteriology, mycoplasmology, mycology, virology
- serology
- histochemistry and immunocytochemistry
- other tests (e.g. electron microscopy, cytology, etc.).

The importance of following standard procedures has already been mentioned. Consistency of method is likely to produce consistency of results and this is vital when investigating an epizootic or attempting to establish a profile of the health status of a wild animal population.

Sampling is an example of where errors can arise. A sample, such as a specimen of faeces, needs to be selected, collected, packaged, transported and processed. If, at any stage during this chain of events, a mistake is made or a delay occurs, the results may be unreliable. Samples will yield erroneous information if they are incorrectly handled or if specimens are treated in different ways (Fig. 4). For example, the use of transport medium for bacteriological samples has much to commend it. However, the use of such media should be consistent and the same medium, stored in the same way, should be used each time. Even simple points, such as whether specimens for toxicology are placed in glass or plastic containers, or whether specimens for electron microscopy are fixed in formaldehyde or glutaraldehyde, can influence the results obtained. The key to all of this is to follow protocols; where these already exist, for example those recommended by the Office International des Epizooties (OIE: World organisation for animal health) for diagnostic samples (26), these should be used. Where there are no such protocols, the investigator should develop his/her own. These should be rehearsed, put in writing and refined as necessary. They are a key part of the ‘Materials and Methods’ of any investigation.

The role of pathological examination

The purpose of post-mortem and laboratory examination is primarily to diagnose infectious or non-infectious disease, particularly that of importance in terms of morbidity or mortality to the species itself, to other wildlife, to domestic livestock or to humans. This, however, is only the tip of the iceberg. Pathological examination can also diagnose subclinical disease, detect underlying pathological lesions which may be affecting survival, reproductive success or other parameters in wildlife, and help in developing reference data on, for example, the normal appearance, weight (and weight ratios) of organs, and permit the establishment of reference collections of tissues and organs which can be used subsequently or made available to other research workers.

Wild animals can serve as important sentinels of environmental infection and pollution but their true value is only apparent when reliable reference material is available. The value in wildlife work of post-mortem and laboratory investigation cannot be over-estimated. The vast majority of 'new' diseases of wildlife are diagnosed initially post mortem and by thorough
examination, with correct collection of samples. These are a vital part in the early recognition of these and other conditions. It is important to retain records, including photographs and notes, because these may be required at a later date. It is also imperative to retain pathological material so that it can be used for subsequent study. When dealing with threatened species, the establishment of a reference collection should be considered. Such collections usually comprise fixed and frozen tissues, blood smears, sera, paraffin blocks, freeze-dried bacterial isolates and other material which are catalogued and stored for future use. The importance of such reference collections has been stressed by several authors in recent years (14).

Examples of diseases in wildlife

Mycobacteriosis

Mycobacteriosis (tuberculosis and related disease), caused by Mycobacterium spp. can affect all vertebrate species, ranging from primates to fish. Bovine tuberculosis is of great economic importance in domestic livestock. Human tuberculosis, often associated with immunodeficiency, has reached epidemic proportions in many parts of the world. Both forms of tuberculosis may affect wild mammals while avian tuberculosis is prevalent in free-living birds (and other non-avian species) as well as in domestic poultry. Bovine tuberculosis is present in wild ungulates in many countries and can spill over into other species, including carnivores: this is currently a major problem in several parts of Africa. In New Zealand the introduced Australian brush-tailed possum (Trichosurus vulpecula) is an important source of infection for domestic livestock. In Europe, especially the United Kingdom (UK), badgers (Meles meles) provide a similar wildlife reservoir for M. bovis and much research has been performed on their possible role in this respect (6).

Clinical signs vary greatly. Many cases are inapparent although subtle signs, such as loss of condition, may be seen.

The pathogenesis of tuberculosis has been studied in detail for over a century and it and other features of disease in domestic mammals are discussed in detail by Huchzermeyer and others in Coetzer et al. (7).

There is usually a primary lesion (focus) in tuberculosis. In ruminants, this is generally in the lung while in poultry and other birds it is in the intestinal tract. In ectothermic vertebrates (reptiles, amphibians and fish), skin lesions are often primary although all organ systems can be involved.

In mammals, lymphatic spread from the primary focus results in caseation of the draining lymph node: this combination of primary focus and node is termed the primary complex. Further dissemination occurs via lymphatics, blood and by direct spread, leading to the development of tubercles, consisting predominantly of histiocytes (Fig. 5). Some tubercles become walled-off by fibrous tissue but others continue to spread. Blood-borne spread can cause the formation of multiple secondary foci (so called miliary tuberculosis).

There are differences in pathogenesis according to species, especially amongst wildlife.

Diagnosis of mycobacteriosis in wild animals is rarely based on clinical findings. Post-mortem diagnosis is often the first indication that the disease is prevalent and in some cases this may follow a period during which animals have been dying or declining in numbers (as happened, for example, with the flamingos in Kenya in the 1970s) (10) (Fig. 6).
Individual wild animals that have to be handled can be tested for mycobacteriosis using a variety of techniques, most of which are not fully effective. Tuberculin testing may be used in mammals, and small, valuable animals can be radiographed. Molecular techniques are being used increasingly, for instance, the enzyme-linked immunosorbent assay (ELISA) tests on the blood or polymerase chain reaction (PCR) on faeces or tissues.

**Rift Valley fever**

Rift Valley fever (RVF) is caused by a Phlebovirus and is transmitted by mosquitoes. Originally described from Kenya a century ago, it has subsequently been reported from many parts of Africa. There was a major epizootic in East Africa in 1997 and 1998 that killed thousands of sheep, goats and camels and more than 90,000 human cases were recorded. The virus is associated with the rains and with wet conditions, such as those induced by the building of dams or the production of rice fields, which favour the vectors.

Rift Valley fever primarily affects ruminants but rodents of different species are highly susceptible; humans and other primates are moderately susceptible. According to Swanepoel and Coetzer quoted in Coetzer *et al.* (7), birds, reptiles and amphibians are refractory. The epizootiology of the disease remains unclear and is the subject of debate. It is possible that the virus is maintained in *Aedes* mosquitoes, in which transovarial transmissions are possible.

Clinical signs of RVF depend upon the strain of virus and the susceptibility of the affected host. Typical observations during an epizootic are pyrexia, nervous signs and abortion in ruminants, with associated disease in humans.

The main pathological lesions are hepatic necrosis with haemorrhages. The liver often has a characteristic yellow colour. Other gross lesions can include haemorrhagic pleural effusion, splenomegaly, enteritis and disseminated haemorrhages at many sites. Histological examination reveals coagulative necrosis of hepatocytes, which may show intranuclear inclusion bodies, but the latter are variable, depending upon age and species of host.

A tentative diagnosis of RVF may be based on haematological findings of leucopenia, raised liver enzyme values and changes consistent with disseminated intravascular coagulopathy (DIC). Diagnosis is confirmed by histology, isolation of virus (using tissue culture or mice), detection of virus antigen in impression smears by immunofluorescence or serology (paired serum samples, using a variety of tests).

The principal differential diagnosis of RVF in domestic animals is Wesselsbron disease but the two can be distinguished on the basis of pathology and by serological and virological studies.

**Rabies**

Rabies, caused by a Rhabdovirus, has been recognised for centuries. It affects endothermic vertebrates — probably all species of mammal and occasionally birds. Lyssa viruses (genus *Lyssavirus*) have attracted increased interest in recent years. The detailed classification of these viruses is subject to debate as at least six species and numerous viral variants exist (29). Lyssa viruses are found in a number of species of bat and can, on occasion, be transmitted to other species. Such spread has jeopardised the ‘rabies-free’ status of some countries such as Australia.

The clinical features of rabies in wildlife are discussed, with extensive references, by Swanepoel in Coetzer *et al.* (7). The disease occurs throughout the world other than in Antarctica and certain other areas, especially islands, from which it has been eradicated.

Gross pathological findings in rabies are minimal: those that are seen are usually secondary to the neurological and behavioural signs shown by affected animals. The virus attacks the central nervous system (CNS) and it is here that characteristic lesions are seen (intracytoplasmic inclusion [Negri] bodies and varying degrees of meningeal congestion, gliosis, perivascular mononuclear ‘cuffing’ and neuronal degeneration) (Fig. 7).

**Fig. 7**

Histological section of a positive case of rabies

Note the intracytoplasmic Negri body

Photo: courtesy of M.E. Cooper
Non-specific lesions, secondary to clinical signs, may include wounds (some self-inflicted), worn pads or hooves and gastric foreign bodies. Animals may be emaciated. Diagnosis of rabies can sometimes be made provisionally on clinical signs but usually laboratory tests such as histology, immunofluorescence, virus isolation or PCR are required for confirmation. Post-mortem examination and removal of organs must be undertaken with due care for personal safety.

Histological examination must be thorough and performed by an experienced pathologist. Recognition of Negri bodies is generally not difficult but they can be variable in shape and size and in felids must be distinguished from lyssa bodies, which are small and eosinophilic. When brain material is not available, or too damaged or autolysed for full interpretation, Negri bodies may be found in certain other organs such as salivary glands, retina and adrenal. Ganglioneuritis may also be apparent.

Vacuolation resembling that seen in the spongiform encephalopathies is sometimes also a feature of rabies.

Although histological examination of brain and other tissues is an important aspect of diagnosis, it can be time-consuming and is only 65%-90% reliable. Touch preparations (impression smears) are no more accurate but provide a rapid technique that can be employed in the field.

Immunofluorescent techniques on brain are generally to be preferred because of their sensitivity and their applicability even to autolysed material. Mouse inoculation was the method of choice for a long time, but immunofluorescence is now used more frequently.

Other methods of diagnosis of rabies include the following:

a) virus isolation in tissue culture
b) reverse transcription and PCR with subsequent nucleotide sequencing or endonuclease restriction mapping
c) use of monoclonal antibodies and indirect immunofluorescence tests.

Serological tests include complement fixation, haemagglutination-inhibition, serum neutralisation, radio-immunoassay and ELISA.

An entire range of conditions is included in the differential diagnoses of rabies. Most are diseases that produce neurological signs, such as canine distemper, cerebral babesiosis, tetanus and various forms of poisoning.

**Spongiform encephalopathies**

Spongiform encephalopathies have assumed increasing importance in recent years. One such disease, scrapie, has been recognised in sheep for centuries and others, such as chronic wasting disease of deer (38, 39) have been the subject of study for decades (37). However, it was the discovery in the UK in 1986 of a new disease of cattle, bovine spongiform encephalopathy (BSE), that resulted in world-wide interest in such conditions (4) (Fig. 8).

![Fig. 8 An early case of scrapie in a sheep exported to Kenya from the United Kingdom](photo)

The animal was severely pruritic and there was localised loss of wool on the rump. Diagnosis was based on histopathological examination of brain

Photo: courtesy of M.E. Cooper

The cause of spongiform encephalopathies appears to be prions (modified host protein molecules, not associated with detectable nucleic acid, that are transmissible).

The pathogenesis of some spongiform encephalopathies has been studied extensively in recent years but much remains to be learned. In mouse models, different patterns are seen, corresponding with 'species' and strains (5, 16). Naturally occurring BSE and feline spongiform encephalopathy (FSE) produce a similar picture (35, 36) characterised by spongiform change, astrocytosis, neuronal loss and the formation of amyloid plaques.

The significance of spongiform encephalopathies in free-living wildlife is largely unknown. Scrapie-like disease has been reported in over twenty captive wild mammals of ten species in zoological gardens in the UK (22, 23). These cases are believed to have contracted the disease either from foodstuffs that contained BSE-contaminated (ruminant-derived) protein or, in the case of the felids (cheetah [*Acinonyx jubatus*], puma [*Felis concolor*] and others), as a result of eating tissue from infected cattle. A spongiform encephalopathy reported from Germany in ostriches (*Struthio camelus*) (30) may or may not be a related disease but it serves as an important warning to wildlife veterinarians and biologists that prion diseases could possibly become widespread in birds as well as mammals. The spread of prions by (or even infection by prions of) scavenging birds, such as vultures, has been postulated and has prompted a ban of the use of ruminant carcasses to feed rehabilitated vultures in Europe (9).
Morbilivirus infections

Members of the genus Morbillivirus (family Paramyxoviridae) cause a number of diseases of mammals, including measles (in humans and other primates), canine distemper, rinderpest, peste des petits ruminants and a variety of conditions of aquatic species.

Morbilivirus infections of aquatic mammals (pinnipeds and cetaceans) have caused epizootics in various parts of the world but were unrecognised in such species prior to 1987 (19). One of the best documented outbreaks of morbillivirus disease amongst pinnipeds was the epizootic in 1988 which killed nearly twenty thousand seals in north-west Europe (18). That outbreak finished in 1989 although there were some subsequent cases. The implication of morbillivirus infection was as a result of the detection of inclusion bodies, morbillivirus antigen in tissues and antibodies to canine distemper. Subsequently a morbillivirus was isolated.

Kennedy provides a useful table of aquatic mammals in which ‘clinical distemper’ has been reported and another, more extensive one, of serological evidence of morbillivirus in aquatic animals (18). The latter includes several species of seal, dolphin, whale and porpoise, as well as polar bear (Ursus maritimus).

Transmission of morbillivirus of marine species is assumed to be by a range of routes (respiratory, faecal, urinary and possibly ocular). The virus is believed to be excreted from all body orifices and possibly through the skin. It has been postulated that some animals may be carriers and a prolonged viraemia may be significant in transmission. The pathology of these morbillivirus infections is still not fully understood. Haematological changes have been reported by some authors but in most cases such studies have not been performed. Natural infection with morbillivirus in seals usually produces pneumonia and other respiratory lesions. There may also be lymph node enlargement. In other species, a range of pathological features have been described ranging from stomatitis to skin and cerebral lesions. Histopathological features may be found in the respiratory tract, CNS and in epithelia. The pulmonary lesions are those of an interstitial pneumonia with associated changes. Inclusion bodies are found in the cytoplasm and nuclei of the respiratory tract and there may also be syncytia. Inclusions may also be seen in other organs, including the bladder, kidney and lymphoid tissues. Morbilliviruses that are neurovirulent can produce lesions of demyelination and other changes in the brain.

The diagnosis of morbillivirus is not usually possible based on clinical signs alone. Cytological examination of touch preparations or smears of secretions may permit the detection of inclusions in cells. At post-mortem examination, lymphoid depletion is seen, together with a range of changes in the respiratory and other tracts. The main diagnostic feature is the presence of acidophilic inclusion bodies in various organs. The disease can be confirmed by immunoperoxidase or immunofluorescence labelling of antigen in sections. Other techniques have also been used, such as the ELISA, as have PCR and other molecular tests. The isolation of morbilliviruses is possible using cell lines from seals and sometimes from other species. Serological tests, such as virus neutralisation and ELISA, will detect changes in titre but cross-reaction amongst morbilliviruses makes this non-specific. In such cases, the use of monoclonal antibodies is preferred.

Diagnosis of morbillivirus infections is based largely upon laboratory tests. These include serology and the detection of virus – either by isolation or by the reverse transcriptase-polymerase chain reaction.

Newcastle disease and other paramyxovirus infections

The family Paramyxoviridae is a large one and, under current taxonomical principles, includes at least six separate genera. Those that affect birds are now considered to be members of the genus Rubulavirus, which includes the virus that causes mumps in primates. Newcastle disease is due to avian paramyxovirus (PMV) (1).

A paramyxovirus of fruit bats or ‘flying foxes’ (Pteropus spp.) was recognised less than ten years ago (34) and is now designated Hendra virus. The virus can kill horses and humans. Another species, Menangle virus, was recently detected serologically in fruit bats (27) and is believed to be involved in disease in domestic pigs and humans.

Newcastle disease is well recognised in domestic poultry and certain other species. It can be associated with a range of clinical signs and pathological lesions, depending upon the strain of virus involved. The alimentary, respiratory and nervous systems are particularly affected. Other factors that may influence morbidity and mortality include the age of the bird, its immune status, route of exposure and external factors such as stressors and temperature. Pigeons can either be infected with traditional strains of Newcastle disease or with PMV-1 variant. The former cause conjunctivitis, diarrhoea and nervous signs; the latter can be asymptomatic. Wild birds show a range of clinical signs when infected with Newcastle disease (Fig. 9).

The pathology of Newcastle disease will depend upon the strain and the duration of the disease. Peracute cases will show relatively few gross lesions other than perhaps, indications of a septicaemia and dehydration. More chronic cases will show haemorrhage and necrosis of the intestine and a range of respiratory lesions such as tracheitis and pneumonia. Gross lesions of the CNS are often not apparent. From time to time, lesions of the skin and eyes may be seen.
The histopathology shows gastrointestinal lesions including necrosis and haemorrhage. The respiratory system is characterised by necrotic and haemorrhagic tracheal lesions and other changes. Histopathological lesions may also be found in the CNS (encephalomyelitis) and in a range of other organs. Diagnosis of the disease is based on a combination of epizootiology, clinical signs, gross post-mortem findings, histopathological findings and laboratory investigation. The latter include isolation of virus, detection of antigen and serology (haemagglutination inhibition) and other tests.

Poxviruses are epitheliotropic. There is initial local multiplication followed in some cases by spread, via the lymph nodes, to the bloodstream (a primary viraemia which in turn causes multiplication in body organs followed by a secondary viraemia).

The epidermal changes are similar in all mammalian species and a little different in birds and reptiles. A macule develops into a papule: these are characterised by swelling and vacuolation of cells in the stratum spinosum and elsewhere. Vesiculation follows (an accumulation of tissue fluid). When vesicles rupture, they form pustules, characterised by the presence of hyperplastic epithelial cells and mixed inflammation. A crust may form as the final stage (Fig. 10).

Poxvirus infections

Members of the family Poxviridae cause disease in vertebrates (Chordopoxvirinae) and invertebrates (Entomopoxvirinae). Many new poxviruses have been recognised in recent years. Amongst species of importance in free-living mammals are the following:

- cowpox (large felids, elephants, okapi [Okapia johnstoni] and rhinos as well as bovids and humans)
- monkeypox (primates and certain other mammals)
- seal poxvirus
- lumpy skin disease (cattle and buffalo [Syncerus caffer])
- myxoma virus (rabbits [Oryctolagus cuniculus]).

Relatively little is known about poxviruses in free-living mammals (28). Birds of many species can be affected by the genus Avipoxvirus and reptiles, including some of economic importance, are subject to their own poxvirus infections.

Poxviruses usually infect their hosts by aerosol, by entry into skin wounds and by the bites of arthropod vectors. Pox diseases are often spread in different ways.

Other changes can include vasculitis, especially in lumpy skin disease, and localised necrosis. Internal pox infections show many of the changes above but there are species and virus-associated differences. The lesions of camel pox were studied and described in detail by Kinne et al. (21).

Diagnosis of poxvirus infections is based on a combination of clinical signs and laboratory tests. The latter can range from simple, inexpensive techniques, such as the examination of impression smears of lesions, through histology and electron microscopy (including direct negative straining of material) to virus isolation and a variety of molecular investigations.

Viral encephalitides

Most of the viral encephalitides are caused by members of the Togaviridae family. Those viruses usually occur in habitats where vertebrate hosts and vector mosquitoes co-exist. Features of their interaction can include overwintering of virus and its amplification. The genus Alphavirus causes disease in horses, birds and humans.
The best known and most significant viral encephalitides are Eastern, Western and Venezuelan equine encephalitis (EEE, WEE and VEE, respectively) which are a particularly important cause of disease in horses and humans in America. However, over twenty other members of the genus Alphavirus occur in other parts of the world, such as Chikungunya in Africa. Such viruses can be isolated from wildlife. Antibodies may be detected but the significance of the infections remains unclear.

The epizootiology of EEE, WEE and VEE is complex and is discussed in detail by Thomson in Coetzer et al. (7). Mosquitoes transmit the viruses but a range of hosts may then be involved, including birds (3), reptiles (2) and possibly amphibians, depending upon the virus.

Clinical signs of Alphavirus infection vary considerably. In the majority of cases, infection is sub-clinical. The equine encephalitides may manifest themselves by pyrexia and by neurological signs, the latter being particularly marked in young animals (and children).

The pathological changes induced by alphaviruses are largely microscopical – oedema, neuronal degeneration, neurophagia and perivascular and interstitial, predominantly mononuclear, infiltration. Sometimes, as in VEE, there are visceral lesions, such as pancreatic necrosis.

Diagnosis is rarely possible on the basis of clinical signs. There are many differential diagnoses, including rabies and various toxocoses. Virus isolation is the prime diagnostic tool using tissue (cell) culture or mice. Serology is also of value: high IgM antibody titres, especially in cerebrospinal fluid, is pathognomonic. The diagnosis of EEE in birds is particularly difficult but has been facilitated recently by the development of an immunohistochemical technique (40).

**West Nile virus infection**

West Nile virus is a Flavivirus that is harboured by birds and transmitted by Culex mosquitoes. Primarily found in Africa, the Mediterranean region and areas of Asia, an outbreak of the virus in 1999 in the USA (32) drew attention to the potential for the virus to spread elsewhere. Since that occurrence in New York, West Nile virus has been detected in several States of the USA, and there are concerns that spread may extend to Central America. Human cases in the USA have been few but at least one person has died.

Clinical signs in humans include pyrexia and sometimes encephalitis. Atypical avian hosts may die, as was the case in the USA, and this can be the first indication that the virus is present. A range of pathological lesions may be seen in such cases. Diagnosis is based on virus isolation and/or evidence of a serological response.

West Nile serves as a reminder of the number of viruses that can, under certain circumstances, cause morbidity and mortality in wildlife and emphasises the role of the latter as environmental sentinels.

**Chytridiomycosis of amphibians**

In recent years, the decline throughout much of the world of many species of amphibian has attracted concern. Various factors have been implicated, both infectious and non-infectious, and there is little doubt that in some instances the causes are multifactorial. One group of agents that has attracted great attention is chytrid fungi (Chytridiomycota) and chytridiomycosis is now a recognised cause of population decline, sometimes death, in amphibians – mainly anurans (frogs and toads) – in parts of Australia, Europe and the USA (24).

Chytrid fungi are generally saprophytic organisms that flourish in aquatic and terrestrial habitats. In amphibians they can cause cutaneous lesions. Clinical signs vary: some animals are found dead but others show skin changes – thickening and discoloration, often excessive ecdysis (shedding). Sometimes there are systemic signs, such as anorexia and lethargy, and death is probably due to dehydration and electrolyte imbalance.

Diagnosis of chytridiomycosis is based primarily upon microscopical examination of skin samples – biopsies or scrapings from live amphibians, pieces of integument from dead animals. Ante-mortem diagnosis in dendrobatid (poison dart) frogs (Dendrobates azureus) has been reported to be practicable by examination of shed skin or skin imprints (25). The key to diagnosis is detection of typical chytrid thalli in superficial epidermis. Histological examination will usually show different forms of the fungus: the best stains for the purpose are periodic acid Schiff (PAS) and the Gomori stain. Associated with the fungi are epidermal degenerative changes and sometimes local inflammation. Transmission electron microscopy (TEM) can also be used to detect and identify the fungus.

It seems likely that chytridiomycosis will prove to be a threat to many more species of amphibians. Wildlife veterinarians and biologists must therefore be aware of its presence and the need for prompt investigation of unexplained deaths.

**Access to laboratories, databases and sources of information (including websites)**

The role of suitably experienced laboratories in the investigation of animal diseases has been discussed in the past by the OIE. Obtaining assistance with diagnosis is not always
easy, especially when the samples originate from wildlife; some laboratories claim little or no experience in this area and may be reluctant to examine such material.

Small countries and those with limited resources can find themselves in a particularly difficult position, especially when they need to send specimens from wildlife overseas. This can present problems for a number of reasons. Transporting samples to other countries is expensive and in many cases such material will require both health and CITES permits (CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora). Obtaining the latter can delay the sending and reception of urgent samples from threatened species (15).

It may also be difficult to know to which laboratories samples might be sent. Those needing to submit material from wildlife for examination are therefore advised to do the following:

1. To seek advice from the OIE with regard to specific reference laboratories for the disease investigations required.

2. To consult existing lists of laboratories and institutions that have expressed willingness to receive material from wild animals (for example, those listed for birds) (8).

3. To enquire of the national veterinary laboratory or government agricultural department in the country concerned.

4. To make use of the numerous organisations that exist which have an interest in wildlife and which, in some cases, have established a network of interested people, for example, the World Association of Wildlife Veterinarians (WAWV) and the Veterinary Specialist Group of the World Conservation Union (IUCN). Many of these can be contacted through websites – for those who have access to the internet – or in the columns of veterinary and other journals.

5. To obtain, at an early stage, information about legal controls, the permits that may be needed and the correct methods of packing and transportation for despatch.

6. To retain frozen, chilled and fixed tissues, whenever possible. These can be used for further investigations, or to provide additional material for extra tests.

Conclusion

Wildlife pathology is an increasingly important subject. Gross post-mortem examination and laboratory investigation of samples provides an opportunity for early diagnosis, for detection of new or emerging conditions and for research on the pathogenesis and epizootiology of infectious disease. However, for pathology to be of maximal value, it needs to be carried out systematically. The retention of material is always necessary so that alternative investigations can be pursued.

There is a need for more research in order to enhance the efficiency of pathological examination of wild animals. Improved field kits are required that can be transported easily and provide reliable in situ results. Methods of collection and transportation of some samples still need to be evaluated, standardised and agreed. Perhaps most important of all, databases and reference values must be established so that those working in this exciting area have access to information to which they can relate their own findings.

A hundred years ago the famous Canadian physician Sir William Osler told his students ‘As is your pathology, so is your practice’ – a reminder of the importance of sound pathological techniques to clinical diagnosis. The same maxim is true of investigative work with wildlife.

Acknowledgements

I am grateful to the OIE and to Dr R.G. Bengis for the invitation to write this chapter and to the colleagues who read and commented on an early draft. The manuscript was typed by Mrs P. Smith and my wife, Mrs M.E. Cooper, provided invaluable support.
Examens diagnostiques appliqués à certaines maladies de la faune sauvage

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Résumé
La détection rapide des maladies infectieuses de la faune sauvage et leur gestion efficace dépendent dans une large mesure du diagnostic sur le terrain. Si le diagnostic clinique est utile dans certains cas, l’examen anatomo-pathologique et le laboratoire restent la pierre angulaire du diagnostic (nécropsie macroscopique et analyses complémentaires en laboratoire). La méthode et les principes qui régissent l’examen post-mortem des lésions macroscopiques sont les mêmes pour toutes les espèces, seule la technique utilisée peut varier considérablement. De même, les prélèvements sont habituellement réalisés selon des principes identiques, l’accent étant mis sur la standardisation, l’utilisation correcte du matériel et les méthodes appropriées de conservation et de transport des prélèvements. Cependant, le type de prélèvement effectué et les tests de laboratoire nécessaires varient selon les circonstances et le diagnostic possible. Il est important dans tous les cas de conserver les organes et tissus qui font l’objet de prélèvements. La discussion porte sur les principes de l’examen anatomo-pathologique pour le diagnostic de certaines maladies particulières, à savoir : la mycobactériose, la fièvre de la Vallée du Rift, la rage, les encéphalopathies spongiformes, les infections à morbillivirus et poxvirus, les encéphalites virales, l’infection due au virus West Nile et la chytridiomycose. Les auteurs soulignent l’importance des enquêtes sur le terrain, dans des conditions d’efficacité et de sécurité.

Mots-clés

Estudios diagnósticos de ciertas enfermedades de la fauna salvaje

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Resumen
El diagnóstico sobre el terreno es un factor muy importante para detectar a tiempo y gestionar con eficacia las enfermedades infecciosas de la fauna salvaje. Aunque a veces el diagnóstico clínico también resulte útil, la piedra angular del trabajo de diagnóstico en la fauna salvaje es el estudio anatomo-patológico y las pruebas de laboratorio complementarias. Pese a eventuales y significativas diferencias de tipo técnico, los métodos y principios de la necropsia macroscópica no varían de una especie a otra. También suelen ser comparables los principios que se aplican al muestreo, entre los que cabe destacar la estandarización de las muestras, el uso correcto del material y la homogeneidad de los métodos de conservación y transporte de las muestras. Sin embargo, según las circunstancias y el diagnóstico que se anticipa, diferirán el tipo de muestra extraída y las pruebas de laboratorio que se le apliquen. En cualquier caso, siempre será importante conservar los órganos y tejidos utilizados para el muestreo.
El autor expone los principios de los exámenes anatomo-patológicos y los aplica a una serie de enfermedades: micobacteriosis, fiebre del Valle del Rift, rabia, encefalopatías esponjiformes, infecciones por morbillivirus y poxvirus, encefalitis víricas, infección por el virus West Nile y quitridiomicosis, para acabar recalculando la importancia de poder realizar con eficacia y seguridad una serie de análisis sobre el terreno.

**Palabras clave**

**References**


