**Zoonotic diseases in companion animals**

Companion animals have been important to humans since the first dog joined man, and the positive aspects of human-animal interaction are well known and have been thoroughly documented.

However, there is a concern relating to this close contact between people and their pets: companion animals carry the risk of transmitting diseases to humans. This group of diseases is termed zoonoses. Zoonotic diseases are diseases being common to, shared by or transmitted between human beings and other vertebrate animals.

How do we handle the challenge of zoonotic diseases while maintaining the positive sides of sharing household with companion animals? Not by diminishing the human-animal interaction, but by increasing the knowledge on the actual diseases. This includes knowledge on disease recognition and management as well as prophylactic measures.

Veterinarians need to be able to recognise zoonotic diseases and the effect of such diseases in animals primarily. But companion animal veterinarians also need knowledge on the effect of these diseases on humans, thus being able to work in cooperation with human doctors on disease control.

The second online issue of EJCAP, the official journal of FECAVA, is dedicated to the topic of zoonotic diseases. You will find a collection of articles written by outstanding European experts in their field. It is our goal that the information gained through these articles will help you in identifying and handle zoonoses in the best way possible.

Ellen Bjerkås
Scientific Editor of EJCAP
Companion animals live in close contact with the human population, and the risk of transmitting zoonotic diseases to humans is therefore significant if the animal itself has been infected. Increased travel activity increases the possibility of transfer of infection between animal populations, and increased risk for contact with new infectious agents. An increasing number of people suffer from immunodeficiencies. Environmental- and climatic conditions cause a change in distribution of vectors in need of special climatic conditions to establish. Exotic species are also to an increasing extent introduced as family pets, which may contribute to a wider panorama of infections. However, the traditional zoonotic diseases are still the most important. Vaccination, proper hygiene measurements and knowledge on preventive measures restrict the risk of transmittance of infections from companion animals. The most significant risk of companion animals in Norway are mostly related to dog and cat bites or other physical injuries. In total the benefit and pleasure of this type of animal husbandry is more important than the fear of zoonotic diseases.

**SUMMARY**

Companion animals live in close contact with the human population, and the risk of transmitting zoonotic diseases to humans is therefore significant if the animal itself has been infected. Increased travel activity increases the possibility of transfer of infection between animal populations, and increased risk for contact with new infectious agents. An increasing number of people suffer from immunodeficiencies. Environmental- and climatic conditions cause a change in distribution of vectors in need of special climatic conditions to establish. Exotic species are also to an increasing extent introduced as family pets, which may contribute to a wider panorama of infections. However, the traditional zoonotic diseases are still the most important. Vaccination, proper hygiene measurements and knowledge on preventive measures restrict the risk of transmittance of infections from companion animals. The most significant risk of companion animals in Norway are mostly related to dog and cat bites or other physical injuries. In total the benefit and pleasure of this type of animal husbandry is more important than the fear of zoonotic diseases.

**Introduction**

Companion animals are of great significance in today’s society. 37% of Norwegian households own a family pet [1]. Of these, 44% include dogs and 50% cats. Stray dogs are a non-existing problem in Norway. Cats are to a major extent kept as companions, although in the cities there may be colonies and stray cats.

In Norway, with a population of about 4.5 million inhabitants, there are about 42,000 horses, 216,000 rabbits, 135,000 captive birds and 11,000 reptiles kept as pets [1]. These animals normally live in close contact with humans, and, conditions permitting, there may be risk of transmittance of zoonotic agents.

The regulations for import of dogs and cats from the EU and EFTA-countries were subject to change in 1994, thus facilitating easier transport of animals between the European countries. Through this, the risk of animals attracting “new” infectious diseases and further transmittance to humans is increased. Infections may be transmitted from animals that suffer from illness, or the animals can be asymptomatic carriers. Agents dependent on a vector may be transferred indirectly from companion animals. Climatic changes cause a change in distribution of invertebrates that may act as vectors in transmitting diseases between species. One example is the cat flea (*Ctenocephalides felis*) that at present is slowly spreading from Denmark through Southern Sweden to Norway.

The use of medicines and increased prevalence of diseases causing immunodeficiency result in humans being more susceptible to infections, and they may develop more serious clinical symptoms if diseased.

Increased knowledge on epidemiology and infections reveal zoonotic significance in more infectious diseases than earlier anticipated. Thus, we must be open to the increased risk of transmittance between species.

This paper describes the most relevant zoonotic diseases that may be transferred from companion animals. Table 1 presents an overview of the zoonoses that should be known to veterinarians. Relevant references may also be found in earlier issues of the Norwegian Veterinary Journal [2-9].

**Dogs and cats**

*Diseases mainly transferred by saliva through bites and scratches*

Many bite wounds caused by dogs or cats develop into
infections. Cat bites are usually associated with a higher risk of wound infection than dog bites. Potential pathogen bacteria can be cultivated from about 90 % of bite wounds caused by a cat or a dog, and in most cases more than one agent is diagnosed. The most common isolates include species of Pasteurella, Streptococcus, Staphylococcus, Neisseria and Moraxella. Other agents are sporadically isolated [10].

Capnocytophaga canimorsus (former Bacteroides ochraceus, DF-2) is a gram negative rod considered to be part of the normal flora of the oral cavity in dogs and cats. The bacteria is rarely cultivated from about 90 % of bite wounds caused by a cat or a dog, and in most cases more than one agent is diagnosed. The most common isolates include species of Pasteurella, Streptococcus, Staphylococcus, Neisseria and Moraxella. Other agents are sporadically isolated [10].

Pasteurella multocida is also part of the normal flora in most vertebrates, including the dog and cat. Pasterurella infections are especially seen after cat bites [10, 13]. Clinical signs may include cellulitis, lymphangitis and lymphadenopathy, in some cases in combination with arthritis [10].

“Cat scratch disease”
The disease is caused by Bartonella henselae (former Rochalimaea henselae) that is a small, bent gram negative rod. Other Bartonella spp. may also cause disease in humans. Most humans that develop “cat scratch disease” are children or young adults, and the disease is considered to be the most common cause of chronic lymphadenopathy in this age group [11]. In some geographic areas where the cat flea (C.felis) is endemic, up to 40 % of the healthy cats may suffer from Bartonella bacteriæmia. Cats with chronic bacteriæmia are contagious through saliva, and there is highest risk of transmittance from young adult cats [10]. The most common clinical symptoms in humans are non-pruritic swelling on the inoculation site and lymphadenopathy. In some cases fever and general malaise occur. In rare cases, acute encephalopathy, liver- and spleen abscesses and pneumopathies may develop [14]. Most infected cats are asymptomatic carriers, but debilitated individuals may develop generalised infection. Dogs are believed also to develop and transfer disease caused by Bartonella spp.

In the period 1998-99 samples were taken from 100 cats in Mid-Norway. None of the cats were seropositive. This finding corresponds with the fact that the disease has not been reported in humans in Norway [15].

Rabies
Rabies is an acute, progressive non-treatable virus encephalitis [2, 3]. Carnivores and bats are the most important reservoirs. Transmittance occurs through saliva, most often through bites from infected animals. The virus follows the nerve pathways to the central nervous system. After virus replication virus is excreted through saliva. The incubation time is from one to three months, however, development of clinical symptoms have occurred days to years after transmittance of infection. The dog represents the most significant reservoir for disease in humans. About 35,000 humans are infected every year as a consequence of dog bites [16]. The infection is maintained through infected fox herds in arctic, tempered and tropical areas, these representing the most important reservoir for infection in Eastern Europe. Hares, rabbits, rodents and birds are considered to have little significance in transmittance of infection. After 1980 more rabies cases have been reported in cats than in dogs in the US [17]. However, cats still not represent the most important reservoir for infections to humans. Because of the long incubation time and increased global travel activity, rabies may be imported to areas formerly free of the disease. Clinical symptoms in humans include fever, anorexia, ataxia, anxiety, altered mental state, paralysis, coma and death. The patient usually dies at latest ten days after appearance of clinical symptoms. Rabies has not been diagnosed in mainland Norway since the beginning of the nineteenth century, but has been diagnosed sporadically in polar fox, reindeer and seal in Svalbard, the most recent cases in 1999 [2, 18]. Vaccine delivered in meat dropped form aeroplanes as feed for foxes has reduced the rabies problem in Europe significantly the last 25 years. The disease is at present more common in Eastern Europe.

Enteric diseases with mainly faecal-oral transmittance
Salmonellosis
The most common sero-variant affecting animals in mainland Norway are S typhimurium. Rodents and other wild animals, including birds, serve as a reservoir for infection [4].

Norwegians are most commonly infected when travelling to other countries, in this cases the sero-variant S.enteritidis is the most common agent isolated, mainly after consumption of contaminated food. The most common sources of infection both for animals and humans in Norway are contaminated food and direct faecal-oral transmittance. Dogs have a relatively low
susceptibility for infection with salmonella bacteria compared to other animals, like horse and cattle, however, salmonellosis has recently been diagnosed in dogs infected through contaminated chewing bones made from slaughter offals. So-called inverse infection, where animals are being infected through owners suffering from salmonellosis in not uncommon.

Clinical signs in animals vary dependent on number of bacteria and immune status in the host animal. Gastroenteritis is the most common manifestation of disease. Bacteriaemia and enterotoxaemia are usually subclinical signs in the gastrointestinal manifestation in dogs and cats, however, serious depression, hypothermia and shock may also be seen with or without gastrointestinal signs. Animals recovering from infection may shed bacteria up till six weeks post infection.

**Camphylobacteriosis**

Camphylobacteriosis may be caused by different species of the camphylobacter- bacteria in dog and cats. The most common sub species to cause diarrhea in humans is *Camphylobacter jejuni* sub species *jejuni*. *C.jejuni* can cause enteric disease in the dog. The dog is also considered to be the most important carrier of *C.upsaliensis*. In a Norwegian survey from 2000-2001, 24 % of 595 dogs and 18 % of 332 cats tested positive for *Camphylobacter* spp. Most of the isolates from both dogs and cats were *C.upsaliensis*. The frequency was equal in animals with and without diarrhoea [19]. This bacteria may also cause diarrhoea in humans.

An increased risk for camphylobacteriosis has been found in humans that keep dogs or cats as pets [20].

**Toxocarosis**

Toxocara canis and *T.cati* are the nematodes of the dogs and cats, respectively, and the most important parasite forming migrating larvae (*larvae migrans*) in humans. Adult dogs and cats usually present with only asymptomatic (“dormant”) infections, while untreated puppies and kittens may be heavily infested.

In connection with pregnancy in the dam the larvae are reactivated. This may cause transplacental transmittance to the foetuses. If the dam and puppies do not undergo antiparasitic treatment during the first two weeks after birth, eggs are shed through faeces. In humans ingesting eggs, the eggs will develop in the intestine. Since humans are not natural hosts, the larvae will migrate and may cause serious damage in many organs, including the retina. Luckily, such cases are rare in Norway. Cats carry a lower significance than dogs for transmitting the parasite to humans [21]. Antiparasitic treatment with regular intervals, plus removal of faeces in the environment reduces the risk of transmittance of the parasite.

**Protozoans**

In giardiosis and cryptosporidiosis acute and chronic small intestinal diarrhoea may develop both in humans and animals. Wild animals serve as potential reservoirs for dogs and cats, which are infected through contaminated feed or drinking water. Most infected dogs and cats do not show signs of disease, however, some animals do develop diarrhoea, anorexia and unkempt fur. Clinical signs may be constantly present or occur periodically. *Giardia* spp. as cause of diarrhoea in puppies and young dogs are occasionally diagnosed. The prevalence of *Giardia* spp. and cryptosporidiae seems relatively high among about 600 dogs included in a study in Norway. The prevalence in cats in Norway has not been studied (I.S.Hamnes, personal information, 2004).

Toxoplasmosis is caused by *Toxoplasma gondii* with cat as definite host. The cat only plays a minor role for direct transfer of the protozoan. However, cats shed oocysts through faeces. These oocysts sporulate within one to five days causing the cat to act as a source of infection. Thus, new faeces does not represent a source of infection, but sporulated oocysts may survive for months to years. Infection to humans through contaminated sand and soil is considered rare compared to ingestion of uncooked meat. Infected cats only shed oocysts for a short period in life. Routine screening of cats for antibodies against *T.gondii* is of limited value, but pregnant women are recommended not to touch cat faeces because of the risk of infection, which may cause abortion or congenital disease in the child [18]. Infected cats are most often asymptomatic, but signs of generalised infection may occur in young or debilitated cats [17].

**Echinococcosis**

*Echinococcus granulosus* and *E.multilocularis* are the tapeworms of dogs and foxes, respectively. The parasite has a life cycle that includes two hosts, and it is the larval stage of the parasite that causes disease. In predators the tapeworm develops to maturation and produces eggs that are spread to the environment. When eggs are ingested by grazing animals, rodents or humans, the larvae are activated and migrate to different organs where cysts develop. These cysts can be large and destroy the surrounding tissue. The cysts can also develop microcysts that spread further. Dogs and cats are invaded when ingesting either prey with tapeworm, in earlier years also through slaughter offal from contaminated reindeer. Today antiparasitic treatment of reindeer is routinely performed and *E.granulosus* is almost extinct in Mainland Norway. However, of ten examined mice (*Microtus epiroticus*) in Svalbard in 2002, two were found positive for *E.multilocularis* [18]. Both types of tapeworm are found in areas in Europe, however, thus antiparasitic treatment of dogs immediately before and after import to Norway is mandatory.

**Diseases mainly transmitted through direct or indirect contact with skin or skin lesions**

**Ringworm**

Ringworm (dermatophytosis) is caused by closely related fungi (dermatophytes) that are only able to infect keratinised tissues in nails and claws, hair and stratum corneum.

The dermatophytes are classified as anthropophilic, zoophilic and geophilic and have humans, animals and soil as their main reservoirs. All dermatophytes able to cause ringworm in animals can also cause disease in humans [9].

The significance of dermatophytes as cause of skin lesions in dogs and cats is not known, but there are indications that the
disease is over diagnosed clinically, especially in dogs. Cats may be an asymptomatic carrier. However, this is considered rare in dogs. In a Norwegian study of skin and hair samples from animals suspected of suffering from ringworm 5% of 780 samples from dogs and 30.8% of 279 samples from cats were found positive [22]. The cat is considered the most important reservoir for infection to humans. Transfer of the fungus occurs either through direct or indirect contact. In Norway, as in other countries, *Microsporum canis* is the dominant agent in ringworm infections in the cat [22, 23]. Stenwig [22] reported that *M. canis* represented 83 of the samples in 86 positive cats. Likewise, E. Christensen (personal information 2003) reported that 33 of 36 positive samples from cats were diagnosed as *M. canis*. Affected cats may show clinical signs, they may be subclinically infected or they may serve as vectors. About 50% of humans exposed to *M. canis* positive cats will show skin lesions. In households with infected cats at least one person will be infected in about 70% of the households. Typical skin lesions in humans are circular, slightly prominent, crustaceous and erythematous [10].

**Scabies**

The mite *Sarcoptes scabei* var. *canis* is most often diagnosed in dogs. However, it is not species specific and the mite has been diagnosed in many other animal species [24]. About 24 hours after contact with an affected dog, humans may develop papulose severely pruritic lesions on the contact area of the skin, most often the arms and body. Spontaneous remission occurs after 12-14 days. It has been shown that the mite can survive on a human being up till six days and may produce eggs in this time period [25].

**Fur mites**

The fur mites, *Cheyletiella yasguri*, *C. blakei*, *C. parasitivorax*, cause varying severity of pruritus and dandruff in dogs, cats and rabbits, however, cats and dogs may also be asymptomatic carriers. In humans, the mites show a “hit-and-run” behaviour, so that repeated direct contact with an infested animal is necessary in order for skin lesions to develop. Typical lesions in humans are papules and/or erythematous maculae, often in groups in the contact area(s); arms, chest and abdomen. Gradually, vesiculae and pustules develop [25].

**Ear mites**

The ear mite, *Otodectes cynotis*, can invade the aural canal in cats, dogs and ferrets and cause otitis externa. Kittens and puppies are most susceptible for invasion. Transfer to humans with development of pruritic, papular lesions have been reported [26, 27].

**Cowpox**

Cowpox virus causes sporadic infection in cats, and cowpox-infection has also been reported in cats in Norway [28]. The natural reservoir is wild rodents. Cats are usually infected through hunting.

**Generalised disease transmitted through direct or indirect contact**

**Leptospirosis**

Leptospirosis is caused by the bacteria *Leptospira interrogans*, a spirochaet that is further subdivided into many serologic variants (serovars), of which many are able to cause disease [7]. In Norway the serovariants *Ichterohaemorrhagiae* and *Canicola* have been connected with disease. The disease has not been reported in humans in Norway for more than 20 years, Thus, infection from endemic areas in other countries is considered the most important risk [6]. Up to the 1950ies leptospirosis was relatively common in dogs in Norway. In 1947 Strande found that 6.5% of dogs hospitalised for internal medicine disease suffered from leptospirosis [29]. During the last years, there have been single cases of leptospirosis in dogs unrelated to import [6]. In other countries in Europe and in USA the disease is still stationary, and increased prevalence may be seen in years with humid weather. Leptospirosis is probably the most widespread zoonosis in the world [30].

Clinical signs of infection in dogs include generalised infection that may range from per acute to more chronic development. Petechiae and bleeding tendency may be seen, as well as icterus, which is more common in the subacute form. Hepatitis may lead to intrahepatic cholestasis with grey faeces as one manifestation.

**Horse**

**Salmonellosis**

Salmonellosis in horses presents with enteritis and septicaemia, with foals and stressed adults being especially susceptible. *S. typhimurium* is the most common serovar isolated from horses in Norway [4].

Clinical signs of salmonellosis in horse may be dramatic, especially in acute enteritis and may even result in the horse dying. Large amounts of contagious, soft stool, often gaseous and excreted under strong pressure represent a significant risk for transfer of bacteria to other animals and to humans. Isolation of infected horses and restricted access for humans taking care of the horses is of utmost importance, and caretakers must be
instructed about risk of infection and necessary steps to avoid transfer of bacteria.

Tuberculosis
Horses are relatively resistant to tuberculosis, but may occasionally be infected with M.bovis, M.tuberculosis or M.avium [5]. Clinical signs are unspcific, and may include diarrhea and respiratory signs. A case of avian tuberculosis was described in a horse in the nineties [31].

Ringworm
Ringworm in horses in Norway is most often caused by Tricophyton equinum or Microsporum equinum [9, 22]. Cases of ringworm may be diagnosed in riding horses and trotters. Control of ringworm outbreaks in stables with sports horses and thus transport of animals between competitions and horse shows may be a challenge. In stables where horses are in frequent contact with humans, many of which are young people, the zoonotic aspect of the disease should be emphasized in the information given.

Birds
In addition to salmonellosis, psittacosis is the most important zoonotic disease related to infection from wild or captive birds. The agent is found in secretion form eyes, nose and from faeces and transmittance is aerogenic or through direct contact. Birds with psittacosis are most often asymptomatic, however, generalised signs of infection may be seen in immune compromised birds. Respiratory symptoms and headache are the most common symptoms in humans [32].

Rabbits, rodents and ferrets
Rabbits can be affected by fur mites (Cheyletiella spp.) and transmit invasion through direct contact – see description for dogs and cats. Rabbits are also susceptible to ringworm (Tricophyton mentagrophytes, M.canis and Microsporum gypseum) and may transfer infection to humans [14]. Tularaemia can be transferred to rabbits from wild rodents through ticks and fleas. Rodents can also suffer from ringworm (T.mentagrophytes).

Salmonellosis can be transferred from rodents to humans. Guinea pigs will most often die if they develop salmonellosis, while rats and mice may have subclinical infection. The most important serovariants are Typhimurium and Enteritidis.

Leptospirosis can be transferred from rats, usually wild, and these are also considered a cause of infection in humans, through contaminated water.

Ferrets may suffer from salmonellosis, campylobacteriosis and ringworm.

Reptiles
Turtles are asymptomatic carriers of salmonella. A carrier frequency of more than 90% has been reported. In a Swedish study on snakes from 1988, salmonella was diagnosed in 41% of the examined animals. Even if many of these salmonella types belong to subgroups that are not pathogenic to humans, the risk of salmonella infection represents a significant problem as all reptiles must be considered potential carriers of infection [34]. The bacteria are shed periodically, thus single samples for diagnosis are of restricted value. The risk of infection is lowest in terrestrial species, snakes and lizards. These animals are rarely exposed to their own faeces, which in addition is hard and rarely excreted due to the slow metabolism. Aquatic turtles and terrapins represent a greater risk, as the water is contaminated by faeces. Children are especially exposed to infection. The animals should not be treated with antibacterial agents, as this may result in resistant bacteria. Reptiles are not recommended as pets for children (M.Heggelund, personal communication).

Vector borne diseases
Many infections causing disease in animals and humans are spread through an invertebrate vector. In Norway, the tick Ixodes ricinus is the most important vector transferring disease between species [8].

Borrelia ( Lyme disease)
Borrelia is caused by the bacteria Borrelia burgdorferi and is probably the most widespread vector borne zoonotic disease in Northern Europe [35]. In humans, the disease leads to general infection, with skin, joint, nervous tissue and heart most often affected. Among animals, dogs and horses are especially susceptible. Serology is frequently used for establishing a diagnosis. A Swedish/Norwegian study of dogs from areas where I.ricinus is endemic, showed 4% and 27% respectively, of tested healthy animals to be seropositive [36,37]. Most seropositive animals do not develop clinical illness, but both acute and chronic disease with inflammatory signs may develop. The prevalence among cats has not been studied.

Horses may also suffer from borreliosis. Clinical signs are similar to what is found in dogs. The diagnosis is most often established in animals with disease, known exposure to ticks and positive serology. A Swedish study showed a seroprevalence for B.burgdorferi of 17%. No difference in seroprevalence was found between healthy horses and horses with signs of disease [36].

Anaplasmosis
Anaplasma phagocytophilum is the present name of bacteria formerly termed Ehrlichia phagocytophila, E.equii and “Human granulocytic ehrlichial agent” (HGE). The intracellular bacteria affect granulocytes in the blood in particular and may be diagnosed through direct microscopy. “Granulocytic ehrlichiosis” in dogs was described from Sweden in 1989. Later studies showed that the dogs had been infected with A.phagocytophilum, the same type causing disease in humans and horses [38]. Clinical signs in affected dogs include fever, depression, lameness plus gastrointestinal and nervous symptoms. Haematology signs include thrombocytopenia, leukopaenia and anaemia. Asymptomatic cases are common. Studies have shown a seroprevalence in Swedish dogs to be 17% and 23% in dogs from Southern Norway. The significance of anaplasmosis in cats is not known, however, natural infection has been described.
"Equine granulocytic ehrlichiosis" was described in USA in 1969. In Sweden the first cases were described in 1990. DNA analysis showed the bacteria to be closely related to *E. phagocytophila* and identical with agents causing human granulocytic ehrlichiosis [38]. Clinical signs in horses are similar to what is described in dogs, however, the infection can also be asymptomatic. The seroprevalence in a Swedish study was 17%, antibody findings in healthy and diseased animals being equal [38].

**Tick borne encephalitis (TBE)**

TBE is caused by flavivirus and is relatively widespread in Eastern Europe and the Baltic states. The disease has been known in humans in Sweden from 1954 with 40-80 new cases diagnosed annually in endemic areas [39]. The disease was diagnosed for the first time in humans in Norway in 1997. 5 cases are described from 1998–2001, all from Trom Island in Southern Norway. Based on this, as serologic study of 317 healthy dogs from this area was carried out. Antibodies against TBE virus were found in 16.4% of the dogs [40]. Based on this, it can be stated that the disease is present in Norway and that dogs may serve as potential carriers. Clinical signs in diseased dogs include fever, and progressing CNS signs. In a study of 545 dogs from Austria, 131 had antibodies against TBE virus and about half of these showed clinical signs of disease [41].

**Leishmaniasis**

Leishmaniasis is caused by an intracellular protozoan. In the Mediterranean area, the dog represents the main reservoir for infection to humans, the parasite being transmitted through a sand fly [3]. The disease has a cutaneous and a visceral form, the latter being caused by *Leishmania donovania infantum* and being endemic in areas in the Mediterranean area. The disease may progress slowly, and it may take years before development of clinical symptoms, although individual differences exist. In both humans and animals the visceral form leads to generalised symptoms of infection, emaciation, muscular atrophy, anaemia, liver- and kidney failure plus failure of other organs. The disease is diagnosed in Norway in animals imported from endemic areas in Italy and Spain. The sand fly does not survive the Norwegian winters, however, thus there is no risk for the disease to become endemic. The parasite may also be transferred to humans through skin contact with infected organs.

**Heartworm (Dirofilariasis)**

*Dirofilaria immitis* is the dog heartworm transferred through a mosquito that demands certain temperature and environmental conditions for survival. The mosquito has spread north in USA, but routine antiparasitic treatment of dogs during the summer months has prevented spreading of the parasite. Single cases of infection causing abortion have been described in humans. In Europe, *Dirofilaria repens* is diagnosed in humans in an increasing number. Noduse develop in skin, subcutis and internal organs. This parasite is also transferred through a mosquito. Studies of dogs from endemic areas in Italy have shown an increase in number of seropositive dogs. It therefore seems reasonable to consider a role from the dog as reservoir and in transfer of the parasite between species [42]. Dogs rarely develop skin nodules, but the parasite migrates through subcutaneous tissues.

**Resistance to antibiotics**

Bacterial resistance to antibiotics is an increasing and challenging problem both in veterinary as well as in human medicine. Antibiotic resistance can develop through genetic adaptation in the bacteria. This occurs to a significant degree through genes causing antibiotic resistance frequently being transferred between bacteria as mobile genetic elements. Mobile resistance genes can be used by many bacteria, not necessarily closely related. This means that widespread use of antibiotics and increase in resistance genes in veterinary medicine can be of significance for the increase in bacteria being resistant to antibiotics and causing disease in humans. These bacteria pick up resistance genes directly from the animal pathogenic bacteria, or resistance genes are delivered through the normal flora in animals or humans as a type of zoonotic problem. Similarly, resistance genes included in animal pathogenic bacteria may be of human origin.

**Discussion and conclusion**

Companion animals are of benefit to humans. One study showed that people owning pets visited the doctor less frequently and had lower blood pressure and blood cholesterol than non-owners [43].

The highest risk in companion animal keeping is connected to the risk of bites and the risk of transfer of diseases from animals to humans. There has also been a focus on the risk of sensibilisation and development of allergy, especially to cats, but this is controversial. More recent studies have shown that children in households without a cat or a dog are at greater risk of developing allergies against these animal species [44].

The risk of transmitting disease from companion animals to humans is related to certain factors, including young animals, dense animal population and poor hygiene, insufficient prophylactic measures, free-roaming animals eating cadavers, contact with stray animals and increased travel activity. Small children, pregnant women and immunodeficient persons form the group at risk, but also veterinarians, nurses and other staff working with animals with infections are exposed. Even if the risk of transferring infection from companion animals is relatively low, the risk of infection can be reduced further. Owners, and especially those in the group at risk must be informed about the potential risk and possible routes of infection, including the life cycles of vectors and agents. This is a task for both veterinarians and doctors.

In short, the risk of transfer of infection can be reduced through

- Good hygiene, in particular hand hygiene
- Removal of faeces in outdoor areas, and regular cleaning of litter boxes
- Antiparasitic treatment of the animals
- Not letting children play in contaminated areas
- Cover sand boxes when not being used
- Avoid scratches and bites
- Not bringing animals into areas with increased risk of infection/invasion unless proper prophylactic measures have been taken

However, in total the benefit of having a pet is higher than the fear of zoonotic disease.
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<td>Faecal-oral transmittance</td>
<td>Dog, cat, most mammals, birds, reptiles</td>
<td>Dog, cat</td>
<td>Normal bacterium in the oral cavity</td>
</tr>
<tr>
<td></td>
<td>Capnocytophaga infection (wound infection, septicaemia)</td>
<td></td>
<td></td>
<td></td>
<td>Dog, cat, rodents, primates, horse, fish</td>
<td>Most often (aerogenic) infection from humans to animals (&quot;inverse zoonosis&quot;)</td>
</tr>
</tbody>
</table>

Tab. 1 Zoonoses that can be transferred from companion animals to humans. Under "affected species" only companion animals are included.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Agent</th>
<th>Affected species</th>
<th>Clinical signs in animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tularaemia</td>
<td>Francisella tularensis</td>
<td>Hare and other wild rodents, dog, cat, other animals</td>
<td>Ulcerative dermatitis, lymphadenopathy, fever, skin lesions with varying morphology, also asymptomatic (mainly cat)</td>
</tr>
<tr>
<td>Mycoses</td>
<td>Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton schoenleinii</td>
<td>Cat, dog, rabbits, rodents, horse</td>
<td>Asymptomatic, dermatitis, lymphadenopathy, fever, contact skin through scratches and bites, or indirect environmental pollutant</td>
</tr>
<tr>
<td>Sporotrichosis</td>
<td>Sporothrix schenckii</td>
<td>Cat</td>
<td>Asymptomatic, dermatitis, lymphadenopathy, fever, contact skin through scratched and bitten</td>
</tr>
<tr>
<td>Parasitoses - protozoa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>Cryptosporidium spp.</td>
<td>Cat</td>
<td>Asymptomatic, dermatitis, lymphadenopathy, fever, faecal-oral transmittance, contact skin</td>
</tr>
<tr>
<td>Giardiosis</td>
<td>Giardia spp.</td>
<td>Dog, cat, bird, reptiles</td>
<td>Asymptomatic, diarrhoea, faecal-oral transmittance, contact skin through contaminated water</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>Toxoplasma gondii</td>
<td>Cat</td>
<td>Asymptomatic, diarrhoea, organ changes due to pressure from cysts and granulomas, faecal-oral transmittance</td>
</tr>
<tr>
<td>Parasitoses - helminthes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echinococcosis</td>
<td>Echinococcus granulosus, Echinococcus multilocularis</td>
<td>Dog, cat, rodents</td>
<td>Asymptomatic, anaemia, diarrhoea, respiratory signs, linear skin lesions, pododermatitis</td>
</tr>
<tr>
<td>Hookworm</td>
<td>Ancylostoma spp., Uncinaria stenocephala, Toxocara canis</td>
<td>Dog, cat, rabbit</td>
<td>Asymptomatic, diarrhoea, organ failure in debilitated humans (dogs), faecal-oral transmittance</td>
</tr>
<tr>
<td>Toxocariasis</td>
<td>Toxocara canis, Toxocara cati</td>
<td>Dog</td>
<td>Asymptomatic, diarrhoea, organ failure in debilitated humans (dogs), faecal-oral transmittance</td>
</tr>
<tr>
<td>Fur mite</td>
<td>Cheyletiella sp.</td>
<td>Dog</td>
<td>Asymptomatic, diarrhoea, respiratory signs, linear skin lesions, pododermatitis</td>
</tr>
<tr>
<td>Cheyletiellosis</td>
<td>Cheyletiella sp.</td>
<td>Dog</td>
<td>Asymptomatic, diarrhoea, respiratory signs, linear skin lesions, pododermatitis</td>
</tr>
<tr>
<td>Disease</td>
<td>Agent</td>
<td>Human aspects</td>
<td>Geographic distribution</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>--------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Scabies (sarcoptosis)</td>
<td><em>Sarcoptes scabiei</em> var. <em>canis</em></td>
<td>Occurs</td>
<td>Global</td>
</tr>
<tr>
<td>Ear mite (otodectosis)</td>
<td><em>Otodectes cynotis</em></td>
<td>Occurs</td>
<td>Global</td>
</tr>
<tr>
<td>Fleas</td>
<td><em>Ctenocephalides felis</em>, <em>Ctenocephalides canis</em></td>
<td>Occurs</td>
<td>Global</td>
</tr>
<tr>
<td><strong>Vector borne zoonoses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBE (tick borne encephalitis)</td>
<td><em>Flaviviridae</em> TBE virus</td>
<td>Occurs, rel. common in the Baltic states</td>
<td>Mid-Eastern Europe, Nordic countries, Baltic states</td>
</tr>
<tr>
<td>Borreliosis</td>
<td><em>Borrelia burgdorferi</em> (bacterium)</td>
<td>Areas where the vector is present</td>
<td>Global</td>
</tr>
<tr>
<td>Anaplasmosis (ehrlichiosis)</td>
<td><em>Anaplasma phagocytophilum</em> m.fl (bacterium)</td>
<td>Areas where the vector is present</td>
<td>Global</td>
</tr>
<tr>
<td>Leishmaniosis</td>
<td><em>Leishmania</em> spp. (protozoan)</td>
<td>Rel. common in areas with sandflies</td>
<td>Mediterranean, Africa, Asia, South-America</td>
</tr>
<tr>
<td>Dirofi lariosis (heartworm)</td>
<td><em>Dirofilaria repens</em></td>
<td>Southern Europa</td>
<td>Mediterranean, Africa, Asia, South-America</td>
</tr>
</tbody>
</table>

The diseases listed below are uncertain with regard to the zoonotic aspect and if they can be transmitted from companion animals to humans.

- Helicobacteriosis, enteric disease (*Helicobacter* spp.) may possibly be transferred through contact with infected organs via cats
- Anaerobiospirillosis, enteric disease (*Anaerobiospirillum* spp.) may be transferred from infected cats
- Yersiniosis, (*Yersinia pseudotuberculosis*) septicaemic form and classical syndrome with organ nodules in guinea pigs and other rodents, rabbits and wild and captive birds may possibly be transferred via dog and cat. *Y. enterocolitica* causes enteric form
- Q-fever, infectious syndrome (*Coxiella burnetti*) may possibly be transferred through contact with infected organs via cat
- Babesiosis, (*Babesia* spp.) may possibly be transferred via ticks and through bites and scratches from dogs
- "Rocky Mountain spotted fever", infectious syndrome (*Rickettsia rickettsiae*) may possibly be transferred from cats to humans through flea bites
References


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with Soft Rubber Outer Edge

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To ensure that you get the real BUSTER Comfort Collar look for this label.
Rabies has the highest case-fatality rate of any currently recognized infectious disease with an estimated 55,000 human deaths per year. The disease is routinely diagnosed in animals based on clinical signs and on epidemiological grounds in rabies-endemic countries. The use of conventional diagnostic tests including the fluorescent antibody test (FAT), rabies tissue-culture infection test (RTCIT) and mouse inoculation test (MIT) can now be complemented with molecular diagnostic tools such as reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridisation (ISH). Negative diagnostic test results do not exclude the clinical diagnosis as these tests are entirely dependent on the quality of the sample supplied. Molecular tools and virus typing are becoming more widely used for the rapid detection and strain identification of rabies viruses from ante-mortem clinical samples. The currently available diagnostic methodologies for both post-mortem and ante-mortem detection of rabies virus are reviewed.

**SUMMARY**

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**Diagnostic tools for the detection of rabies virus**

L. M. McElhinney(1,3), A. R. Fooks(1,3), A. D. Radford(1,2)

**Introduction**

Animal rabies is endemic throughout all continents with the exception of several islands and peninsulas including Australia and New Zealand, an increasing number of European countries and Antarctica. Having eliminated rabies, countries that are striving to maintain their rabies free status, usually do so at considerable cost and with a continual risk of re-importation [1,2].

Rabies viruses are members of the Lyssavirus genus, within the Rhabdoviridae family. The lyssaviruses comprise seven related virus groups (genotypes 1 - 7). These include, classical rabies virus (RABV, genotype 1), Lagos bat virus (LBV, genotype 2), Mokola virus (MOKV, genotype 3), Duvenhaque (DUVV, genotype 4), European bat lyssavirus type 1 (EBLV-1, genotype 5), European bat lyssavirus type 2 (EBLV-2, genotype 6) and Australian bat lyssavirus (ABL, genotype 7). With one exception, (MOKV), all genotypes have been isolated from bats (Tab. 1) [3]. Four additional rabies-related viruses, isolated from bats in Eurasia (Aravan virus, Khujand virus, Irkut virus and West Caucasian Bat Virus), have recently been proposed as new members of the Lyssavirus genus [4-6].

Rabies virus is largely maintained in two ecologically inter-related disease cycles; urban and sylvatic (wildlife). Urban rabies, primarily in dogs and cats, has been eliminated in an increasing number of developed countries via parenteral vaccination programmes. However, sylvatic rabies remains widespread throughout parts of Europe, Africa and North America and is maintained in a variety of species including the red fox, raccoon-dog, raccoon, skunks and bats. Urban canine rabies remains widespread throughout developing countries and human mortality from endemic canine rabies was calculated to be 55,000 deaths per year with 56% of the deaths estimated to occur in Asia and 44% in Africa [7]. The total global cost of rabies prevention is estimated to exceed $1 billion per year. However, the prevalence and cost of rabies is believed to be significantly underestimated due to poor reporting and surveillance [7].

In Europe, sylvatic rabies (predominantly fox rabies) has been significantly controlled by the successful implementation of oral rabies vaccination (ORV) campaigns. The majority of the western European countries are now free of Classical rabies (RABV), with reported rabies restricted to relatively rarer bat
Diagnostic tools for the detection of rabies virus - L. M. McElhinney, A. R. Fooks, A.D. Radford

or imported cases. The most recent European country to be declared rabies free was Germany (September 2008). Rabies Bulletin Europe (WHO) reported 9563 cases of rabies in Europe in 2007 (includes figures for Russian Federation, Ukraine and Turkey). These cases were associated with red foxes (4515 cases, 47.2%), cats (1411 cases, 14.8%), dogs (1394 cases, 14.6%), cattle (1288 cases, 13.5%), raccoon-dog (364 cases, 3.8%), bat (26 cases, 0.3%) and humans (9 cases, 0.1%). Although fox rabies is the principal vector in Europe as a whole, the raccoon-dog plays a significant role in the epidemiology of rabies in the Baltic countries where numbers of infected raccoon-dogs can exceed that of foxes. The cattle and human cases are generally considered to represent dead end host spill-over events. Dog and cat rabies is still prevalent in some Eastern European countries, Belarus, Ukraine, Russia and Turkey.

In 2000, the UK Government introduced a system whereby eligible dogs and cats from qualifying countries could enter or return to the UK without being subjected to quarantine. The UK Pet Travel Scheme (PETS) comprised of identification, vaccination and serological testing combined with tick and tapeworm treatment prior to entry. The scheme was extended to include further qualifying countries and in 2004 the EU Pet Travel Scheme succeeded it. The EU PETS allowed for the transportation of ferrets, a greater list of qualifying countries but no obligation to confirm seroconversion in the vaccinated animal or administer treatment for the cestode *Echinococcus multilocularis* (exotic to the UK). The UK, Malta, Sweden and Ireland retain the authority to demand serological testing under an EU derogation in place until June 2010. In addition, treatment for ticks and tapeworm is required for entry into Ireland, Malta and the United Kingdom, while Sweden requires tapeworm treatment and not treatment for ticks. Approximately 546,908 pets have entered the UK between February 2002 and August 2008 (http://defraweb/animalh/quarantine/pets/procedures/stats.htm). Commercially traded eligible animals are transported without quarantine under the Balai Directive (Council Directive 92/65/EEC of 13 July 1992) as amended (e.g. regulation 998/2003).

Rabies cases in Europe are principally attributed to three of the Lyssavirus genotypes, namely genotype 1 (RABV, classical rabies) and to a lesser extent genotypes 5 and 6 (European bat lyssaviruses type-1 and -2). The 26 insectivorous bat cases reported in 2007 were due to European Bat Lyssaviruses (EBLV-1 or -2). The majority (>95%) of the reported EBLV-1 cases in European bats have been identified in one bat species, *Eptesicus serotinus* (Serotine bat). The rarer EBLV-2 cases (2%) are associated with *Myotis* species, *M.daubentonii* (Daubenton’s bat) and *M.dascyneme* (pond bat). EBLV-1 has been reported in several countries across Europe (not UK) whereas EBLV-2 has only been confirmed in the UK, Netherlands, Switzerland and Germany.

Spill-over of EBLV-1 into sheep has occurred on two separate occasions in Denmark, into a stone marten in Germany, domestic cats in France and one confirmed and possibly other unconfirmed human cases [8]. EBLV-2 has been recorded in two

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phylogroup</th>
<th>Species</th>
<th>Abbrev. (ICTV)</th>
<th>Distribution</th>
<th>Potential vector(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Rabies virus</td>
<td>RABV</td>
<td>World (except several islands)</td>
<td>carnivores (world) bats (Americas)</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Lagos bat virus</td>
<td>LBV</td>
<td>Africa</td>
<td>frugivorous bats</td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>Mokola virus</td>
<td>MOKV</td>
<td>Africa</td>
<td>unknown (isolated from shrews)</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>Duven-hage virus</td>
<td>DUVV</td>
<td>Africa</td>
<td>insectivorous bats</td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>European bat lyssavirus 1</td>
<td>EBLV-1</td>
<td>Europe</td>
<td>insectivorous bats (Eptesicus serotinus)</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>European bat lyssavirus 2</td>
<td>EBLV-2</td>
<td>Europe</td>
<td>insectivorous bats (Myotis sp)</td>
</tr>
<tr>
<td>7</td>
<td>I</td>
<td>Australian bat lyssavirus</td>
<td>ABLV</td>
<td>Australia</td>
<td>frugivorous/insectivorous bats</td>
</tr>
<tr>
<td>Proposed</td>
<td>I</td>
<td>Aravan virus</td>
<td>ARAV</td>
<td>Central Asia</td>
<td>insectivorous bat (isolated from Myotis blythii)</td>
</tr>
<tr>
<td>Proposed</td>
<td>I</td>
<td>Khujand virus</td>
<td>KHUV</td>
<td>Central Asia</td>
<td>insectivorous bat (isolated from Myotis mystacinus)</td>
</tr>
<tr>
<td>Proposed</td>
<td>I</td>
<td>Irkut virus</td>
<td>IRKV</td>
<td>East Siberia</td>
<td>insectivorous bat (isolated from Murina leucogaster)</td>
</tr>
<tr>
<td>Proposed</td>
<td>II or III</td>
<td>West Caucasian bat virus</td>
<td>WCBV</td>
<td>Caucasus region</td>
<td>insectivorous bat (isolated from Miniopterus schreibersi)</td>
</tr>
</tbody>
</table>

Tab. 1 Classification of Lyssaviruses.
human cases (Finland 1985 and Scotland 2002) but no other spill-over event has been reported. There has been no evidence of transmission of EBLV by any other terrestrial species; hence to date all spill-over events have been dead-end.

Human and animal rabies is usually acquired via the transdermal inoculation of infected saliva in the bite of a rabid animal but on rare occasions human rabies can be transmitted via aerosols or inoculation of infected saliva in the bite of a rabid animal but on rare occasions human rabies can be transmitted via aerosols or corneal and organ transplantation [9] or via routes other than bites [10-13]. In the majority of human cases, the exposure to an infected animal is known and the disease can be prevented by timely post exposure treatment. The successful administration of anti-rabies prophylaxis is enhanced by speedy and accurate diagnosis.

Clinical Signs

The long and variable incubation period is a key feature of rabies. It relates to the time that the neurotropic virus is protected from immune responses within peripheral cells and nerves. The incubation period can vary between 15 days to 6 years (average 2–3 months) depending on the strain, species, viral dose and the proximity of the site of virus entry to the CNS. A short incubation period would be expected for a deep bite near to the head of the victim. Most clinically infected species excrete rabies virus in the saliva. Experimental data suggests that different species exhibit varying times of virus excretion before the onset of clinical signs (cats <24hours, dogs<13 days, foxes <29 days post infection) [14].

Prophylaxis and treatment

Vaccination is the mainstay of control in at-risk human and animal populations. In humans, following suspect exposure, prophylaxis including repeated vaccinations with or without immunoglobulin therapy is usually administered. In cats and dogs, there is little evidence to show the effectiveness of such post-exposure prophylaxis, and this is not generally recommended. Animals that are bitten by potentially infected animals should be immediately reported to the relevant authority.

Without intensive care interventions, death occurs within 1–10 days of neurological signs. In cats and dogs, the clinical phase can be relatively rapid at 3–4 days [15,16]. Although the mortality for clinical rabies is believed to be 100%, sporadically cases of survival are reported. At least five humans that had all received either pre- or post-exposure prophylaxis are believed to have recovered after developing clinical signs of rabies [7]. There is some doubt as to whether the clinical signs in some of these cases could be attributable to vaccine-induced encephalitis rather than rabies infection. However, in 2004, an unvaccinated teenager in Wisconsin, USA, was bitten by a bat, developed early signs of disease, yet became the first person to recover from the disease after experimental therapy was initiated. Rabies biologicals (vaccine or rabies immunoglobulin), usually administered as post exposure treatment, were not given to this patient. She developed fever, hypersalivation, parasthesiae of the bitten hand, progressive cranial nerve paralysis and leg weakness. Rabies was diagnosed by the detection of a rare early but significant antibody response (6 days after the onset of signs) which encouraged the clinicians to attempt a drug-induced coma and a cocktail of anti-viral drugs. No virus or antigen was detected. Her treatment included the induction of coma with the anaesthetic ketamine and antiviral agents ribavirin and amantadine. This appeared to be successful and the patient recovered with only minor residual neurological deficits [17]. Despite a number of attempts to follow the costly procedure (known as the Milwaukee Protocol), this remarkable success has not been repeated. It is not clear if the success was due to the viral strain (assumed to be an American bat strain), development of paralytic rabies (lack of damaging hydrophobic spasms) or the immune status of the patient (rare early neutralising antibody production) [18].

Clinical Diagnosis

Rabies is a notifiable disease in the EU. Clinical signs are not characteristic, especially early in the course of disease. Clearly the history may raise the index of suspicion e.g. travel especially smuggling, contact with infected wildlife including bats, lack of vaccination. When presented with a suspect case, owners and veterinarians must contact the competent authority in their country.

Clinical diagnosis is based on the observation of clinical signs, the first of which usually appear after the variable incubation period and vary depending upon species. Differential diagnosis can be difficult due to common sequelae resulting from various diseases including transmissible spongiform encephalopathies, tetanus, listeriosis, poisoning, Aujeszky’s disease and other viral non-suppurative encephalitides. Paralytic rabies is often mistaken for Guillain-Barré Syndrome [19, 20]. In addition, secondary infections can mask the presence of rabies leading to misdiagnosis [21]. A report by Laothamatas et al. [22] demonstrated that Magnetic Resonance Imaging (MRI) could not differentiate the two forms of human rabies (paralytic and furious) as both share a similar MRI pattern. However, such a pattern in a non-comatose patient with suspected encephalitis may prove useful in distinguishing rabies from other viral encephalitides.

In the clinical course of rabies, three stages are classically described; prodromal, excitement (furious) and paralytic (dumb) (Tab. 2). However, it is important to note that not all stages are observed in individual cases. The first clinical symptom is usually neuropathic pain at the site of infection (bite wound) due to viral replication in dorsal root ganglia and ganglionitis. This is usually accompanied by non-specific signs typical of a viral encephalitis. Following the non-specific prodromal phase, either or both the excitement and/or paralytic forms of the disease may be observed in a particular species, with disease commonly progressing to the paralytic form. Cats are more likely to develop furious rabies than dogs [23]. In some cases, no clinical signs are observed and rabies virus has been identified as the cause of sudden death.

In a recent study, Laothamatas et al. compared furious and paralytic rabies in dogs using neuroimaging (MRI) and RT-PCR (RABV RNA and brain cytokine mRNA) [24]. They concluded that the early stage of furious rabies in dogs is characterized...
by severe virus neuroinvasiveness and moderate inflammation whereas paralytic rabies was associated with a delayed viral neuroinvasion and a more intense inflammation. However, during the late stage of infection, little difference was observed in the brains of furious and paralytic rabid dogs.

In humans, classical signs of brain involvement include spasms in response to tactile, auditory, visual or olfactory stimuli (e.g. aerophobia and hydrophobia). Such signs occur during the clinical phase in almost all rabid patients in whom excitation (furious rabies) is prominent and are interspersed with periods of lucidity, agitation, and confusion. However, excitation is less evident in paralytic rabies. Atypical rabies or non-classic rabies is increasingly observed and may result in underreporting.

There are no gross lesions characteristic for rabies visible at autopsy. In most animals, microscopic non-specific lesions suggestive of viral encephalomyelitis with ganglioneuritis may be observed in the nerve centres, as well as histolymphocytic cuffs and gliosis. The most significant lesions are usually in the cervical spinal cord, hypothalamus and pons. The only specific lesions consist of intracytoplasmic eosinophil inclusions (Negri bodies) corresponding to the aggregation of developing rabies virus particles. These oval inclusions, measuring from 4 to 5 µm, are usually located in Ammon’s horn.

Consequently, diagnosis can only be confirmed by laboratory tests preferably conducted post mortem on central nervous system (CNS) tissue removed from the cranium. Diagnostic techniques for rabies in animals have been internationally standardised [25]. The hippocampus, cerebellum and the medulla oblongata are the recommended tissues of choice. The head of the suspect animal is generally submitted for laboratory diagnosis. However, if this is impractical, for example for large numbers of carcasses, the ‘straw technique’ may be considered, whereby brain material is collected by passing a plastic straw through the occipital foramen [25].

### Rabies virus antigen detection techniques

In the past decade, a diverse number of methods have been published for the detection and identification of rabies viruses in clinical specimens. However, the most commonly used diagnostic test is the fluorescent antibody test (FAT) which detects virus antigen in the brain using fluorescently labelled anti-rabies antibodies [26] and is recommended by both WHO and OIE. It may be used to confirm the presence of rabies virus nucleocapsid protein in the original sample (brain smear) or sub-passaged material (see virus isolation below). The FAT gives reliable results on fresh specimens within a few hours in 95–99% of cases [25]. However, the sensitivity of the FAT is dependent on the quality of the specimen, conjugate, equipment and the skills of the diagnostic staff. The sensitivity of FAT can be affected by autolysis, lyssavirus species and the accuracy of dissection of the brain and may be lowered in samples from vaccinated animals.

For direct rabies diagnosis, smears prepared from hippocampus, cerebellum or medulla oblongata are fixed in high-grade cold acetone and then stained with a drop of a specific antibody conjugated to fluorescein isothiocyanate. In the FAT, the specific aggregates of nucleocapsid are identified by their specific apple-green fluorescence.

The enzyme-linked immunosorbent assay (ELISA) and rapid rabies enzyme immunodiagnosis (RREID) methodologies have been shown to be of benefit for large scale surveillance having the potential to be automated [27, 28]. These methods detected the presence of EBLV-1 antigen in a cat in France in 2007 when conventional FAT failed (H. Bourhy, personal communication).

### Rabies virus isolation

The OIE recommends the use of a confirmatory virus isolation test, particularly when FAT results are equivocal and for human exposure cases.

---

**Tab. 2 Clinical stages of rabies.** Clinical progression is variable and although most end in the paralytic phase, some do not show many signs associated with the excitement phase.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prodromal</td>
<td>Pyrexia, altered personality, increased nervousness, loss of fear, disorientation, loss of appetite, hyperactivity, hypersensitivity to noise or light, rubbing or biting the site of inoculation (wound), tremor, dyspnoea.</td>
</tr>
<tr>
<td>Excitement (furious)</td>
<td>Increased aggression, abnormal vocalisations (high pitch howl in canines, characteristic bellowing in ruminants), ‘fly snapping’ at imaginary targets, wandering long distances, hyper sexuality, head butting (particularly ruminants), staring eyes, partial paralysis of tongue and lower jaw with drooling of saliva, ‘bone in throat’ syndrome. Acute colic with abdominal straining (horses and ruminants).</td>
</tr>
<tr>
<td>Paralytic (dumb)</td>
<td>Hind limb paralysis, generalised paralysis, dysphagia, respiratory arrest, inability to drink or swallow, excessive salivation, hydrophobia (only in man), convulsions, coma, death.</td>
</tr>
</tbody>
</table>
The mouse inoculation test (MIT) involves the intracerebral inoculation of mice with a clarified supernatant of a homogenate of brain material (cortex, Ammon's horn, cerebellum, medulla oblongata). Clinical signs (positive result) can be observed as soon as six to eight days for RABV but have been known to take up to 20 days for EBLV-2 [29]. Mice should be observed for a period of 28 days. This in vivo test is time-consuming, expensive and involves the use of animals. The OIE recommends that it should be avoided for routine diagnosis if validated in vitro methods are established within the laboratory. In vitro virus isolation tests such as the Rabies Tissues Culture Inoculation Test (RTCIT) involve the inoculation of the sample into a neuroblastoma cell line. Positive results are commonly obtained within 2-4 days. The FAT is then used to confirm the presence of rabies antigen in both infected mice or cell monolayers. One advantage of the above in vivo and in vitro assays includes the amplification of the virus isolate for future typing and analysis.

Histopathology

Rabies diagnosis based upon the detection of Negri bodies and histological tests such as Sellers’s or Mann’s tests, are no longer routinely performed by the majority of diagnostic laboratories, as they are considered unreliable, particularly for decomposed material.

A protocol suitable for the detection of classical rabies virus and European bat lyssaviruses type 1 and 2 has more recently been described [30]. A robust, highly sensitive and specific in situ hybridisation technique, employing digoxigenin labelled riboprobes was used for the detection of lyssavirus RNA in mouse-infected brain tissue. Using this method, both genomic and messenger RNA were detected. The ability to detect messenger RNA is indicative of the presence of replicating virus.

Nucleic acid based methods

Molecular based tools are becoming more widely acceptable and accessible for the diagnosis of rabies. The use of the reverse transcriptase polymerase chain reaction (RT-PCR), nested or hemi-nested RT-PCR and other PCR based techniques are increasingly reported but are not currently recommended by the WHO for routine post mortem diagnosis of rabies. However, in laboratories with strict quality control procedures in place and demonstrable experience and expertise, these molecular techniques have been successfully applied for confirmatory diagnosis and epidemiological surveys. Reverse transcription (RT)-PCR has been reported to confirm rabies diagnosis intra vitam in suspect human cases, when conventional diagnostic methods have failed and post-mortem material is not available [31]. Rabies virus RNA can be detected in a range of biological fluids and samples (e.g. saliva, cerebrospinal fluid, tears, skin biopsies and urine). Owing to the intermittent shedding of virus, serial samples of fluids such as saliva and urine should be tested but negative results should not be used to exclude a diagnosis of rabies. This was demonstrated in 2001 in the UK when multiple ante mortem saliva specimens taken during the clinical phase of infection in a Nigerian patient failed to yield rabies viral RNA but rabies infection was confirmed using FAT on subsequent post mortem brain specimens [32]. All positive PCR products should be sequenced to confirm the origin of the virus and rule out possible contamination. Such studies are increasingly reported in the literature and are giving new insights into the epidemiology and evolution of this most important virus.

Several RT-PCR methods have been reported over the past decade for the detection of lyssaviruses. These methods invariably involve multiple transfers of nucleic acids between different tubes and coupled with the high sensitivity of PCR methodologies, any small amount of contamination will undoubtedly produce false-positive results. Attempts have been made to adapt RT-PCR to reduce manipulations thereby reducing contamination risks. The visualisation of PCR products by gel electrophoresis exposes facilities and operators to large quantities of amplified material and thus many adaptations have been directed at replacing this step. New and improved rapid diagnostic tools for rabies including PCR ELISA [33] and Taqman technology [34-36] have recently been developed for diagnostic purposes.

A rapid rabies TaqMan assay that distinguishes between classical rabies virus (genotype 1) and European bat lyssaviruses 1 and 2 (genotypes 5 and 6) has recently been reported [37]. This sensitive and specific assay is performed in a single step in a closed tube system, thereby dramatically decreasing the risk of contamination and allows for the genotyping of unknown isolates. Such real time assays can be applied quantitatively and the use of an internal control (e.g. β-actin RNA or 18s ribosomal RNA) enables the quality of the isolated template to be assessed, thereby minimising the chance of false negative results associated with poor sample quality. Real time Taqman assays have been successfully employed in the rapid diagnosis and genotype confirmation of EBLV-2 infected Daubenton’s bats in the UK [38]. The assay was more recently used for the intra vitam detection and genotyping of a human rabies case in the UK in July 2005 within five hours of sample receipt [20] and for the detection of a rabies infected puppy in UK quarantine in 2008 [39].

In 2001 Wacharapluesadee and Hemachudha reported the use of a rapid automated nucleic acid sequence-based amplification (NASBA) technique which was successfully applied to the saliva and CSF of four living patients in Thailand with rabies and detected rabies viral RNA as early as two days after onset of symptoms [40]. This technique differs from RT-PCR in that the viral RNA is directly amplified under isothermal conditions and detected by an automated reader. It is relatively easy to use and the whole process from extraction to detection can take as little as four hours using automated equipment. This technique has also been adapted to investigate rabies virus replication in situ [41].

Serology

Post-infective rabies antibodies are rarely detectable before death, allowing antibody detection methods such as the rapid fluorescent focus inhibition test (RFFIT) or the fluorescent antibody virus neutralisation (FAVN) test to determine post-
vaccinal immunity in vaccinated animals. In previously non-
immunised humans, virus neutralisation assays are sometimes
successful in detecting rabies specific antibodies in the CSF or
serum and thus confirming exposure to a lyssavirus. Neutralising
antibodies, if present in serum tend to appear on average eight
days after clinical signs occur whereas rabies antibodies are
infrequently found in CSF.

Virus neutralisation (VN) assays in cell cultures are more
customarily used to determine levels of immunity in vaccinated
animals (domestic and wildlife). The FAVN and RFFIT are the OIE
prescribed tests for international trade of companion animals. In
both cases, a given quantity of the virus is mixed with different
dilutions of the serum to be tested and the neutralising limit
dilution is determined. Virus escaping neutralisation by the test
antiserum is identified by fluorescence. The results are expressed
in international units (IU) calibrated from standard reference
materials available from the OIE Reference Laboratory in AFSSA,
Nancy, France (for animal tests) or from the WHO (for human
tests). Due to the nature of both tests, dedicated biosecure
facilities and highly trained vaccinated staff are required. Enzyme
linked immunosorbent assays (ELISAs) suitable for the detection
of rabies virus-specific antibodies in serum samples from
companion animals and humans have recently been developed
[42-45]. The companion animal ELISAs have been accepted by
the OIE as a screening tool or alternative to the FAVN but await
approval for the EU Pet Travel Scheme. The major advantages of
the ELISA test are that it can be completed in four hours, does
not require the use of live virus and can be performed without
the need for specialised laboratory containment. This contrasts
with the four day turnaround using conventional rabies antibody
neutralisation assays.

Recently, a virus neutralisation based assay has been developed
utilising lentiviral pseudotypes as antigen [46]. The pseudotype
viruses were constructed using G-protein cDNA sequences from
rabies Challenge Virus Standard-11 (CVS-11), European bat
lyssavirus type-1 or -2 (EBLV-1 and -2), cloned and co-expressed
with HIV/MLV gag-pol and GFP/luciferase in human epithelial
cells. The pseudotypes have three major advantages over the
conventional virus neutralisation assays: they can be handled in
biohazard level 2 laboratories, the use of reporter genes such
as green fluorescent protein (GFP), luciferase or β-galactosidase
allows the assay to be used at low cost in laboratories throughout
the world and the pseudotype assay only requires minimal
volumes (<10µl) of sera. The applicability of the pseudotype
viruses is currently being validated in a collaborative study
involving a number of Rabies Reference Laboratories.

Validation of Diagnostic Assays

Rabies is a notifiable disease in most developed countries and
as such, standardised diagnostic procedures are prescribed by
the OIE. Due to the high sensitivity of PCR based methods,
there is concern that appropriate procedures aimed at reducing
the risk of contamination will not be fully adhered to, thereby
generating false positive results. Consequently, the WHO
expert committee agreed that molecular methods should not
be globally recommended [7]. However, they did recognise the
potential benefits of such methodologies in the hands of trained
staff in appropriately equipped laboratories, both in terms of
diagnosis and molecular epidemiological surveys. As molecular
methodologies are adopted and adapted in increasingly more
laboratories in both developed and developing countries, global
recognition of the importance of quality assurance is essential.
In order to ensure confidence in diagnostic test results, the
OIE published guidelines for quality assurance in veterinary
laboratories (http://www.oie.int/eng/publicat/ouvrages/A_112.
hmt) based on the requirements of the internationally recognised

With respect to serology for international trade, annual proficiency
schemes coordinated by the OIE and Community reference
laboratory AFFSA, Nancy, Malzeville, France, have been in operation
for a number of years. Inter-laboratory ring trials of diagnostic
techniques in European rabies laboratories will also be coordinated
by AFFSA in the near future. Rabies diagnostic proficiency schemes
are in operation in the USA and have highlighted the need for the
standardisation of methodologies. The Centers for Disease Control
and Prevention (CDC) have published a protocol for the FAT on
their website to encourage harmonisation (http://www.cdc.gov/
rncidod/dvrd/rabies/professional/publications/FDA
diagnosis/DFA protocol-b.htm). A report by Rudd et al. emphasised the dramatic
effects that small modifications to the FAT protocol, such as a
change in mountant composition, can have on the specificity or
sensitivity of the test [47].

All laboratories should endeavour to validate their procedures
using the array of virus strains likely to be locally encountered
and not rely on the positive control material (e.g. laboratory
adapted strain) for test validation. Test validation exercises must
be repeated upon the introduction of any modifications to the
test, however minor, and also if the range of lyssaviruses to be
detected is expanded to include, for example, newly isolated
diverging bat strains. As increasing numbers of laboratories
strive to achieve quality assurance standards, the requirement to
participate in inter-laboratory proficiency schemes will facilitate
a more harmonised approach to rabies diagnosis and a greater
confidence in epidemiological data.

Conclusion

Rabies is one of the best known and most feared of viral
infections. It is notifiable and suspect cases must be reported to
competent authorities that are responsible for further diagnosis.
Diagnostic tests based on antigen detection, virus isolation and
histopathology have been the mainstay of diagnosis. However,
these are relatively slow and insensitive, and in some cases require
the use of live animals. There is now an increasing pressure towards
the development and use of modern molecular technologies.
These promise great advances with increased sensitivity providing
for ante-mortem diagnosis. By allowing sequence analysis, they
have also revolutionised our understanding of the evolution and
epidemiology of these viruses, challenging concepts of what
it is to be “rabies free”. Inter-laboratory collaboration and test
standardisation will allow for a better understanding of the global
presence of rabies viruses, and is likely to be necessary to facilitate
their greater control.
Acknowledgements

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Canine leishmaniosis -
a challenging zoonosis

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SUMMARY

*Leishmania infantum* is the causative agent of canine leishmaniosis (CaNL) and human visceral leishmaniasis in the Mediterranean basin and some other parts of the world. Dogs are the major reservoir for this infection. Surveys have revealed that dog infection rates reach 70% in some foci in the Mediterranean basin. Canine leishmaniosis is a good example of a disease in which infection does not equal clinical illness due to the high prevalence of subclinical infection. Population studies have shown that a low proportion of the canine population develops a severe disease while another fraction has persistent subclinical infection. Susceptibility to CaNL has been associated with genetic factors and mutations in several loci. The main clinical signs associated with CaNL are skin lesions, lymphadenomegaly, splenomegaly, ocular abnormalities, abnormal nails growth (onychogryphosis) and poor body condition. Additional findings include: epistaxis, renal failure, decreased appetite, polyuria and polydypsia, vomiting, melena and lameness. The diagnosis of CaNL is performed by microscopic detection of parasites, serology and PCR. Several drugs including allopurinol, meglumine antimoniate, amphotericin B, and miltefosine are used for treatment of CaNL. However, although most dogs recover following therapy, complete elimination of the parasite is usually not achieved and infected dogs may relapse following treatment. Prevention of CaNL includes the use of topical insecticides against sand flies, such as pyrethroid collars, spot-on formulations and sprays. In addition, new canine vaccines are being developed and commercially introduced or evaluated in several countries.

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Introduction

Leishmaniosis has a long history of association with mankind. The disease is known to have been present in Africa and India since at least the mid-eighteenth century [1]. In 1903, Leishman and Donovan separately described the protozoan now termed *Leishmania donovani* in splenic tissue from human patients in India [2, 3]. In 1908, Nicolle and Comte in Tunisia reported infection by *Leishmania* in dogs for the first time [4].

*Leishmania* infections caused by different *Leishmania* species are present in a variety of biogeographical regions of the Old and New Worlds. These are responsible for the wide spectrum of clinical illnesses observed in people. Human leishmaniasis is the third most important vector-borne disease after malaria and lymphatic filariasis. Based on clinical signs in humans, the disease can be divided into cutaneous, mucocutaneous and visceral forms. An estimated 12 million human cases of leishmaniosis exist worldwide, with an estimated number of 1.5-2 million new cases occurring annually: 1-1.5 million cases of cutaneous leishmaniosis and 500,000 cases of visceral leishmaniosis [5]. Epidemiologically, two different situations occur: (i) The zoonotic form of visceral leishmaniosis found in the Mediterranean basin and South America, with the dog as the main source of *L. infantum* infection for the female sand fly; and (ii) the anthropopotic form found in East Africa, Bangladesh, India and Nepal where *L. donovani* transmission is passed from person to person through the sand fly vector [5]. Anthropopotic visceral leishmaniosis caused by *L. donovani* is responsible for a large part of the fatalities due to the visceral disease in people [6].

Visceral leishmaniosis caused by *L. infantum* in the Mediterranean basin was traditionally predominantly a disease of young children

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and the name of the causative agent of this disease reflects the predilection to infants. Malnutrition has been recognized as risk factor for infantile leishmaniosis, and may explain why this disease is more prevalent among children in poor countries as compared with affluent ones despite high prevalence rates in the dog populations [6]. With the appearance of the AIDS epidemic, adult HIV+ patients are now the predominant risk group for human visceral leishmaniosis in Southern Europe. The co-infection of HIV and leishmaniosis reported from more than 33 countries where these infections geographically overlap has been described as a “deadly gridlock” that does not respond well to therapy [7, 8].

CaNL is a major zoonotic disease endemic in more than 70 countries in the world. It is present in regions of southern Europe, Africa, Asia, South and Central America [9] and has recently emerged in the USA [10, 11]. CaNL is also an important concern in non-endemic countries where imported disease constitutes a veterinary and public health problem [12]. Dogs are the main reservoir for human visceral leishmaniosis and the disease is usually fatal if not treated in people and dogs. Phlebotomine sand flies are the proven vectors of Leishmania infantum, the causative agent of CaNL in the Old World and for its New World synonym Leishmania chagasi. The majority of epidemiological studies about the prevalence and incidence of Leishmania infection in dogs have been based on serologic surveys. The seroprevalence in the Mediterranean basin ranges from 10% to 40% depending on the region [13]. Surveys employing other methods to calculate the prevalence of Leishmania infection by detecting Leishmania DNA in different tissues [14-16] or by detecting specific Leishmania cellular immunity (17-19) have revealed even higher infection rates approaching 70% in some foci. It has been estimated based on seroprevalence studies from Italy, Spain, France and Portugal that 2.5 million dogs in these countries are infected [20] and L. infantum infection is spreading north in Europe reaching the foothills of the Alps in Northern Italy [21]. The number of infected dogs in South America is also estimated in millions with high infection rates in some areas of Brazil and Venezuela [22].

Public health considerations
Transmission of L. infantum from dogs or wildlife animal reservoirs via sand flies is the main route for human infection. People in endemic regions share the same habitat and are frequently in close physical contact with infected dogs [23]. Several studies have investigated the association between canine and human leishmaniosis in the same region and examined to what degree infection in dogs poses a risk for human disease [23]. These studies reported that (i) increased prevalence in canine population is associated with increased incidence of human leishmaniosis [22, 24], (ii) poor socioeconomical conditions are risk factors for the association between canine and human infections [22] and (iii) dog density and infected dog ownership are risk factors for infantile human leishmaniosis [25, 26]. Therefore, effective control of canine leishmaniosis could lead to a decrease in human leishmaniosis in endemic regions. Control measures of CaNL could include effective vaccines, topical insecticides, and environmental control of sand flies.

Transmission of canine leishmaniosis
Female sand flies from the genera Phlebotomus (Old World) or Lutzomyia (New World) are the principal agents of transmission of Leishmania in humans and dogs [27]. The activity of the adult sand flies is crepuscular and nocturnal from early spring to late autumn in the Mediterranean basin and all year round in South America [27].

Leishmania is a diphasic parasite that completes its life cycle in two hosts, a sand fly which harbors the flagellated extracellular promastigotes and a mammal where the intracellular amastigote parasite forms develop. Dogs are infected by Leishmania promastigotes deposited in the skin during the bites of infected female sand fly vectors. The promastigotes invade host cells and replicate as intracellular amastigotes. The transmission of Leishmania metacyclic promastigotes by phlebotomine sandflies is extensively reviewed elsewhere [28].

An important medical and epidemiological question is whether dogs found positive to the Leishmania parasite with different techniques such as serology and/or PCR are also infective to phlebotomine sand flies. This information would provide the best indicator of the burden of infectiousness in a dog population in an endemic area. Seropositive dogs with and without clinical signs are infectious to sand flies [29-31]. The rate of infected sand flies increased with the appearance and severity of the clinical signs [30] and with the decrease in CD4+ T cells counts [32]. No studies have been published on transmission from seronegative subclinically infected dogs. Further studies are needed to ascertain the potential of seronegative subclinically infected dogs to transmit Leishmania to vector sand flies.

Other less common transmission routes have been reported in dogs. The transmission of L. infantum through blood products has been reported in dogs that received blood transfusions from infected donors in North America [33, 34]. Vertical in-utero transmission from dam to its offspring has been documented in one study [35] but disputed by other authors [36]. In addition, ticks and fleas have been proposed as alternative vectors of Leishmania transmission but evidence of such transmission is lacking [37, 38]. Direct transmission without involvement of a hematophageous vector has been suspected in some cases of infection in areas where vectors of the disease are apparently absent [11].

Disease versus infection by L. infantum
The classical stages of an infectious disease process include the initial infection, an incubation period and a clinical disease. Numerous studies on these stages in relation to Leishmania have indicated that Leishmania infection and disease are not always considered synonymous in rodents, humans and dogs. The concept that all dogs infected with Leishmania infantum will eventually develop severe clinical leishmaniosis after a variable incubation period [39, 40] has been disproved. Specific anti-Leishmania cellular immunity was demonstrated in apparently healthy dogs naturally infected with Leishmania [18, 41]. Leishmania infantum infection in dogs can display a broad
spectrum of immune responses and clinical manifestations from a clinically healthy condition to a severe clinical disease. This is similar to findings in human infection where clinical disease represents one pole and subclinical infection the other pole of infection [42].

Leishmania infection in dogs may vary between a subclinical infection and a severe fatal disease [9]. In dogs, the two opposite extreme poles of this spectrum are characterized by: (i) protective immunity that is T cell mediated and (ii) disease susceptibility that is associated with the production of a marked humoral non-protective immune response and a reduced or depressed cell mediated immunity (CMI) [9, 13, 44]. For example, clinical disease can range from a mild papular dermatitis associated with specific cellular immunity and low humoral responses [43] to a severe/fatal disease characterized by renal pathology with glomerulonephritis due to immune complex deposition mainly with an extensive humoral response and high parasite loads [45-47].

Population studies in Leishmania-endemic areas have shown that a low proportion of the canine population develops a severe disease and another fraction has subclinical infection. The immune responses mounted by dogs at the time of infection and thereafter appear to be the most important factor in determining if they will develop a lasting infection and whether and when it will progress from a subclinical state into a disease condition. Animals that are predisposed and will develop severe disease are considered "susceptible". Dogs able to control/restrict the infection and remain constantly subclinical, have been termed "clinically resistant" [18, 19]. However, subclinical infection is not necessarily permanent and factors such as immunosuppressive conditions or concomitant disease could break the equilibrium and lead to the progression of clinical disease in dogs as observed in human patients with AIDS and Leishmania co-infection [7].

Dogs with severe disease or progressing toward overt disease have high antibody levels, high parasite load in numerous tissues [48, 49] but decreased or absent leishmanial specific lymphocyte proliferation and delayed type hypersensitivity (DTH) reaction [41, 50, 51]. Conversely, healthy infected dogs (resistant) produce specific lymphocyte proliferation, strong DTH reaction, variable anti-parasite antibody levels [17-19, 41] and lower parasite loads when compared with sick or susceptible dogs [48, 49].

Specific immune responses play a major role in susceptibility and resistance to infection. An experimental model of cutaneous leishmaniosis in mice infected with L. major has shown that a susceptible mice strain (BALB/c) which typically develops a T helper type 2 cellular response will succumb to infection with secretion of specific cytokines such as IL-4 and IL-10, and production of a significant antibody response. Another mice strain (C3H) that responds with a different set of cytokines typical of a T helper type 1 response such as IFN-γ; IL-2, is resistant to infection [52]. This general model appears to be only partially valid for CaNL and human visceral disease where studies of the immune response have shown mixed Th-1 and Th-2 types of reaction. An update on canine immune responses in L. infantum infection is reviewed elsewhere [9, 44].

Immune-mediated mechanisms are responsible for much of the pathological findings in CaNL. Circulating immune complexes [53] and antinuclear antibodies [54, 55] have been detected in animals with CaNL. Glomerulonephritis associated with the deposition of immune complexes in the kidneys is a hallmark of CaNL [45-47]. Vasculitis induced by immune complexes which activate the complements cascade is an important pathological mechanism responsible for tissue necrosis and accountable for dermal, visceral and ocular lesions found in this disease [56-58].

It is not known for certain what mechanisms in dogs are responsible for protection or susceptibility, nor the way factors such as age, gender, nutrition, host genetics, coinfections and concomitant disease, immunosuppressive conditions, cytokine environment, parasitic burden, nature of Leishmania antigens or different Leishmania strains, previous infections and way of
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alopecia which can be generalized or localized over the face, ears and limbs (Fig. 1 and 2), (ii) ulcerative dermatitis, nodular dermatitis, (iii) mucocutaneous proliferative dermatitis and (iv) papular dermatitis [43].

The most common ocular manifestations are anterior uveitis, blepharitis (exfoliative, ulcerative, or nodular) and keratoconjunctivitis, either common or sicca [70-72]. About 25% of dogs with clinical leishmaniosis have ocular and periocular lesions including keratoconjunctivitis and uveitis [72]. Ocular consequences of systemic hypertension such as retinal detachment and/or hemorrhages, retinal arterial tortuosity and hyphema are present in the disease but not diagnosed frequently [73].

The main clinicopathological findings of canine leishmaniosis are hyperglobulinemia (polyclonal gammaglobulinemia), hypoalbuminemia, decreased albumin/globulin ratio, mild to severe proteinuria, non-regenerative anemia, renal azotemia, elevated liver enzyme activities, thrombocytopenia and thrombocytopenia [68-70, 74, 75].

Some degree of renal pathology is present in most dogs with CaNL and subsequent renal failure due to immune-complex glomerulonephritis eventually develops and is believed to be the main cause of death in dogs with CaNL [45-47]. Epistaxis, ocular abnormalities or renal failure may be the only presenting clinical findings in CaNL and this disease should be considered among the differential diagnoses for these conditions in endemic areas or in dogs that have traveled or were imported from an endemic region. Marked hyperglobulinemia with no apparent cause in dogs from endemic regions should also be investigated for CaNL.

It is sometimes difficult to define if dogs have clinical disease due to CaNL because they may be infected with the parasite without clinical signs and be ill due to other causes, or because other diseases may present similar clinical signs. In the past,

Clinical findings in canine leishmaniosis

The clinical features of leishmaniosis vary widely as a consequence of the numerous pathogenic mechanisms of the disease process, the different organs affected, and because of the diversity of immune responses of individual hosts, whether humans or other mammals [66].

The main clinical findings found on physical examination in classical CaNL include skin lesions (Fig. 1 and 2), local or generalized lymphadenomegaly, loss of body weight, exercise intolerance, decreased appetite, lethargy, splenomegaly, polyuria and polydypsia, ocular lesions, epistaxis, onychogryposis, lameness, vomiting and diarrhea [67-70].

Skin lesions are the most frequent manifestation of CaNL in dogs brought for treatment due to the disease. Several dermatological entities have been described [39]: (i) exfoliative dermatitis with

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and ultrasound can assist in raising the suspicion index for this disease. Numerous diagnostic techniques have been developed to help in the diagnosis of canine leishmaniosis (Tab. 1). It is important to understand the basis of each diagnostic test, the limitations and the appropriate clinical interpretation.

Leishmania amastigotes can be demonstrated by cytology from cutaneous lesions (Fig. 3), spleen (Fig. 4), lymph nodes (Fig. 5), bone marrow [13, 78] and less commonly in other tissues [79-81] or body fluids such as joint, cerebrospinal and abdominal fluids [82-86] stained with Giemsa, or modified Wright’s stain, or a quick commercial stain. Detection of amastigotes by cytology is frequently unrewarding due to a low to moderate number of detectable parasites present even in dogs with a full blown clinical disease [87, 88].

Leishmania parasites may also be viewed in histopathologic formalin-fixed, paraffin-embedded biopsy sections of the skin or other infected organs. Leishmania parasites should be suspected in pyogranulomatous, granulomatous or lymphoplasmacytic inflammations in different tissues [79, 80, 89-91] or lymph node reactive hyperplasia [92, 93] on cytological or histological preparations. Definite identification of parasites within tissue macrophages may be difficult and an immunohistochemical staining method can be employed to detect or verify the presence of Leishmania in the tissue [94].

Various serological methods for the detection of anti-Leishmania antibodies have been developed. These include the indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), direct agglutination assays (DAT) and western blotting [95]. In general, good sensitivities and specificities are gained with these methods for the diagnosis of clinical CaNL [23]. High antibody titers are usually associated with disease and a high parasite density [49, 96] and, for this reason; they are conclusive of a diagnosis of leishmaniosis. However, the presence of lower antibody levels is not necessarily indicative of patent disease and needs to be confirmed by other diagnostic method such as PCR, cytology or histology [23, 77]. Serological cross-reactivity with different pathogens is possible with some serological tests, especially those based on whole parasite antigen. Cross reactivity has been reported with other species of Leishmania [97-99], and Trypanosoma cruzi [98].
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Evaluation for dogs [23]. Immunotherapy is also under extensive investigation in dogs with promising results [116, 117].

Keeping and treating infected dogs presents a dilemma to owners, veterinarians and public health officials especially in areas where suitable vectors are found, because of the risk of transmission to people and pets in the community. Treated dogs often remain carriers of the disease and commonly experience clinical relapses. However, it appears that they are less infectious to sandflies [118, 119]. The use of adequate treatment is advocated for the control of canine leishmaniasis and to reduce risks of L. infantum transmission [119]. Owners must receive a thorough and realistic explanation about the disease, its zoonotic potential, the prognosis for their dog, and what should be expected from treatment.

Prevention of canine leishmaniosis

The use of topical insecticides against CaNL [120] in collars [121] or spot-on formulation has been shown to be effective in reducing disease transmission [122, 123]. Deltamethrin-impregnated collar significantly reduced the number of dog sand fly bites under experimental conditions [124, 125] and decreased infection transmission in field studies [121]. In a study supported by the WHO in Iran, collaring of dogs in intervention and control villages significantly reduced the seroconversion rate in dogs and in children living in the intervention villages [126]. A commercial vaccine against CaNL has recently been approved in Brazil [127, 128] and several vaccine candidates are under experimental [129] or field evaluation in Europe [130].

Therapy

Anti-leishmanial treatment often achieves only clinical improvement in dogs with leishmaniosis and it is frequently not associated with the elimination of the parasite. The main drugs used against canine leishmaniosis include the pentavalent antimony meglumine antimoniate which selectively inhibits leishmanial glycolysis and fatty acid oxidation and allopurinol that acts by inhibiting protein translation through interfering with RNA synthesis (Tab. 2). The combination of antimony meglumine antimoniate and allopurinol is the most common treatment protocol used against canine leishmaniosis in Europe [115]. Amphotericin B, which acts by binding to ergosterol in the parasite’s cell membrane and altering its permeability is also used but it is highly nephrotoxic. New drugs are currently under evaluation for dogs [23].

Keeping and treating infected dogs presents a dilemma to owners, veterinarians and public health officials especially in areas where suitable vectors are found, because of the risk of transmission to people and pets in the community. Treated dogs often remain carriers of the disease and commonly experience clinical relapses. However, it appears that they are less infectious to sandflies [118, 119]. The use of adequate treatment is advocated for the control of canine leishmaniasis and to reduce risks of L. infantum transmission [119]. Owners must receive a thorough and realistic explanation about the disease, its zoonotic potential, the prognosis for their dog, and what should be expected from treatment.

Prevention of canine leishmaniosis

The use of topical insecticides against CaNL [120] in collars [121] or spot-on formulation has been shown to be effective in reducing disease transmission [122, 123]. Deltamethrin-impregnated collar significantly reduced the number of dog sand fly bites under experimental conditions [124, 125] and decreased infection transmission in field studies [121]. In a study supported by the WHO in Iran, collaring of dogs in intervention and control villages significantly reduced the seroconversion rate in dogs and in children living in the intervention villages [126]. A commercial vaccine against CaNL has recently been approved in Brazil [127, 128] and several vaccine candidates are under experimental [129] or field evaluation in Europe [130].

References


<table>
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<tr>
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<td>2. Allopurinol</td>
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<tr>
<td>3. Combination of meglumine antimoniate and allopurinol</td>
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<td>4. Amphotericin B</td>
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<td>4. Marbofloxacin</td>
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<tr>
<td>5. Ketoconazole</td>
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Tab. 2 First and second line drugs for the treatment of canine leishmaniosis.
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Toxoplasmosis

*F. van Knapen,*(1) *P.A.M. Overgaauw*(2)

**Introduction**

*Toxoplasma gondii* is an obligate intracellular protozoan parasite and part of the Sporozoa family. There are three known stages of this parasite with different appearances.

During an active *Toxoplasma* infection the free parasites (tachyzoites) replicate by division in the host cell until this cell is lysed (Fig. 1). Extracellular parasites released by the host cell lysis actively invade new host cells and cause severe tissue damage. There is no preference for cell types or organ systems. The tachyzoites are approximately 10 microns long and 2 to 3 microns wide and have a characteristic moon shape.

A second stage is characterized by tissue- and organ cysts of 10-100 microns in diameter; the cysts have a clear wall in which hundreds of slim parasites without significant metabolism are piled up (so called rest phase or latent toxoplasmosis; (Fig. 2). These cysts remain present for many years and even stay infectious for a long period after the host has died (for example in meat).

A third stage of *T. gondii* is only found in cat species (Felidae) and more specifically in the genera Felix and Lynx.

**Epidemiology**

The consumption of, tissue cysts infected, undercooked meat [2], especially from herbivores, is the most common route of infection in man and carnivorous animals. Herbivores infect themselves with oocysts from the environment. Significant variation in frequency of toxoplasmosis infected animals has been reported from different studies and countries. Especially farm animals grazing outdoors may become infected, while production animals housed indoors during their (short) life are usually not infected with *Toxoplasma*. Herbivores and vegetarians can also get infected by the intake of sporulated oocysts shedded by cats (via soil, hands, vegetables etc.).

Cats only shed oocysts once during their lifetime, and only during a limited period of time (2-3 weeks) when they have lost their passive immunity and have started to hunt or to eat raw meat (ingestion of tissue cysts). Most of the spontaneous excretors found in surveys are younger than 6 months of age.
During the excretion period they build up enough immunity to resist a new infection. There are some exceptions to this, i.e. primary infection at a very young age, long-term treatment with corticosteroids, secondary infection with Cystoisospora, etc. In these cases even mature cats may start shedding oocysts again [4, 6]. Infection of a non-immunized cat with oocysts results in a significant antibody response, although no oocysts will be shedded [10]. In the Netherlands 60% of all one-year old cats have passed this period, thus they do not longer play an important role in the epidemiology of toxoplasmosis (Van Knapen, 1974 and 1993, unpublished data). Not in all countries seroconversion is seen in the first year of the cat’s life, as this is also depending on outdoor access, contact with other cats and the source of feed they obtained [9, 3]. This results in a persistent contamination of the environment where (young) cats deposit their faeces. In man it is estimated that 30-60% of all adults have been infected with Toxoplasma. Depending on the genetic type, the amount and virulence of the ingested parasites, clinical symptoms can be expected within 2 to 3 weeks after infection. The enteroepithelial phase of T. gondii is not usually associated with enteric disturbance [13]. The intermediate tissue cyst stage, however, may produce clinical signs in the cat as well as a variety of other animals, including the dog. The clinical signs are in that case non-specific and reflect damage to the organ systems infected, including the central nervous system, musculature, liver, lungs and the eye.

**Pathogenesis, pathology and clinical features in man**

During an acute invasion of Toxoplasma-parasites (proliferative phase, tachyzoites), there is mild to major tissue damage (necrosis). Histology shows inflammatory infiltrates consisting of round cells with free parasites and cyst formation on the borders. Probably due to a combination of cellular and humoral immune responses, the parasites are forced into a resting stage (tissue cysts). This latent toxoplasmosis is characterized by more or less round cysts with a firm wall, around which (for example in the brain) no inflammatory response is visible (Fig. 2).

Since Toxoplasma-parasites show no cell type preference, the clinical signs are variable. An adult person has enough immunity to resist an infection and far most infections proceed without any clinical symptoms. Occasionally parasitaemia is seen when chronic (latent) infections are reactivated. Special attention should be paid when a latent infection flares up again due to immunosuppression. This can be the result of another (viral) infection, or from a long-term treatment with for example corticosteroids or cytostatics.

When clinical symptoms occur after an acute, acquired toxoplasmosis infection, most evident signs are lymphadenitis, fever and malaise. In rare cases a severe hepatitis, splenitis, pneumonia, polymyositis or even meningo-encephalitis can occur.

![Fig. 1 Toxoplasma tachyzoites one hour and 24 hours after infecting a macrophage cell line.](image1)

![Fig. 2 Toxoplasma tissue cyst containing numerous bradyzoites in brain material.](image2)
A special form of toxoplasmosis is congenital toxoplasmosis. If a woman receives her first exposure to *Toxoplasma* while pregnant, the uterus and the unborn foetus(es) can become infected. In early pregnancy this can lead to severe malformation of the foetus leading to abortion or to malformations that are not compatible with life shortly after birth. A majority of congenital infections however go undiagnosed for years after birth, before clinical symptoms related to congenital toxoplasmosis are found (mental retardation, ocular defects) [8].

**Diagnosis in man**

Despite the high incidence of infections, clinical toxoplasmosis is rare. Because the clinical signs of toxoplasmosis resemble other infectious diseases, laboratory tests are necessary for a diagnosis. Detection of specific antibodies alone is not enough, because many individuals (people and animals) already have an antibody titer. A new infection can be distinguished by the detection of increasing amounts of antibodies (seroconversion) of the different isotypes (IgG, IgM, IgA) or by circulating antigens. The detection of free parasites (tachyzoites) in combination with clinical symptoms can confirm an infection (for example in biopsy or abortion material). The detection of tissue cysts (just like antibodies alone) does not confirm an active infection. PCR-tests are available for examination of the foetus, the placenta or amniotic fluid.

**Diagnosis in animals**

Since clinical symptoms are mostly absent, the only way to demonstrate toxoplasmosis in animals is by serological testing. Seropositivity is an indication for past experience and adult animals of all kinds show moderate to high seroprevalences. In cats the excretion of oocysts can be detected by examination of the faeces. Oocysts are only shed during a short period of time, so this examination is not useful when looking for the source of clinical toxoplasmosis in a family.

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**The role of dogs and cats in human toxoplasmosis**

It is obvious that the major source of human *Toxoplasma* infection is the consumption of raw or improperly cooked meat [2, 15]. However, oocysts shed by cats may be a source of soil transmitted disease in man (children) like this is the source for herbivorous livestock and rodents to become infected. Indeed Taylor et al. [14] indicated a parallel in seroprevalence studies in Ireland of specific *Toxocara* and *Toxoplasma* antibodies in children between 4-18 years of age, suggesting that contaminated soil may have been a common source. Sporulated oocysts certainly contaminate the direct environment of man (garden, sandpits) although very little research is done in this field. Having a cat has never been associated with a higher risk of getting a *Toxoplasma* infection. On the other hand, however, having a dog does in some studies correlate with an increased risk of having antibodies to *Toxoplasma* [7]. Dogs definitely do not play a biological role in the transmission of *Toxoplasma*. Soil transmitted infections, like *Toxocara* have recently been demonstrated in the fur of dogs, probably because of dogs behaviour on playing grounds [11, 12].

If there is indication of soil transmitted infections through dogs’ fur, it cannot be excluded that the same is true for toxoplasmosis. Therefore hygiene precautions (e.g. hand washing) should be taken after contact with dogs as a preventive measure for toxoplasmosis.

**Prevention**

Toxoplasmosis in man can be prevented by not eating raw or undercooked meat (do not even taste it!). Especially pregnant women should be careful because of the possibility of transplacental transmission. Furthermore, contact with sporulated oocysts should be avoided. This can be done by cleaning the cat litter box daily (within that time no sporulation has taken place), but far more important is it to pay extra attention to personal hygiene when gardening (do not eat or smoke while gardening, wear gloves) and do not eat unwashed, raw vegetables. Wash hands after contact with dogs.

Toxoplasmosis in cats can be prevented by feeding them solely pre-fabricated food or cooked meat and by preventing cats from hunting and eating prey animals. Small rodents and birds form an important source of infection in cats, just like oocysts infected soil (licked from their fur).

There are no disinfectants effective against *Toxoplasma* oocysts. Boiling water or steam kill them immediately.
References


Toxoplasmosis – an update

G. Miró, A. Montoya, M. Fisher, I. Fuentes

Introduction

Toxoplasmosis is an important parasitic disease caused by the protozoan Toxoplasma gondii. The parasite was first discovered by Nicolle and Manceaux in 1908, in the gundi (Ctenodactylus gundi), a North African rodent. The name Toxoplasma gondii ("toxon" = arc; "plasma" = form in Greek) is derived from its crescent shape and from the animal from which was first isolated. The complete life cycle of T. gondii was not fully described until 1970 [1,2].

The first record of canine toxoplasmosis was made by Mello (1910), who described an acute toxoplasmosis in a dog in Turin, Italy. The dog presented with fever, anorexia, anemia, dyspnoea and haemorrhagic diarrhoea. T. gondii was identified in sanguineous exudates and in nodules in the lungs [3]. The first feline toxoplasmosis case was not reported until 1942, by Olafson and Monlux. After a brief illness characterized by anorexia, fever and a cough, the cat died. At necropsy, tumour-like enlargements of the mesenteric lymph nodes, ulceration of the intestinal mucosa, and multiple nodules in the lungs were observed. T. gondii was identified in the lymph nodes and lungs [4].

Life cycle of Toxoplasma gondii

The complex life cycle of Toxoplasma gondii involves two phases. The sexual phase takes part in the Felidae family, and the asexual phase in any warm-blooded animal, i.e. mammals (including humans) and birds.

There are three infective stages:
- Sporulated oocysts from infected cats. The oocysts are excreted unsporulated and uninfective when passed in feces. Sporulation occurs in the environment after 1-5 days (dependent on temperature and moisture; e.g. 1 day at 24-25°C, 5 days at 15°C and 21 days at 11°C).
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- Bradyzoites (“brady” = slow in Greek) from tissue cysts.
- Tachyzoites (“tachos” = speed in Greek), the rapidly dividing forms that multiplies in any cell of the intermediate host and non-intestinal epithelial cells of the definitive hosts.

The sexual phase or entero-epithelial cycle occurs in the cat’s intestine after ingestion of any infective form. Zoites penetrate the epithelial cells of the small intestine and initiate a sequence of numerous generations (termed Types A to E) [5], whereafter a variable number of schizogonies, microgamonts and macrogamonts are formed. The microgamonts fertilize the macrogamonts and oocysts are formed. Unsporulated oocysts are shed with the feces into the environment, with time to sporulation depending on environmental temperature and humidity. Sporulated oocysts are more resistant to the environmental and chemical conditions than are unsporulated oocysts; their thermic tolerance range being – 4 to 55ºC. In liquid medium they can survive for several years [6] (Fig. 1).

The asexual phase or extra-intestinal cycle occurs in any warm-blooded animal after infection by any infectious stage. After ingestion, sporozoites are free in the intestinal lumen and divide by an asexual process (endodyogeny). Tachyzoites multiply rapidly, invading different cells during active infection. Once host immunity develops the process slows down and during chronic infection and cysts containing bradyzoites are formed [7].

The prepatent period is around 18 days in cats fed infectious oocysts as compared with 3 to 10 days in cats that are fed tissues cysts [8].

Epidemiology

Cats and wild felidae play a crucial role in the epidemiology of *T. gondii*, because they are the only hosts that can shed oocysts in the environment (Fig. 2).

All nonfeline hosts are intermediate hosts that harbor tissue cysts. It is generally assumed that cats probably play a major role in transmitting the parasite through faecal contamination of soil, food or water. In studies carried out so far, there is no toxoplasmosis in areas where there are no cats [9]. However, Prestud et al. (2007), reported other possible sources of infection due to *T. gondii* in the Arctic [10].

Cats and dogs can be infected by *T. gondii* by different routes:
- Ingestion of water, food, vegetables contaminated with sporulated oocysts from cats faeces.
- Ingestion of infected tissues with tissue cysts containing
Pathogenesis

In the majority of acute infections acquired after ingestion of tissue cysts or sporulated oocysts, the main infection route is the intestine. Tachyzoites multiply actively, and are disseminating through the lymphatic system to the different organs where they produce cell necrosis caused by the intracellular growth of the parasites. Heavily infected animals may die during this phase. During this time parasites can be excreted through different biological fluids (faeces, urine, etc.), but these tachyzoites are very labile and are easily destroyed. For that reason transmission to other hosts is very unlikely, even when the animals are very close together. This fact has been demonstrated under experimental conditions (e.g. sick mice living in the same cage).

The sub-acute phase of the disease is characterized by the appearance of IgA antibodies specific for *T. gondii* enterocyte stages, which eliminate tachyzoite replication in the intestinal phase but not those localized in the nervous system.

The chronic form starts with tachyzoites beginning to disappear from visceral tissues and is characterized by persistence of bradyzoites within cysts. These may be long-lasting; up to 10 months in the dog and 3 years in rats and pigeons, or maybe for life. This phase is associated with a systemic immune response, which inhibits tachyzoites proliferation in blood and tissues (liver, spleen, lungs, etc.) [27].

**Clinical signs**

*T. gondii* is the most important coccidian of the cat as it is the cause of one of the most significant zoonoses. However, clinical signs related to infection are normally not severe in the feline host, although cats may have diarrhoea alternating with normal faeces. Oocysts excretion from an infected cat occurs 3-10 days post infection, and is maintained for a period up to 3 weeks. Cats can also be asymptomatic during this phase [28].

The reason why infected dogs or cats develop clinical disease is unknown but may depend on factors such as: age, sex, strain of *T. gondii* infection rate, infection acquisition (post-natally acquired infection is less severe than pre-natally acquired infection), stress, concomitant illness (retrovirus, FIP, mycoplasmosis, distemper, leishmaniasis, ehrlichiosis, etc) [29-33] or immunosuppression (glucocorticoid or cyclosporine therapy) [34-36].

**Cat**

When infected cats act as intermediate hosts they may suffer a more or less severe clinical picture (clinical toxoplasmosis) such as: fever (40 to 41°C), lethargy, dyspnoea, lymphadenomegaly, vomiting, diarrhoea, icterus, respiratory tract disease, neurologic signs (stupor, ataxia, seizures, circling, partial or total blindness, etc.) (Fig. 3), ocular signs (anterior uveitis, retinchoroiditis etc.) (Fig. 4). Cats with ocular lesions have a higher seroprevalence than cats with normal eyes [37]. These animals with toxoplasmosis have circulating immunocomplexes that may play an important role in development of ocular lesions, and ocular signs may occur without polysystemic clinical signs of disease [38].

<table>
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<th>Reference</th>
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<td>IFAT</td>
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<td>42</td>
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DT: Sabin Feldman Dye Test; IFAT: Indirect Fluorescent Antibody Test; ELISA: Enzyme-linked Immunosorbenent Assay; MAT: Modified Agglutination Test; LAT: Latex Agglutination Test.

Tab. 1 Seroprevalence to *T. gondii* in cats from Europe.

bradyzoites from mammals and birds. This is the most frequent form of infection.

- Ingestion of infectious oocysts directly from faeces: dogs ingesting cat litter could serve as mechanical vectors for transmission to humans as they shed ingested sporulated oocysts in their stool [11].
- Congential infection: parasitaemia during pregnancy can cause transfer and spread of tachyzoites to the fetus [12, 13].

Other minor important routes of transmission include tachyzoites shed in milk and ingestion through sucking [14], and blood transfusion.

There are numerous studies on the seroprevalence of *T. gondii* in felines reported from many countries in Europe, however these studies are difficult to compare due to the differences in the diagnostic techniques used, in the size and origin of the samples, and time of the study conducted (Tab. 1). In all of these reports the frequency of finding oocysts in faeces is low, however, probably because oocysts are shed during a short period of time [15].

In general, the cats that have a major influence in the epidemiology of the disease are naive and farm cats. This fact is explained by the preying habits of this group and their diet that includes wild birds, rodents and *Toxoplasma*-infected placentas and stillborn fetuses [15, 16]. The higher seroprevalence reported in adult cats indicates that the risk of exposure to *T. gondii* increases with age. Sex is not considered to be a determining factor of infection [17]. Nonetheless, some authors suggest a higher prevalence in males associated with their territorial habits, as they have a wider area of operation than females [18].
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All these clinical signs may persist for several days up to several months. Theoretically any organ may be infected, thus clinical signs are very variable; even though generalized infections are quite rare in cats. Central nervous system toxoplasmosis is not common in cats, with only 7% of histology confirmed cases of toxoplasmosis showing neurological signs [39].

The disease is more severe in cats co-infected with retroviruses (mainly FIV) [29, 30] or feline infectious peritonitis (FIP) [33].

Epidemiological studies have shown that clinical toxoplasmosis is more prevalent in stray adult cats or owner cats than have access to prey. This higher prevalence is correlated with a higher risk of being infected, but not with age or sex.

Likewise, clinical toxoplasmosis is much more severe in kittens that have acquired the infection by the congenital route where the disease can be severe and fatal. Clinical illness varies with the stage of gestation at the time of infection, and some newborn kittens may even shed oocysts [12]. In these cases, animals may be stillborn or die within a few hours of birth [40]. The most typical clinical signs are anorexia, lethargy and sudden death. At necropsy the liver is the most frequently affected organ with diffuse hepatitis (more than 75% of the liver is destroyed due to T. gondii infection) followed by gross lesions in the lungs (diffuse edema and congestion).

Encephalitis in newborn kittens has been described, animals may sleep continuously or present with signs of hyperaesthesia with atypical crying and incoordination. These clinical signs prevent feeding and affected kittens can die within few days. Cases of infertility in queens have also been described.

Diagnosis

Lappin (1990) suggested three criteria for diagnosing clinical toxoplasmosis: (i) clinical signs of toxoplasmosis, (ii) serological evidence of recent or active infection, and (iii) response to anti-Toxoplasma drugs [41].

Clinical signs, clinico-pathological findings and complementary examinations

Toxoplasmosis should be suspected in dogs and cats with anorexia, fever, dyspnoea, abdominal discomfort, hepatitis with icterus, pancreatitis, anterior uveitis, retinochoroiditis and central nervous system (CNS) disease manifestations. Thoracic radiographs and abdominal ultrasonography may aid in the diagnosis of toxoplasmosis in suspected cases.

The main haematological and biochemical abnormalities that have been reported are non regenerative anaemia, neutrophilic leucocytosis, lymphocytosis and eosinophilia. Severe leucopenia could be present in animals during the acute phase of the disease [42]. Leucocytosis is mainly seen in the recovery phase.

Dog

Mild infections are almost always asymptomatic, but in severe cases clinical signs in dogs include: respiratory disorders (in 50% of the cases), digestive disorders (in 25%) and neurological disorders (in 25%). This is common in young dogs with generalized toxoplasmosis and on some occasions several clinical signs may occur concurrently (unpublished observation). Because of this, it is important to consider other diseases, including distemper and neosporosis as differential diagnosis.

The onset of clinical toxoplasmosis may be insidious, with clinical signs such as fever, anorexia, dyspnoea, vomiting, diarrhoea, seizures and ataxia. In contrast to the frequency of ocular toxoplasmosis that occurs in cats, there are few cases described in dogs, but occasional cases appear, with clinical signs being similar to what is seen in the cat.

Fig. 3 Adult cat with clinical toxoplasmosis.

Fig. 4 Uveitis due to toxoplasmosis in a cat (Courtesy Dr. A. Rodríguez).
A biochemical profile characterized by hypoproteinaemia and hypoalbuminaemia is typical of the acute phase, while hypergammaglobulinemia has been reported in cats with chronic toxoplasmosis.

Dogs with hepatic necrosis present with increased serum levels of alanine aminotransferase (ALT) and alkaline phosphate (ALP), while cats that develop colangiohepatitis or hepatic lipidosis show high serum bilirubin levels.

Animals with acute pancreatitis due to *T. gondii* infection may show increased serum amylase and lipase activities.

**Faecal examination in cats**

Most cats with clinical toxoplasmosis will not be excreting oocysts at the time of presentation [43]. In cats, *Toxoplasma* oocysts (10 x 12 μm) can be identified in feces using any of the standard fecal flotation techniques. However, *T. gondii* oocysts in feces have been detected in less than 1% of the cats that have been examined in numerous studies, because oocysts usually are excreted by cats during a short period of time (1-2 weeks) after first infection and because the oocyst size (10x12μm) makes microscopic analysis difficult (Fig. 5). Moreover, oocysts shedding is not associated with clinical signs such as diarrhea [15, 44].

*T. gondii* oocysts are morphometrically indistinguishable from oocysts of *Hammondia hammondi* and *Besnoitia* sp. Oocysts of these coccidians can be differentiated by in vitro sporulation and subsequent mouse inoculation for xenodiagnosis [45].

**Serological tests**

There are many tests for diagnosing toxoplasmosis: the Sabin Feldman Dye Test, the indirect fluorescent antibody (IFAT), enzyme-linked immunosorbent assay (ELISA), agglutination procedures and Western Blot immunoassay being the most commonly used tests (Fig. 6).

IgM antibodies are detected within 1-2 weeks post-infection and titers remain high for 12-16 weeks. An IgM titer > 1:64 with negative IgG indicates active infection. When IgM antibodies remain persistently it could be associated with reactivation of a chronic infection after repeated exposure to *T. gondii*, glucocorticoid therapy or concomitant infectious diseases (FIV). Since the IgM titer may remain increased for long periods, the interpretation of elevated IgM can be difficult [46].

IgG antibodies do not develop until about 2 weeks post-infection and remain at high levels for several years, even for life. Diagnosis of active toxoplasmosis using IgG test requires a fourfold or greater increase in IgG antibody titer during 2-3 weeks.

Detection of *T. gondii* antibodies in aqueous humor and cerebrospinal fluid have been used as an aid in the diagnosis of dogs and cats with toxoplasmic encephalitis or uveitis.

Whilst some serological tests have been developed for the detection of ocular and CNS toxoplasmosis, they are not conclusive, nonetheless they maybe helpful in some cases [47, 48].

**Fig. 5 Unsporulated oocyst of T. gondii (x 40).**

**Fig. 6 Indirect Fluorescent Antibody Test (IFAT) to detect Toxoplasma infection.**
Lastly, the detection of antibodies to diagnose neonatal toxoplasmosis is controversial. Dubey et al., 1995, showed that transplacental transfer of *T. gondii* does occur in cats but the frequency is not well known [12], so if the queen is seronegative and lives indoors the kittens will not have transplacental transmission. If the queen acquires the infection during pregnancy, both kittens and queen will present with high IgG titers.

Direct detection of *T. gondii*

Cytologic examination of bronchoalveolar washes, aqueous humour, cerebrospinal fluid (CSF), lymph node, peritoneal or thoracic fluid aspirates can be useful to detect tachyzoites of *T. gondii*. A diagnosis may be established by microscopic examination of impression smears stained with Giemsa.

Molecular methods of diagnosis such as polymerase chain reaction (PCR) from samples including blood, aspirates, placentas and amniotic fluid are being used in human medicine to diagnose toxoplasmosis. The PCR can detect 1-10 tachyzoites in CSF and aqueous humor and as little as 5 tachyzoites in blood samples [49-51]. Results of PCR testing in veterinary medicine indicate that it can be an aid in diagnosis, as suggested by the most recent studies done by Montoya 2006 [52] who identified *T. gondii* DNA by nested-PCR and real time PCR from feline brain and blood samples (Fig. 7).

Treatment

Treatment is recommended in cats and dogs with clinical toxoplasmosis and works against replication of *T. gondii*. However, the available drugs are not completely effective in killing the parasite (Tab. 2). Clindamycin is the first line drug for treatment of disseminated toxoplasmosis in both species. Animals can also be treated with a combination of sulfonamides and pyrimethamine, although they are contraindicated in pregnant queens and bitches. Spiramycin can be used in pregnant females [43].

Clinical signs of systemic illness usually begin to resolve within 24-48 hours after institution of therapy.

Bone marrow suppression can occur with the use of pyrimethamine or trimethoprim sulfonamide combinations and can be prevented or corrected with the addition of folic acid (5 mg per day) or yeast (100 mg/kg) to the cat’s diet [43].

Treatment of suspected ocular toxoplasmosis includes controlling anterior uveitis if present with topical anti-inflammatory agents (prednisolone acetate or dexamethasone) and the administration of systemic antitoxoplasmic drug [52].

*T. gondii* oocysts shedding by cats can be suppressed by coccidiostatics such as toltrazuril, clazuril and sulfonamides [54].

Lastly, seropositive cats must be monitored through a routine serologic test, at least once per year, in order to detect eventual seroconversion caused by reactivation from a chronic phase, but specific treatment is not needed in asymptomatic cats.

![Fig. 7 Sensitivity determination of B1 direct PCR (A) and nested-PCR (B).](image)

Tab. 2. Treatment of toxoplasmosis in dogs and cats.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dose-rate</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin hydrochloride</td>
<td>Per os</td>
<td>10-20 mg/kg BID</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Clindamycin phosphate</td>
<td>IM, IV</td>
<td>12.5-25 mg/kg BID</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Pyrimethamine plus trimethoprin-sulfonamide</td>
<td>Per os</td>
<td>0.25-0.5 mg/kg BID (pyrimethamine) 30 mg/kg BID (TMP-sulfonamides)</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>Per os</td>
<td>8-10 mg/kg BID</td>
<td>10 days</td>
</tr>
<tr>
<td>Toltrazuril/Clazuril</td>
<td>Per os</td>
<td>5-10 mg/kg SID</td>
<td>5-10 days</td>
</tr>
</tbody>
</table>
**Prevention**

It is very important to know the *T. gondii* life cycle and the epidemiology of the disease to define the control measures in order to reduce feline infection and environmental contamination with oocysts. The following measures are recommended:

- Do not feed cats with raw or rare meat. If raw meat is used, it can be frozen or gamma-ray irradiated to kill tissue cysts
- Change litter boxes daily to avoid oocysts sporulation
- Do not allow cats to bring their prey into the house
- Perform annual fecal and serologic examination of cats at risk
- Cats should be prevented from entering buildings where food producing animals are housed or feed is stored
- Animal blood donors should be screened before transfusion
- Do not allow dogs to consume cat feces
- For killing parasites, use chemical disinfection with ammonia 10% for 10 minutes, for the litter trays and all surfaces, or immerse in boiling soapy water or use steam cleaning
- Control different invertebrates which may act as mechanical vectors such as: bed bugs, earthworms, cockroaches, etc. [55, 56].

Although commercial vaccines are still not available, a candidate T-263 (bradyzoites of a mutant strain) has been designed against *Toxoplasma* infection in cats. Cats developed immunity without concomitant shedding of infectious oocysts following oral administration of a vaccine containing T-263 bradyzoites [57, 58]. The vaccine can only be administered to healthy cats, and it is not recommended in pregnant females [43].

**Human toxoplasmosis and public health considerations**

Human toxoplasmosis has a wide geographical distribution and is estimated to affect around two billion people worldwide [27]. There is a considerable range in seroprevalence (7.5% - 95%) in different parts of the world and indeed between different population groups within the same country [59]. In Europe seroprevalence varies from less than 20% in northern Europe to more than 60% in southern Europe [60] (Fig. 8).

*Toxoplasma gondii* is transmitted to humans via a variety of routes: ingestion of raw or undercooked contaminated meat; exposure to sporulated oocysts in cat litter or soil, from unwashed fruit, vegetables, gardening or contaminated water; or congenital transmission when a primary maternal infection is passed transplacentally to the foetus. Additionally, toxoplasmosis may be acquired by organ transplant or laboratory accident [62, 63].

The majorities of cases in immunocompetent people are asymptomatic or produce only mild symptoms, but result in life-long chronic infection with parasites inside tissue cysts. Reactivation of latent infection may therefore lead to severe and life-threatening disease in immunocompromised individuals. Toxoplasmosis is the most prevalent disorder affecting the brain in HIV-patients, causing toxoplasmic encephalitis (TE). It is the second most common AIDS-related opportunistic infection and occurs in 10% to 50% of patients with AIDS who are seropositive to *Toxoplasma* and have a low CD4+T lymphocyte count. Since the introduction of highly active antiretroviral therapy in 1996, the incidence rates have decreased in some regions. However, toxoplasmosis is a large health problem in many countries [64]. Serological tests are useful in detecting infection, but they cannot distinguish between reactivated and latent infections and may give false negative results for immunocompromised patients. Therefore, PCR detection of active parasites needs to be performed [65].

Congenital toxoplasmosis occurs if the mother is infected for the first time during gestation. The parasite reaches the foetus transplacentally and the outcome of the infection depends on the virulence of the strain, on the woman's immune response and the period of pregnancy. Risk of congenital toxoplasmosis is lower if infection occurs during the first trimester (10%-25%) than if it occurs during the second or the third trimester (60%-90%) [66]. Clinical manifestation of toxoplasmosis in foetuses and neonates vary from mild disease to severe signs (abortion, hydrocephalus, chorioretinitis, intracranial calcification, hepatosplenomegaly). Most infected neonates are asymptomatic at birth but may present much later with ocular alteration or mental and psychomotor disorders.

The diagnosis of *T. gondii* infection is most commonly established by detecting IgG and IgM antibodies in the blood; however, these tests cannot estimate the time of infection precisely enough to properly manage the risk to the foetus of a maternal infection. A positive IgM test result may reflect an acute infection; however, *Toxoplasma*-specific IgM antibodies may be found for up to several years, and thus may lead to erroneous interpretation. Recently, it has been suggested that the combination of a sensitive test for *Toxoplasma*-specific IgM antibodies and measurement of the avidity of IgG antibodies for *T. gondii* had the highest predictive value with regard to the time of infection [60]. Diagnostics by PCR is also useful. Prevention of human toxoplasmosis mainly concerns avoiding infection. Health education may decrease the incidence during pregnancy by 60% [67]. Education programs, prenatal and
screening of neonates to identify and treat congenital infection, together with animal rearing and production methods designed to reduce T. gondii contamination of meat, are principal interventions. Toxoplasma infection in humans may be prevented by the following measures: cooking meat to a sufficient temperature to kill Toxoplasma (internal temperature higher than 67°C; microwaving does not kill T. gondii cysts); peeling or thoroughly washing fruits and vegetables before eating; steam cleaning cooking surfaces and utensils after they have been in contact with raw meat, poultry or unwashed fruits or vegetables; pregnant women or immunocompromised individuals should avoid changing cat litter, but if done, gloves should be used and the hands should be washed thoroughly afterwards [68]. Children’s sandboxes should be covered to prevent cats from defecating in them and animal care technicians who clean cats and the hands should be washed thoroughly afterwards [68]. Avoid changing cat litter, but if done, gloves should be used and pregnant women or immunocompromised individuals should avoid changing cat litter, but if done, gloves should be used and the hands should be washed thoroughly afterwards [68]. Children’s sandboxes should be covered to prevent cats from defecating in them and animal care technicians who clean cats should wear protective clothing and masks [36].

If all of these recommended measures are put in practice the risk of toxoplasmosis to human beings decreases dramatically. If all of these recommended measures are put in practice the risk of toxoplasmosis to human beings decreases dramatically. If all of these recommended measures are put in practice the risk of toxoplasmosis to human beings decreases dramatically. If all of these recommended measures are put in practice the risk of toxoplasmosis to human beings decreases dramatically.

References


Echinococcus multilocularis in veterinary practice in Europe

E. R. Morgan

Summary

The tapeworm Echinococcus multilocularis is relevant to veterinary practice primarily because dogs are important definitive hosts, shedding eggs that pose a significant zoonotic threat. Dogs can also occasionally become infected as intermediate hosts and develop severe disease. The major role of the veterinary clinician is to ensure adequate de-worming treatment in dogs in endemic areas, and to apply protocols aimed at reducing the risk of introducing the parasite into new areas through animal movement. In both cases, up to date knowledge of the apparently changing geographical range and epidemiology is crucial.

Introduction

Echinococcus multilocularis is an important zoonosis in several parts of the world. In Europe, veterinary practitioners will find themselves in one of three areas: those endemic for the parasite, those with recently recognised cases of alveolar echinococcosis (AE), and those currently free of the parasite, for which exclusion is a priority. The disease is therefore relevant for all, especially since its distribution and epidemiology appears to be undergoing a period of change.

The parasite

Canids, including dogs and foxes, are the usual definitive hosts. Adult worms are small (2-5mm in length, Fig. 1) and live in the small intestine, where they attach by embedding their anterior section in the mucosa. Following infection as the definitive host, dogs produce eggs after about a month, and continue to do so for up to 4 months [1]. There is some evidence for partial acquired immunity, but this is not sufficient to prevent future infection following re-exposure. The parasite's biotic potential is similar in dogs and foxes, but infections in cats produce very few eggs and cats are therefore not of overall significance in maintaining parasite populations [2].

Eggs are produced into the faeces, where they are immediately infective, and can survive for many months in the environment [1]. Normal summer and winter temperatures in Europe are not sufficient to render the eggs non-infective. After ingestion by the intermediate host, usually a rodent, the eggs hatch and migrate to the tissues. The liver is the most common site for further development, and cysts are formed, with a germinal epithelium surrounded by a carbohydrate-rich outer layer which apparently protects the parasite from the host immune response. Asexual reproduction produces more immature parasite stages, known as protoscoleces, into the fluid interior of the cyst [1]. Aggregations of cysts form, with ill-defined boundaries, and ingestion of an infected intermediate host leads to a variable number of adult parasites in the gut of the definitive host.

Alveolar echinococcosis (AE)

Infection in the gut of the definitive host is benign, and dogs can tolerate burdens of many thousands of worms without apparent ill effect [1]. Cysts in the intermediate host, however, can be highly pathogenic. Inadvertent infection of humans leads to alveolar echinococcosis (AE). Unlike its sister species Echinococcus granulosus, cysts in humans are poorly circumscribed, and expand by local tissue infiltration and possibly distant metastasis. The liver is the most common site for the cysts, and by the time tissue destruction is sufficient to provoke clinical signs, complete surgical excision is invariably difficult or impossible [1]. Although advances in diagnosis and treatment have reduced case fatality from its previous rate of more than...
95%, the disease still carries a poor prognosis, and surgery is followed by life-long parasitostatic anthelmintic chemotherapy [3]. Although cases in humans are not common, the severity of disease and the ease with which sources of infection in companion animals can be eliminated places great responsibility on the practising veterinarian for protection of public health.

In recent years a number of cases of AE have also been reported in which domestic animals act as intermediate hosts and develop cysts, usually in the liver. This has been observed in pigs, several species of primate, and dogs [1]. In at least one case a dog has acted as both definitive and intermediate host at the same time. This raises questions over whether autoinfection can occur, with rupture of cysts into the intestine or hatching of eggs before leaving the host. However, the most likely general source of infection is ingestion of eggs from the environment.

AE in dogs follows a similar course to that in humans, although cysts develop more quickly [1]. Cranial abdominal mass is the main presenting sign, and may be accompanied by ascites, diarrhea, vomiting, dyspnoea, exercise intolerance and weight loss. Clinical investigation may reveal signs of liver failure if the functional capacity of the organ is sufficiently reduced. Ultrasound examination is suggestive, with the main differential diagnosis being neoplasia, and exploratory laparotomy reveals a large polycystic mass in the cranial abdomen with liver involvement. Cytology on material collected by fine needle aspirate is unlikely to be helpful, but examination of this material by antigen-ELISA, if available, is diagnostic. Treatment options are euthanasia, or surgical resection of the mass. This is rarely completely achievable due to its locally invasive nature, and might involve removal of entire liver lobes, with inadvertent rupture of the cyst leading to major complications. Surgery is followed by albendazole treatment (daily 10 mg / kg per os). Provided treatment is maintained, remission periods of up to 3.5 years have been recorded, with no instances of clinical recurrence in dogs undergoing chemotherapy after surgery [1].

Clinical cases in non-travelled dogs are an indication that the parasite is completing its life cycle in the area, and people are at risk. This should trigger surveillance and re-evaluation of local recommendations for treatment of dogs.

**Current distribution and epidemiology**

The approximate current distribution of *E. multilocularis* in Europe is shown in Fig. 2. There is some concern that the parasite is spreading, although sparse objective records especially in the East, improved diagnostic tests in recent years, and increased awareness leading to more diagnostic effort, make this difficult to prove. Cases of autochthonous AE and infection in foxes have been recorded in several new areas in the past few years. In some endemic areas, the parasite has colonised urban fox populations [4], placing increasing numbers of people at risk. There is evidence for real increases in parasite biomass within core endemic areas [5] as well as on the edge of the known range [6], which could be linked to increases in fox populations as a result of successful control of rabies [5].

In the natural or sylvatic cycle, foxes act as the definitive host and a range of rodents as the intermediate hosts. Raccoon dogs are also competent definitive hosts and may be of epidemiological importance in northern areas [5]. The most important intermediate hosts are microtine voles rather than rats or mice, although other species including wood mice and musk rats can also be infected. The risk to humans comes from inadvertent ingestion of eggs from infected faeces, with subsequent cyst development. Contamination of fingers or food with eggs in areas frequented by foxes therefore poses a risk of infection, and people working outdoors and in contact with the soil are more likely to encounter the parasite. However, since human contact with dogs is much closer than with foxes, spillover of infection into domestic dog populations constitutes a great multiplication of risk. This would occur when dogs ingest rodents or rodent remains in an area in which foxes are present and the sylvatic cycle is operating. In this context, the increasing urbanisation of fox populations in many European cities is of justified concern [4]. Work in Switzerland suggests that the suburban fringes, where elevated fox numbers coincide with high densities of intermediate hosts and green areas frequented by people and their dogs, are the area of highest risk for transmission. Dogs can also act as a mechanical carrier of infective eggs, for example after rolling in fox faeces.

**Diagnosis and treatment in the definitive host**

Eggs are easily recognised on salt flotation of faeces, but cannot be differentiated from those of other taeniid tapeworm species. Definitive diagnosis is by identification of the adult parasite using sedimantation at necropsy. *In vivo*, expulsion of tapeworms and enumeration of burdens, for epidemiological research purposes, can be achieved by arecoline purge. In practice, however, detection of parasite antigen by ELISA, or DNA in faeces by PCR, provide accurate diagnosis [1, 4].
Praziquantel is the drug of choice for prophylactic treatment in the definitive host. However, it has a very short persistence time with a half-life of only a few hours, so repeated administration every month is needed to ensure that no egg excretion occurs. Since this provokes problems of owner compliance, a more selective approach has been suggested, focusing on dogs that have the closest contact with humans or the greatest opportunity to eat rodents [1]. Routine screening by faecal flotation would also identify infected dogs, although false positives would be caused by other taeniid infections, and false negatives by low test sensitivity. Screening also presents a zoonotic hazard to practice laboratory staff. Routine treatment of all dogs in at-risk areas is therefore the safest strategy. When recommending worming regimes to clients, veterinarians in endemic areas should be wary of combination products targeted to Toxocara canis and Dirofilaria immitis along with ectoparasites such as fleas, which do not necessarily have any effect against tapeworms. Diagnosis of patent infections in dogs should prompt the owners and their families to seek medical advice, and it is recommended that they are screened for specific antibodies [1]. The pathogenesis in humans is chronic and years can go by between infection and the appearance of clinical signs, with prognosis deteriorating as the cysts grow. Early diagnosis in people at high risk of infection is therefore beneficial.

Dogs travelling from endemic to uninfected areas could carry the parasite, leading to establishment in new countries [7]. Consequently, cross-border transport of dogs in some cases carries a legal requirement for treatment. The protocol is a compromise between the gut transit time (ensuring that all eggs are voided before arrival in the destination country) and the chance of re-infection (which is in theory possible almost immediately after treatment). Currently, the favoured strategy is a single dose of praziquantel between 24 and 48 hours before entry, and there are so far no confirmed reports of introduction of E. multilocularis by dogs so treated. However, there is political pressure to weaken requirements for such treatment in the interests of European harmonisation and free movement, and given the severity of disease in humans the veterinary profession has a responsibility to ensure that changes to regulations for cross-border animal movement takes due account of the risks for disease transmission. Infection of dogs visiting an endemic area can further be reduced by avoiding opportunities for ingestion of rodents or carrion.

**Prospects for control in endemic areas**

In most parts of its range, E. multilocularis is maintained in a sylvatic cycle, with foxes and voles as the major hosts, although there is evidence in Asia that dogs can take over as the main definitive host [1]. Whether or not dogs can maintain the parasite cycle in the absence of foxes, infection of dogs undoubtedly leads to increased risk of human disease. In hyper-endemic areas, the lifetime incidence of E. multilocularis infection in dogs could be as high as 80% [4]. Routine treatment of dogs with praziquantel at an appropriate dose and frequency (or, in future,
other proven drugs) and proper disposal of dog faeces should therefore form the cornerstone of control.

Control in foxes or in rodents has been considered [8]. Culling foxes is unlikely to be a realistic option, not only because of logistical and ethical problems, but also because the ensuing disruption could be counterproductive. Disturbance of stable family groups with defended territories is likely to lead to increased dispersal, especially by young foxes, which carry the highest worm burdens. This could lead both to geographical spread of infection and increased opportunity for contact with humans and domestic animals. Experiences with badger culling to control bovine tuberculosis in the UK should prompt caution when considering wildlife culling as a means of disease control, especially in territorial species [9]. In practice, a sustained decrease in fox populations might reduce the force of infection, but given the high reproductive potential of foxes is unlikely to be achievable by conventional means. A more successful strategy has employed treatment, with five- to ten-fold reductions in the prevalence of *E. multilocularis* in fox populations given praziquantel-laced baits over a 2 year period [4]. Treatment of urban and suburban foxes in this way could directly reduce zoonotic risk, as well as the chances of spillover infections in domestic dogs. However, such treatment should be strategic and take into account the population dynamics of foxes and the parasite, and the spatial and dynamic complexities of transmission [10].

Conclusions

The severity of zoonotic disease caused by *E. multilocularis* compels the practising veterinary surgeon to take serious responsibility for control of infection in dogs. This can be achieved by routine anthelmintic treatment in endemic areas, and strategic treatments in dogs moving from endemic to uninfected countries. Veterinary efforts to control this parasite will benefit from awareness among practitioners of its life cycle, epidemiology and diagnosis, risk factors for human and canine infection, and the presentation and diagnosis of disease in animals acting as intermediate hosts. They should also be aware of the apparent changes in epidemiology in terms of urban fox populations and expansion of the known geographical distribution.
This paper reviews the current knowledge about an important zoonotic dog and cat parasite, *Toxocara*. A good understanding of the epidemiology is required so that effective prevention of infection in man, dogs and cats can be possible. Education of the dog and cat owner will be significant in prevention. In this review, the epidemiology, clinical symptoms, diagnosis, prevention and control in the dog, the cat and the human will be discussed. Uniform guidelines for deworming dogs and cats will be given.

**SUMMARY**

This paper was commissioned by FECAVA for publication in EJCAP.

**Introduction**

*Toxocarosis of dogs and cats*

*Toxocara canis* and *Toxocara cati* are roundworms of dogs and cats and the reported infection rates in Western Europe vary from 3.5% to 17% for *T. canis* in dogs and 8% to 76% for *T. cati* in cats [8, 15, 22, 35, 39, 41, 52]. The prevalence of patent *Toxocara* infections is highest in young dogs and cats and much less common in adult animals. *Toxocara* infection follows ingestion of embryonated *Toxocara* eggs or larvae in a paratenic host. Migration of larvae can lead to (overt) clinical disease (toxocarosis) in the (paratenic) host.

**Epidemiology**

*Infection of the dog and cat*

Adult worms in the intestinal tract of infected dogs and cats shed large numbers of eggs into the environment (Fig. 2) via the faeces where they embryonate (Fig. 3) and maybe ingested by natural hosts as well as paratenic hosts. In the intestine the larvae hatch (Fig. 3) and migrate throughout the body via blood vessels. This is called visceral larva migrans or VLM (Fig. 4 and 5). In young animals larvae migrate from the lungs up the trachea and after swallowing, mature in the intestinal tract. In paratenic hosts and most adult dogs and cats that have some degree of acquired immunity, the larvae undergo somatic migration and remain as somatic larvae in the tissues. After predation of *Toxocara* infected paratenic hosts by dogs or cats, larvae will be released and in most cases develop directly into adult worms in the intestinal tract. This may explain the higher *Toxocara* prevalence rate in adult cats, which catch prey animals more often than dogs. In the pregnant bitch, ‘dormant’ somatic larvae are reactivated and migrate in the bitch across the placenta to infect the fetuses. New-born puppies and kittens also acquire infection through ingestion of larvae in the milk [49, 59, 60].

*Environment*

*Toxocara* eggs are unembryonated and not infectious when passed into the environment in the faeces of dogs and cats. Within a period of 3 weeks to several months, depending on soil type and climatic conditions such as temperature and humidity, eggs will develop to an infectious stage that can survive for at least one year under optimal circumstances. Studies from all over the world demonstrated high rates (10-30%) of soil contamination with *Toxocara* eggs in backyards, sandpits, parks, playgrounds, lake beaches, and other public places [37]. In a survey in the Netherlands, the presence of *T. canis* eggs in public parks was comparable with reports from other European cities, but most of the investigated sand-boxes were polluted with *T. cati* eggs [27].

**Infection routes**

*Tracheal migration*

After young dogs ingest infective *Toxocara* eggs, larvae migrate through the liver, the vascular system and the lungs to the trachea. After swallowing, they complete their development in the stomach and small intestine. Eggs first appear in the faeces 4 to 5 weeks post-infection [49]. Depending on previous exposure to infection, the migratory pathway and deworming
Toxocarosis, an important zoonosis - P.A.M. Overgaauw, F. van Knapen

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Fig. 1 Toxocara egg 400x (Photo Felix Yaya).

[12], in pups of one to two months, the probability that newly hatched T. canis larvae will develop into adult ascarids may fall to a lower level, while the probability of somatic migration progressively increases. This is called age resistance and the mechanism operates partly within the lungs. Age resistance seems to be low in older cats. An explanation is not known [69].

Although the prevalence of T. canis is highest in young dogs, a certain proportion of the adult canine population can also be infected [12, 35, 43, 53], mainly by ingestion of infective Toxocara eggs from contaminated soil.

Somatic migration
After ingestion of infective Toxocara eggs, larvae will migrate actively by penetration of the tissues and invasion of all parts of the body. Gradually somatic larvae accumulate in the tissues, persisting for long periods in a manner similar to that seen in paratenic hosts. Larvae of T. cati prefer to migrate to the muscles, while T. canis larvae were more commonly found in the central nervous system.

Transplacental migration
Nearly 100% of puppies are infected by somatic larvae in utero from day 42 of the gestation [33]. This so called transplacental migration or intra-uterine infection is the most important mode of transmission in dogs. In cats, prenatal infection via the placenta does not occur. The larvae in pregnant bitches are probably reactivated by the changing hormonal status of the bitch during pregnancy. Within hours of birth, the larvae that were present in the liver of the neonate, migrate to the lungs and undergo a tracheal migration. Adult worms can be found at two weeks of age and large numbers of eggs may be passed in the faeces after a minimum period of 16 days [32].

Transmammary transmission
After activation, somatic Toxocara larvae in dogs and cats will also be transmitted via the colostrum and the milk (transmammary transmission, lactogenic or milk-borne infection). Following ingestion by the offspring, the larvae undergo development without tracheal migration. Larvae are found to pass in the bitch’s milk for at least 38 days after parturition [72]. This route is less important than intra-uterine transmission in the puppy, but it is the primary mode of infection in the kitten. Kittens infected by lactogenic transmission will show faecal egg excretion after 7 weeks.

Fig. 2 Embryonating Toxocara egg 400x (Photo RVC).

Infection of the dam by the offspring
Infection of the lactating bitch will occur mainly by ingestion of immature fourth-stage larvae from vomit or faeces from the puppies. Larvae develop to adults without a tracheal migration. Toxocara eggs shed in the faeces of puppies or kittens can be ingested by the mother, where they pass through the digestive tract, causing a false-positive diagnosis of Toxocara infection upon faecal examination. There is no development of intestinal infection with T. canis from somatic larvae at other times during gestation and the bitch is not at higher risk of Toxocara infection during metoestrus [44].

Transmission through paratenic hosts
Paratenesis is the mode of infection of some larval nematodes like Toxocara, ensuring its continuing survival by its distribution in prey species [21]. This route of infection exists because of the development of somatic larvae in paratenic hosts, including vertebrates such as rodents and birds or invertebrates such as earthworms and insects (e.g. flies). After ingestion of an infected paratenic host, the larvae develop directly in the intestine. Cats catch and eat more prey animals than dogs and this may be the explanation why higher infection rates are found.

Fig. 3 Hatching Toxocara larvae 400x (Photo RVC).
and remain infective for years. Since no practical methods exist for reducing environmental egg burdens, prevention of initial contamination of the environment is most important. This can be achieved by taking measures such as eliminating patent infections in dogs and cats, preventing defecation by pets in public areas (Fig. 8), taking hygienic precautions and education of the public [19]. Household garden soil was found to be a potentially greater source of *Toxocara* infection than soil in public green areas [25]. A decrease in contamination can be achieved by methods including: restriction of uncontrolled dogs and cats, cleaning up faeces from soil and on pavements by dog owners, preventing access of dogs and cats to public places (especially children’s playgrounds) and by use of strategic anthelmintic treatment of dogs and cats with emphasis on worming puppies, kittens, nursing bitches and queens.

**Anthelmintic treatment strategy**

The most serious and concentrated sources of infection are found in bitches nursing a litter and puppies aged between 3 weeks and 6 months. A major aim of long-term prophylactic treatment programmes is to suppress *T. canis* egg-output throughout the whole of puppyhood using a multidose schedule. Puppies should be treated with appropriate anthelmintics at the age of 2 weeks and because milk transmission occurs continuously for at least 5 weeks post partum, repeated treatments are necessary [3]. Larvae that reach the intestine need at least 2 weeks to mature and start passing eggs, therefore the treatment should be repeated every 14 days. The treatment schedule therefore requires deworming at 4, 6 and 8 weeks of age and then monthly until 6 months of age. Because prenatal infection does not occur in kittens, fortnightly treatment can begin at 3 weeks of age. Nursing bitches and queens should be treated concurrently with their offspring since they may develop patent infections along with their young.

Control in older dogs and cats can be achieved by periodic treatments of dogs and cats with anthelmintics, or by treatments prescribed based on the results of periodic diagnostic faecal examinations. Annual or twice annual treatments have been shown not to have a significant impact on preventing patent infection within a population, so a treatment frequency of at least 4 times

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**Clinical symptoms**

The clinical symptoms depend on the age of the animal and on the number, location and stage of development of the worms. *Toxocara* infection is highest in puppies and kittens up to 6 months of age. After birth, puppies can suffer from pneumonia associated with the tracheal migration and die within 2 to 3 days. At an age of 2 to 3 weeks, puppies can show emaciation and digestive disturbances, caused by mature worms in the stomach and intestine (Fig. 6 and 7). Diarrhoea, constipation, vomiting, coughing and nasal discharge can be found at clinical examination. Distension of the abdomen (‘potbelly’) can occur, probably as result of gas formation caused by dysbacteriosis [41].

Kittens are older when worms are maturing (adult from day 28 and egg producing from day 49 after birth) and tracheal migration with related symptoms does not occur. Therefore, kittens have a better chance to grow and in the meantime develop better bodily condition before problems may be seen. For this reason clinical symptoms similar to those in puppies are usually inapparent.

**Diagnosis**

Patent *Toxocara* infection in dogs and cats can be tentatively diagnosed from the medical history, particularly the use or otherwise of an appropriate anthelmintic schedule, and the clinical symptoms. Confirmation of the diagnosis can be obtained by finding dark brown coloured eggs with thick pitted shells in faecal samples (Fig. 1). The direct faecal smear technique is not a sensitive test and generally should never be used for recovering eggs from faecal samples. Examination of faeces by a floatation technique is a useful method for detecting helminths [7].

**Control measures**

There are two reasons for *Toxocara* control: to prevent human infection and to reduce the risk of infection to pets. *Toxocara* eggs are very resistant to adverse environmental conditions
Toxocarosis, an important zoonosis - P.A.M. Overgaauw, F. van Knippenberg

per year is proposed as a general recommendation. Anthelmintics at the recommended doses are not highly effective against inhibited somatic larvae [11] and treatment of bitches before mating and two weeks before the anticipated whelping date has no useful effect on prenatal transmission [10, 14]. Therefore it is generally not advised to deworm pregnant dogs and cats.

In the past, uniform guidelines for the control and treatment of parasites in pet animals were developed and published by CAPC in the US (www.capcvet.org) and recently by ESCCAP in Europe (www.esccap.org). The guidelines give overviews of worms, their significance and suggest rational control measures for the most important species in order to prevent animal and/or human infection.

**Hygiene**

For dogs and cats, hygiene can be achieved by removing the faeces and by thorough cleaning of kennels. A 20% solution of commercial bleach can be used. This does not kill the eggs, but removes their sticky outer protein coat. These decorticated eggs are then easier to remove from inaccessible areas. Any worms vomited or passed in faeces should be destroyed and predation and scavenging on carcasses by dogs and cats should be prevented.

**Education**

Dog and cat owners can help to avoid contamination of the environment with *Toxocara* eggs and the exposure of other persons to unnecessary risks of toxocarosis infections. Proper information about this zoonosis and the social concept of responsible pet ownership is required. Pet owners should be advised about deworming schemes, effective anthelmintics and to prevent their animals from defecating on children’s playgrounds [57].

Information disseminated to pet owners should include a description of *T. canis* and *T. cati* and how they affect their hosts; clearing of prenatal and transmammary transmission; how *Toxocara* can be transmitted to and produce damage in humans; how prevention can most effectively be achieved; how pet owners should routinely collect and safely dispose of their pets’ faeces, especially from children’s play areas and finally advice for children not to play in potentially contaminated environments. Although veterinarians should be the most appropriate sources of information for their clients regarding the dangers and the control of toxocarosis, surveys have demonstrated that client education on this issue is lacking [23, 42].

**Human toxocarosis**

**Infection routes**

Toxocarosis is a public health problem. Man acts as an unnatural host in which *Toxocara* larvae will not develop but will migrate and survive for a long time. The mode of transmission to humans is by oral ingestion of infective *Toxocara* eggs from contaminated soil (sapro-zoonosis), from unwashed hands or consumption of raw vegetables [19]. Some infections may occur from ingestion of larvae in under-cooked organ and muscle tissue of infected paratenic hosts such as chickens, cattle and sheep [2, 38, 55, 61, 62].

Recent studies indicate the fur of as an important source of *Toxocara* eggs for infection after direct contact [1, 54, 71]. Direct contact with dogs and cats that harbour a patent *Toxocara* infection is usually not considered a risk, since the eggs need to mature several weeks before they are infective [41, 45, 46]. Moreover, *Toxocara* eggs are very sticky and therefore difficult to remove from the coat of a dog or cat and this makes ingestion of a sufficient number of eggs unlikely. A low percentage of these eggs were embryonated in the studies and even in the worst case scenario of highly contaminated fur, it is necessary to ingest several grams of heavily contaminated hair to get infected [48].

**The importance of Toxocara cati in human toxocarosis**

The role of *T. cati* as a zoonotic parasite is not always clearly recognised. Despite the fact that differentiation between *T. canis* and *T. cati* infections is not performed in surveys, the majority of reported human cases of toxocarosis in the past have been associated with *T. canis* and not with *T. cati* [13]. The greater
abundance of T. canis eggs in park soil and playgrounds might be considered the reason for this, but sand-boxes and garden soil (Fig. 8) are more often polluted with T. cati eggs [27]. The large number of common antigenic fractions shared between T. canis and T. cati and the similarity in the mode of infection are indications that there is no difference in zoonotic risk. Furthermore, in Islamic countries, dogs are avoided for religious reasons, while cats are favoured pets. The seroprevalence of human toxocarosis in these countries is considerable as well [58]. The role of T. cati in human toxocarosis should therefore not be underestimated [40, 58].

Infection risk to children
Children are more frequently infected than adults and VLM with more severe clinical symptoms is mainly found in children of 1 to 3 years of age. This can be explained because young children play and have closer contact with potentially contaminated soil in yards and sand-pits. In addition, children may often put their fingers into their mouth and sometimes eat dirt.

Clinical symptoms
Visceral larva migrans
After ingestion of infective Toxocara eggs by a human, Toxocara larvae hatch in the stomach and migrate into the mucosa of the upper small intestine and disperse throughout the body via blood and lymphatic vessels. A more marked, inflammatory, immune response is called visceral larva migrans syndrome or VLM. This multisystem invasion can be associated with varied, non-specific clinical symptoms as a result of the host’s immune response.

VLM is mainly diagnosed in children between 1 to 7 years of age (mean age 2 years) and is characterised by persistent eosinophilia, leukocytosis, an elevated GT γ level and hypergamma-globulinaemia [56].

Eosinophilia is seen more often in children than in adults [68] and a relationship between Toxocara seroprevalence and the incidence of chronic airway disorder (asthma), elevation of serum IgE concentration, the presence of allergen-specific IgE and eosinophilia is established. The occurrence of asthma or recurrent bronchitis and hospitalization due to asthma were significantly related to seroprevalence, while eczema tended to be more frequent. It was concluded that allergic phenomena in children who are predisposed to asthma, are more frequently manifested after Toxocara infection [4]. Toxocara is not a causative agent but contributes to the development of atopic diseases and the allergic manifestation of asthma [51].

General clinical symptoms often include malaise, fever, abdominal complaints (vague upper abdominal discomfort attributed to hepatomegaly), wheezing or coughing [17]. Toxocara infection should be considered in the differential diagnosis of any child with a persistent and unexplained eosinophilia or recurrent abdominal pain. Chronic ‘idiopathic’ urticaria, chronic pruritus, and miscellaneous eczema in adults and children are found strongly associated with toxocarosis [16, 50].

Severe clinical symptoms are reported including life-threatening pneumonia after massive infection [28], eosinophilic meningo-encephalitis in children [36], and thrombosis of the aorta [67].

Ocular larva migrans
Migrating Toxocara larvae can induce granulomatous retinal lesions, which are characterised by complaints of loss of visual acuity, squint and ‘seeing lights’ (Fig. 9). This is called Ocular Larva Migrans syndrome (OLM) [6, 63]. In a minority of cases, total blindness of one or both eyes can result. The mean age of patients with OLM is 8 years, but it is diagnosed in adults as well [31, 56]. Ocular larva migrans is usually caused by no more than a single larva.

Covert toxocarosis
A third clinical syndrome, called ‘covert toxocarasis’ (CT), was found in patients with a vague complex of non-specific clinical symptoms, which do not fall within the categories of VLM or OLM. Symptoms such as hepatomegaly, cough, sleep disturbances, abdominal pain, headaches and behavioural changes have been associated with raised Toxocara antibodies.
The diagnosis ‘Idiopathic Abdominal Pain of Childhood’ is usually made in children.

Cerebral toxocarosis

There are some indications that larval involvement in the human brain may have subtle public health implications, such as changes to cognitive function in children [26]. A large study to determine OLM in 120,000 Irish schoolchildren, ranging in age from 3 to 19 years, revealed a strong association between having had a convulsion and ocular toxocarosis [20].

Diagnosis

Direct diagnosis of Toxocara infection is not easy because patients do not excrete parasite material such as eggs or larvae. Serodiagnostic techniques (ELISA) are the most reliable tools to detect antibodies and circulating antigens [58]. Anti-Toxocara antibodies measured by ELISA were found to persist for up to 2.8 years in infected adults and their presence alone does not distinguish between current and past infections. It should therefore be accompanied by other laboratory tests for a blood eosinophil count and total serum IgE [34]. Seroprevalence for Toxocara in some reports varied between 4.6 to 7.3% in children in the USA [24], 2.5% in Germany to 83% for children in the Caribbean [65]. In the Netherlands the prevalence was found to be 19% on average: between 4% and 15% in people younger than 30 years and 30% in adults older than 45 years [5]. Regularly re-infection of adults is probably the cause of the higher prevalence. Titres fall gradually over a period of about three years but should be considered as a balance between the fading memory of the immune system and its stimulation by continuing ingestion of viable ova or reactivation of dormant larvae. A review of cases of toxocarosis (VLM and OLM) from all over the world revealed that more than half of the patients were less than three years old, one fifth were adults and 60% were males [9]. For OLM, serum antibodies are not diagnostic but the presence of intraocular antibodies appears more promising as a diagnostic aid [63].

On CT or MR imaging, hepatic lesions may be seen as multiple, ill-defined, oval lesions that measure 1.0-1.5 cm in diameter. On sonography, the lesions appear as multiple, small, hypoechoic lesions in the liver parenchyma [30]. Magnetic resonance imaging (MRI) can be used in patients with neurological syndromes to detect granulomas located cortically or subcortically [34].

Control measures

Preventive measures

A zoonotic disease like toxocarosis can be prevented for the most part. Control is important from the point of view of welfare, for the quality of human life and also for the economic costs to society [66]. Prevention of toxocarosis is possible by the institution of certain measures: appropriate health care for pets including regular anthelmintic treatments; reducing the number of uncontrolled and stray pets; preventing contamination of the environment with faeces; and promoting responsible pet ownership [40, 57, 70]. To increase the awareness of potential zoonotic hazards, particularly amongst pet owners, veterinary practitioners, general practitioners and public health agencies should provide sufficient information and advice for appropriate measures to be taken to minimize the risk of infection. Several reports, however, have indicated a significant lack of knowledge within the professions [23, 42, 47]. All authors concluded that continuing education with emphasis on the zoonotic risks is still strongly recommended.

Recommendations include the following: be careful when in contact with young dogs and cats; wash hands before eating and after contact with animals; deworm dogs and cats regularly especially puppies and kittens; prevent children from eating earth and from playing on areas soiled with animal faeces; remove pet faeces; keep children’s nails clipped.

Treatment of patients

Patients with severe Toxocara infections can be treated with systemic acting and larvicidal anthelmintics [34]. Clinicians, however, should balance the risk of therapy with the severity of the disease, because treatment can lead to severe hypersensitivity reactions caused by dying larvae. Especially in OLM cases the anthelmintic dose should be increased gradually over a period of days and accompanied by the concomitant administration of steroids [34].

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Update on avian chlamydioidosis and its public health significance

D. Vanrompay

SUMMARY

The present review gives updated information on *Chlamyphila psittaci* strain classification, epidemiology, transmission, clinical disease, new diagnostic tests, public health significance, present legislation on psittacosis and recommendations for prevention and control of the disease.

**Cp. psittaci** strain classification

In 1999, the family *Chlamydiaceae* was reclassified into two genera and nine species [7]. The genus *Chlamydia* (*C*) includes *C. trachomatis* (human), *C. suis* (swine), and *C. muridarum* (mouse, hamster). The genus *Chlamydophila* (*Cp*) includes *Cp. psittaci* (avian), *Cp. felis* (cats), *Cp. abortus* (sheep, goats, cattle), *Cp. caviae* (guinea pigs), *Cp. pneumoniae* (human) and *Cp. pecorum* (ruminants).

All known avian strains belong to the species *Chlamydophila psittaci*, which includes six avian serovars A through F, and two mammalian isolates WC and M56 isolated from epizootics in cattle and muskrats, respectively. Serotyping of avian strains is performed by a micro-immunofluorescence test using monoclonal antibodies against serovar-specific epitopes of the major outer membrane protein (MOMP) [1, 42]. The avian serovars are relatively host-specific. Serovars A and B are usually associated with psittacine birds and pigeons, respectively. Serovar C has primarily been isolated from ducks and geese, and serovar D mainly from turkeys. Serovar F was isolated from a psittacine bird and from turkeys. The host range of serovar E is the most diverse of the strains: it has been isolated from pigeons, rats, ducks, turkeys and occasionally from humans. All serovars should be considered to be readily transmissible to humans.

At present, analysis of the MOMP gene encoding the outer membrane protein A (*ompA*) is more often used to characterize avian *Cp. psittaci* strains as molecular techniques can differentiate *Cp. psittaci* strains more precisely and serovar-specific monoclonal antibodies are not commercially available restricting their use by veterinary clinical laboratories. Analysis of the *ompA* gene by sequencing, genotyping real-time PCR [10] or genotyping micro array [33] allows for the detection of genotypes A to F as well as the recently discovered genotype E/B [11]. These genotypes correspond to MOMP serovars A to F. Monoclonal antibodies cannot distinguish genotype E/B from B and E. Genotype E/B was mainly isolated from ducks.

**Epidemiology**

The psittacosis outbreaks of 1929-1930 and 1930-1938 were attributed to psittacine birds. However, in the next years it became clear that *Cp. psittaci* infections were not limited to psittacine birds, but could affect other bird species. In 1939, the bacterium was isolated from two South African racing pigeons and soon, additional pigeon isolates were obtained, this time in California. At that time, psittacosis in two New York citizens could be attributed to contact with infected feral pigeons. In 1942, serological evidence showed ducks and turkeys to be frequently infected and within the next 3 years, human infections due to contact with infected ducks were reported in California and New York. In the early 1950s, *Cp. psittaci* could be isolated from turkeys and from humans in contact with infected turkeys, during severe respiratory outbreaks in the US turkey industry. The incidence of severe *Cp. psittaci* epidemics in US poultry declined during the 1960s. However, avian chlamydioidosis...
herons, egrets, pigeons, blackbirds, grackles, house sparrows, and killdeer, all of which freely intermingle with domestic birds. Seagulls and egrets could be asymptomatic carriers of highly virulent strains of Cp. psittaci excreting a high number of bacteria.

Transmission

Cp. psittaci is excreted in faeces and nasal discharges. Faecal shedding occurs intermittently and can be activated by stress caused by nutritional deficiencies, prolonged transport, overcrowding, chilling, breeding, egg-laying, treatment or handling. During natural infection, bacterial excretion periods vary according to strain virulence, infection dose and host immune status. However, shedding may occur for several months. Transmission of chlamydiae is mainly by inhalation of contaminated material and sometimes by ingestion. Large numbers of Cp. psittaci can be found in respiratory tract exudate and faecal material from infected birds. In turkeys, the lateral nasal glands become infected early and remain infected for more than 60 days. Choanal/oropharyngeal swabs are more consistent for isolation of the agent than faecal swabs, especially during the early stages of infection.

Avian species, including domestic poultry sharing aquatic or moist soil habitats with wild infected aquatic birds may become infected via contaminated water. Granivorous birds like pigeons, doves, pheasants and house sparrows may become infected by dust inhalation in faecal contaminated barnyards and grain storage sites. The consumption of infected carcasses may transmit Cp. psittaci to host species that are predators or scavengers of other birds. Transmission of Cp. psittaci in the nest is possible. In many species, such as Columbiformes, cormorants, egrets, and herons, transmission from parent to young may occur through feeding, by regurgitation, while contamination of the nesting site with infective exudates or faeces may be important in other species such as snow geese, gulls and shorebirds. Cp. psittaci can be transmitted from bird to bird by blood-sucking ectoparasites.
Clinical disease

Depending on the chlamydial strain and the avian host, chlamydiae cause pericarditis, air sacculitis (Fig. 1), pneumonia (Fig. 2), lateral nasal adenitis, peritonitis, hepatitis, and splenitis. Generalized infections result in fever, anorexia, lethargy, diarrhoea, and occasionally shock and death. Chlamydiosis is a very common chronic infection of psittacine birds. Infections cause conjunctivitis, enteritis, air sacculitis, pneumonitis, and hepatosplenomegaly. Droppings are often green to yellow-green. Many of the birds become chronically infected but show no clinical signs until stressed. These birds often shed chlamydiae intermittently and serve as a source of infection for humans and other birds.

Recent developments in diagnosis

Sample collection and storage of specimens

The laboratory diagnosis of avian chlamydiosis includes isolation or identification of the organism from the host preferably by recently developed molecular techniques. Because *Cp. psittaci* requires living cells to multiply, isolation requires inoculation of cell cultures or specific pathogen free embryonated chicken eggs in a biosafety level 3 laboratory, as the infection can be aerogenically transmitted to humans. Specimens should be collected aseptically, as contaminant bacteria may interfere with isolation. A special transport medium [35] should be used consisting of a sterile sucrose–phosphate–glutamate (SPG) buffer (succrose, 74.6 g/liter; KH₂PO₄, 0.512 g/liter; KH₂PO₄, 1.237 g/liter; and L-glutamic acid, 0.721 g/liter), supplemented with foetal calf serum (10%), vancomycin and streptomycin (100 mg/ml), and nystatin and gentamicin (50 mg/ml). The medium also serves for freezing swabs and tissues at -80°C in order to preserve chlamydial viability. The stability of chlamydiae during storage depends upon the material in which it is contained. Chlamydiae in tissue specimens or yolk-sac suspension can be preserved indefinitely by storage at -80°C [2]. Swabs should be frozen in transport medium. At 4°C, the organism can survive with gradual loss of infectivity for 30 days or longer when in heavily infected tissues or in transport medium.

For isolation the following samples can be collected: pharyngeal/choanal slit swabs, lung tissue, thickened exudate-coated air sacs and free exudates, thickened pericardial tissues and exudates, sections of enlarged spleen and liver, intestinal mucosa at sites of hyperemia, colon contents, conjunctival and nasal discharges, whole blood and peritoneal exudates. In live birds, pharyngeal/choanal slit swabs are preferred rather than faeces or cloacal swabs, as faecal shedding is intermittent. In dead birds, samples of the respiratory tract are most suitable.

The same tissues can be collected for molecular diagnosis. The preservation of DNA from degradation is critically important. DeGraves et al., [5] suggest all specimens for PCR analysis should be collected in a DNA stabilization reagent. Such reagents are commercially available; for instance the RNA/DNA Stabilization Reagent for Blood/Bone Marrow® from Roche Applied Science. This reagent is based on the denaturation of proteins in a concentrated solution of guanidinium isothiocyanate and another agent, for the inactivation of ribonuclease during RNA isolations. However, it also works perfectly well for DNA stabilization.

Polymerase chain reaction

Molecular methods, particularly the polymerase chain reaction (PCR), permit rapid detection and identification of *Chlamydia psittaci* from clinical specimens. Two different genomic target regions are usually used for amplification, namely the ribosomal RNA gene region [7, 23, 24] and the gene encoding the MOMP antigen designated *ompA* [15]. In some cases, concentration of target organisms by physical means increases the assays' sensitivity, but it is important to ascertain the enrichment effect [5]. Such methods include immunomagnetic, centrifugal, or filter concentration. Several in-house-made and commercial DNA extraction methods have been described [16, 31, 47]. Alternatively, commercial DNA extraction kits can be used for the extraction of chlamydial DNA. In our hands, the QIAamp® DNA Mini Kit performed well for pharyngeal swabs while the High Pure PCR Template Preparation Kit performed well for cloacal swabs and faeces.

Recently, two highly sensitive nested PCR assays have been developed to detect *Chlamydia psittaci* in avian samples [31, 41]. The method of Sachse and Hotzel [31] is based on a first amplification generating a genus-specific *ompA* product (576-597 bp) followed by a second amplification using one species-specific and one genus-specific primer generating a *Chlamydia psittaci*-specific amplicon (389-404 bp). PCR results are visualized by agarose gel electrophoresis using ethidium bromide containing gels. The sensitivity of the nested enzyme immunoassay (PCR-EIA) was established at 1 to 0.1 infection forming unit (IFU). Van Loock et al., [41] developed a nested PCR-EIA. The fluorescein–biotin labeled PCR products were immobilized on streptavidin-coated microtitre plates and detected with anti-fluorescein peroxidase conjugate and a colorimetric substrate, although detection by use of a fluorometer is also possible using this method. The sensitivity of the nested PCR-EIA was established at 0.1 infection-forming units (IFU). Specificity was 100%. An internal inhibition control
was included to rule out the presence of inhibitors of DNA amplification.

A number of real-time PCR tests have been developed. Real-time PCR systems have the advantage that their sensitivity approaches that of the nested PCR systems, and they require no additional pipetting or handling of the PCR product following the initial set-up of the PCR mix. This reduces both the time needed for the test and the incidence of contamination. Recently a SYBR Green-based real-time PCR was developed targeting the rDNA ribosomal spacer of *Chlamydia psittaci* [10]. The test could detect 10rDNA copies/μL DNA extract. Geens et al. [10] also developed a genotyping real-time PCR which detects all known *ompA Chlamydia psittaci* genotypes.

**Micro arrays**

Micro arrays can be coupled with PCR where they serve as a set of parallel dot-blots to enhance product detection and identification of bacterial isolates. Sachse et al., [32] developed a DNA micro array-based detection and identification method for *Chlamydia* and *Chlamyphila spp*. The test was developed using the ArrayTube platform (CLONDIAG®-chip technologies). Unique species-specific hybridization patterns were obtained for all nine species of the family *Chlamydiaceae*. The assay proved suitable for species identification of chlamydia cell cultures and showed a potential for direct detection of these bacteria from tissue samples. Recently, an *ompA*-based *Cp. psittaci* genotyping micro array has been developed [33].

**Serology**

Despite the introduction of very sensitive and specific tests like PCR, the idea of serological diagnosis still lingers in the minds of several veterinary clinicians. However, serology alone is not particularly useful for diagnosis of a patent disease in birds because of the high seroprevalence of this infection in asymptomatic birds and the long-term (up to several months) persistence of anti-chlamydial antibodies. In most bird species, there is a high background rate of anti-chlamydial antibodies, and until we have more information on the disease pattern in certain bird species in relation to direct identification of the bacteria and serology, we are unable to comment on the real significance of antibody titres obtained. Thus, to determine if a single bird is infected, serology should always be used in conjunction with bacterial antigen or gene detection or paired sera should be examined. The latter can not be applied for immediate clinical evaluation. Treatment with antibiotics may delay and/or diminish the antibody response. Serology is very helpful in epidemiological research. Recently, a sensitive and specific recombinant ELISA has been developed based on the detection of antibodies against the chlamydial major outer membrane protein [46].

**Treatment**

Tetracyclines are the drugs of choice and can be administered orally (medicated feed or by mouth if the bird does not accept the medicated diet) or parenterally (injections). Parakeets, budgerigars, finches, canaries and rice birds can be treated through the use of commercially available bird seed impregnated with chlortetracycline (CTC) (0.5 mg CTC/g of seed). For large psittacine birds, feed containing 1% CTC (10 mg per g diet) is recommended. CTC medicated diets are not available in every country. A medicated mash diet for psittacine birds can be prepared by cooking 2 pounds of rice, 2 pounds of hen scratch feed and 3 pints of water for 15 minutes, adding CTC to the cooled mash. Pigeons can be fed with uncooked hen feed supplemented with CTC (5000 ppm). To reduce interference with absorption the calcium content of the diet has to be reduced to <0.7%. Doxycycline is the drug of choice for administration by mouth (25 to 50 mg/kg bw) and parenteral (intramuscular) treatment (75-100 mg/kg bw) every 5-7 days for the first 4 weeks and subsequently every 5 days for the duration of the treatment (45-day period).

**Public health significance**

*Cp. psittaci* can infect humans and should be handled carefully under conditions of bio-containment. Humans most often become infected by inhalation when urine, respiratory secretions or dried faeces of infected birds is dispersed in the air as very fine droplets or dust particles. Other sources of exposure include mouth-to-beak contact, a bite from an infected bird or handling the plumage and tissues of infected birds. Therefore, post-mortem examinations of infected birds and handling of cultures should be done in laminar flow hoods or with proper protective equipment. Human infection can result from transient exposures. The disease is of public health significance because of the popularity of psittacine birds as pets and the increased placement of these birds in child-care facilities and in homes for the aged. Not only direct contact with birds poses risk of psittacosis but also the rural environment and activities such as gardening that may expose individuals to the infectious agent [8, 38]. Person-to-person transmission of psittacosis is possible but it is believed to be rare [18].

The incubation period in humans is usually 5–14 days or longer. Human infections vary from asymptomatic to severe systemic disease with interstitial pneumonia and occasionally encephalitis. The disease is rarely fatal in properly treated patients; therefore, early diagnosis is important. Symptoms usually include headache, chills, malaise and myalgia, with or without signs of respiratory involvement. Pulmonary involvement is common. Several severe psittacosis (pneumonia) cases have recently been documented [13, 26, 36] and *Cp. psittaci* has been associated with ocular lymphoma [9, 48]. However, many psittacosis cases in humans are presented with either mild respiratory symptoms or as asymptomatic infections [16, 44]. The public health significance of these infections is unknown.

Diagnosis is usually established through testing paired sera or only one serum sample by use of the micro-immunofluorescence (MIF) test which is more sensitive and specific than the complement fixation (CF) test. However, the MIF test presents cross-reactivity with other chlamydial species. As in birds, culture or the more sensitive molecular methods can be used to specifically detect *Cp. psittaci*. Tetracyclines are the drugs of choice for therapy of human psittacosis. Doxycycline or tetracycline is usually administered (not
in pregnant women and children < 9 years), where erythromycin constitutes a second choice. The duration of treatment depends on the drug. Tetracyclines should be given for at least 14 days. In most countries, psittacosis is a notifiable disease.

Prevention and control

Vaccine development

Recently, attention has been turned to DNA vaccination and recombinant adenoviral vaccines as a protective means against chlamydial infections [45, 49]. DNA vaccination has been more successful at inducing protective immune responses in turkeys infected with Cp. psittaci [45], than in sheep or mouse models infected with Cp. abortus [22]. Importantly, DNA vaccination can be performed in the presence of maternal antibodies [39]. However, additional research is needed to further enhance the immunogenicity of DNA vaccines and to lower the vaccination costs.

There are currently no vaccines available against avian chlamydiosis although research on MOMP-based DNA vaccination in SPF turkeys has been very promising. DNA vaccination significantly reduces clinical signs and chlamydial excretion, though it did not give full protection. Until an effective vaccine is produced, risk reduction strategies and treatment will have to be applied. Worldwide, psittacosis prevention and control recommendations and legislation are mainly focused on the decrease of human morbidity. Recommended control measures: 1) maintenance of accurate records by the seller of all bird transactions. Records should mention when the birds are sold, the name, address and phone number of the customer, date of purchase, species and band number if applicable; 2) Birds with signs compatible with chlamydiosis should not be purchased or sold; 3) Before adding new birds to a group, the birds should be quarantined and treated; 4) accurate preventive husbandry (prevent feathers, food and other materials moving from one cage to another, daily cleaning, sufficient exhaust ventilation to prevent accumulation of aerosols); 5) special husbandry during infection (isolation, non-dusty litter, keep circulation of feathers and dust to a minimum by frequent wet-mopping with disinfectants, cleaning and disinfection); 6) disinfection (1:1000 dilution of quaternary ammonium compounds, 70% isopropyl alcohol, 1% Lysol, 1:100 dilution of household bleach or chlorophenols can be used); 7) personal protection (HEPA mask in high risk situations). During quarantine it is suspected or confirmed that psittaciformes are infected with Cp. psittaci, all birds of the consignment must be treated by a method approved by the competent authority and the quarantine must be prolonged for at least two months following the last recorded case". Thus, these requirements did not involve bird species other than poultry nor were there any rules for the management of psittacosis within the EU. Therefore, Decisions 2000/666/EC and 2005/760/EC were repealed and a new Commission Regulation, EC no 318/2007 has been in place since 1 July 2007 after being published in the Official Journal of the EU. This regulation lays down the animal health conditions for imports of certain birds from third countries and parts thereof into the EU. This Regulation also lays down the quarantine conditions. For instance: 1) approved quarantine facilities and centres; 2) direct transport of birds to quarantine stations; 3) attestation by the importers or their agents; 4) quarantine for at least 30 days; 5) examination, sampling and testing to be carried out by an official veterinarian; 6) actions in case of chlamydiosis suspicion, which are treatment of all birds and prolonged quarantine for at least two months following the date of the last recorded case. Importantly, the Regulation allows for imports of birds only from approved breeding establishments. Thus, for birds other than poultry, only birds bred in captivity with an individual identification number and accompanied by an animal health certificate are allowed for importation into the EU.

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The role of *Bartonella* spp. in veterinary and human medicine with special emphasis on pathogenicity mechanisms

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**SUMMARY**

*Bartonella* spp. are important pathogens in human and veterinary medicine. Currently, *B. henselae* is considered to be the most relevant zoonotic *Bartonella* species responsible for cat scratch disease (CSD), bacillary angiomatosis and peliosis hepatis. Besides *B. henselae*, several other species have been isolated from cats, dogs and humans suffering from a variety of clinical manifestations ranging from mild infections to severe disease. The analysis of the underlying pathogenicity mechanisms will result in better understanding of the diseases and consequent vector control might allow the prevention of such *Bartonella*-infections.

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**Introduction**

Bartonellosis is considered an emerging zoonosis with increasing frequency. While cats often remain asymptomatic carriers for *Bartonella* spp., dogs may develop clinical signs that are similar to those observed in humans [21]. Due to their way of transmission in cats and dogs via fleas and presumably also ticks [12], bartonellosis is counted into the field of canine vector borne diseases (CVBD).

The ability to cause vasculoproliferative disorders or intraerythrocytic bacteraemia is a unique feature of the genus *Bartonella*. The pathogens are present in a broad spectrum of mammals including cats, dogs, ruminants and rodents which might either suffer from these infections [25] or serve as asymptomatic reservoir hosts for zoonotic infections [13]. Elucidating the course of *Bartonella* infections in animals, arthropods and humans will help to prevent such infections in future; vector control in cats and dogs might also prevent transmission to humans as well as disease in the pets themselves.

*Bartonella* infections of cats, dogs and humans

Infectious diseases caused by *Bartonella* have been described for more than 1,000 years. Historically, infections with *B. bacilliformis* (which is endemic in South America) are known since the dynasty of the Inca. *B. quintana* was even detected in 4,000-year-old human tissue [15]. However, only 18 years ago, *B. henselae*, the most important human pathogenic species, was discovered by David Relman who identified this pathogen in 1990 by molecular techniques [41].

Cats have been confirmed as a reservoir for *B. henselae*. Besides this species, cats are also the main reservoir for *B. clarridgeiae* and are presumably the reservoir of *B. koehlerae* which has been isolated from the blood of cats in the USA [16] and was also detected in cat fleas in France [46]. Mostly, cats with serological, cultural or PCR-based evidence of an exposure to a certain *Bartonella* spp. show no particular signs of infection. Nevertheless, *B. henselae* infections of cats have been associated with a variety of clinical manifestations, including, e.g., uveitis [31, 32].

The most prevalent *Bartonella* species in dogs, *B. vinsonii* subsp. *berkhoffii*, was initially isolated from a dog with endocarditis and intermittent epistaxis in 1993 [5, 30]. Meanwhile, this pathogen has also been associated with cardiac arrhythmias, myocarditis, granulomatous rhinitis, anterior uveitis, and choroiditis [4, 5, 36, 38, 39]. *B. henselae* [17, 19, 23, 29, 47], *B. clarridgeiae* [10,
The role of Bartonella spp. in veterinary and human medicine - V. A. J. Kempf & F. Krämer

23], B. washoensis [11], B. elizabethae [3] and B. quintana [25] have also been associated with pathology and clinical signs in dogs, including endocarditis, hepatic disease and sudden death. Furthermore, B. henselae has been implicated in peliosis hepatis [29], granulomatous hepatis [23], and granulomatous sialodeniitis [47]. Interestingly, B. henselae and B. quintana were also detected in the blood or lymph nodes of dogs suffering from lymphoma [19].

Today, the clinically most important humanpathogenic species are B. henselae, B. quintana and B. bacilliformis. A synopsis of Bartonella spp., their reservoir hosts, vectors and the spectrum of human diseases are given in Tab. 1.

Cat scratch disease (B. henselae, B. clarridgeiae)
Cat scratch disease (CSD) is caused by B. henselae (less frequently by B. clarridgeiae). The pathogens are transmitted by bites or scratches of infected cats (Fig. 1). Usually, 2–3 weeks after infection, a unilateral lymphadenitis in the lymph

Tab. 1 Bartonella spp.: reservoirs, vectors, human diseases (modified table, taken from ref. 13).

<table>
<thead>
<tr>
<th>Bartonella spp.</th>
<th>Reservoir</th>
<th>Vector</th>
<th>Human diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human-specific spp.:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. bacilliformis</td>
<td>human</td>
<td>sandfly</td>
<td>Carrión’s disease, Oroya fever, verruga peruana</td>
</tr>
<tr>
<td>B. quintana</td>
<td>human</td>
<td>body louse (cat flea, ticks)</td>
<td>trench fever, endocarditis, bacillary angiomatosis</td>
</tr>
<tr>
<td>B. rochalimae</td>
<td>unknown</td>
<td>unknown</td>
<td>bacteraemia, fever</td>
</tr>
<tr>
<td><strong>Zoonotic spp.:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. clarridgeiae</td>
<td>cat</td>
<td>cat flea</td>
<td>cat scratch disease</td>
</tr>
<tr>
<td>B. elizabethae</td>
<td>rat</td>
<td>unknown</td>
<td>endocarditis, neuroretinitis</td>
</tr>
<tr>
<td>B. grahamii</td>
<td>mouse, vole</td>
<td>unknown</td>
<td>neuroretinitis</td>
</tr>
<tr>
<td>B. henselae</td>
<td>cat</td>
<td>cat flea (ticks?)</td>
<td>cat scratch disease, bacillary angiomatosis, endocarditis, neuroretinitis, bacteraemia</td>
</tr>
<tr>
<td>B. koehlerae</td>
<td>cat</td>
<td>unknown</td>
<td>endocarditis</td>
</tr>
<tr>
<td>B. vinsonii subsp. arupensis</td>
<td>mouse</td>
<td>tick</td>
<td>bacteraemia, fever, endocarditis (?)</td>
</tr>
<tr>
<td>B. vinsonii subsp. berkoffii</td>
<td>dog</td>
<td>tick</td>
<td>endocarditis</td>
</tr>
<tr>
<td>B. washoensis</td>
<td>ground squirrel</td>
<td>unknown</td>
<td>myocarditis, endocarditis (?)</td>
</tr>
<tr>
<td><strong>Animal-specific spp.:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. alsatica</td>
<td>rabbit</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. birtlesii</td>
<td>mouse</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. bovis (= B. weissii)</td>
<td>cattle, cat</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. capreoli</td>
<td>roe deer</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. chomelii</td>
<td>cattle</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. doshiae</td>
<td>vole</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. peromysci</td>
<td>deer, mouse</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. phoceensis</td>
<td>rat</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. rattimassiliensis</td>
<td>rat</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. schoenbuchensis</td>
<td>roe deer</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. talpae</td>
<td>vole</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. taylori</td>
<td>mouse, vole</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. tribocorum</td>
<td>rat</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>B. vinsonii subsp. vinsonii</td>
<td>vole</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Fig. 1 Inoculation site (forearm) of a patient suffering from a B. henselae-caused CSD.
draining region near the site of the scratch or bite occurs (Fig. 2). In ~10% of CSD, infected lymph nodes may form a fistula through the skin draining pus. CSD is a common cause of chronic lymph node swelling in children, and these patients might also suffer from fever, headache or splenomegaly. In rare cases, ocular-glandular involvement (Parinaud’s syndrome), encephalopathy, neuroretinitis or osteomyelitis can occur. Usually, the disease is self-limiting and patients do not require antibiotic treatment. Serological testing is the best evaluated method for the laboratory diagnosis of CSD. Relapsing or systemic infections might be treated with macrolides (e.g., erythromycin).

Cats have been identified as the primary reservoir for \( B. henselae \), and the domesticated species is most often associated with the transmission of the infection to humans (mainly children) by scratches or bites [3]. Between cats, the organism is transmitted by the cat flea \( Ctenocephalides felis \) [9]. Recent studies have shown a strong correlation between the prevalence of \( B. henselae \) in cats and fleas from these cats [33].

Dogs are also suggested to represent a reservoir for \( B. henselae \) as the pathogen was found to be the causative agent in a case of human osteomyelitis following a dog scratch [28]. Furthermore, a dog owner suffering from lymphadenopathy (seroreactive for \( B. henselae \)) was reported and \( B. henselae \) DNA was amplified from gingival swaps of the dog [53]. Currently, four \( Bartonella \) species have been detected in dog saliva [18]. From these findings, a transmission of \( Bartonella \) from dogs to humans cannot be excluded and it might be assumed that vector control (flea and tick) reduces the risk of transmission to dogs (e.g., via ticks [12]) and, thus, possibly from dogs to humans.

**Bacteria-induced neoangiogenesis: bacillary angiomatosis, peliosis hepatitis (\( B. henselae, B. quintana \))**

The ability to induce tumorous vasculoproliferative disorders is a unique feature of pathogens of the genus \( Bartonella \). In humans, mainly immunosuppressed patients (e.g. AIDS patients) suffer from the vasculoproliferative disorders bacillary angiomatosis (cutaneous manifestations) (Fig. 3) or peliosis hepatitis (hepatic manifestations). Both diseases are histologically characterized as lobulated proliferations of mainly capillary-sized vessels and are understood as the result of a chronic infection with \( B. henselae \) or \( B. quintana \). The pathogen is detectable in the vasculoproliferative lesions, and bacterial eradication by antibiotic treatment results in a complete regression of the angiomatosus tumors.

**Bloodstream infections: Trench fever (\( B. quintana \))** \( B. quintana \), the agent of “Trench fever”, caused large epidemics in Europe during the First and Second World Wars. Trench fever reemerged at the end of the last century with increasing frequency. \( B. quintana \) bloodstream infections were reported in homeless people or patients with chronic alcoholism [7, 52]. The disease is characterized by a sudden onset and subsequent periodical feverish relapses (“five-day fever”) accompanied by an intraerythrocytic bacteraemia. The presence of \( B. quintana \) in the bone marrow (erythroblasts) has been described [45]. Humans are the only known natural reservoir host for \( B. quintana \); body lice transmit the pathogen from patient to patient. Interestingly, the pathogen has recently been detected in cat fleas [46] and ticks [8]. \( B. quintana \) has also been detected in two dogs suffering from endocarditis [25].

Recently, \( B. henselae \) and \( B. vinsonii \) subsp. \( berkoffii \) were detected in the peripheral blood of immunocompetent patients suffering from chronic neurological symptoms including ataxia, seizures and tremor [6].

**Fig. 2** Typical histology of CSD in a lymph node of a 52-year-old patient showing granulomatous inflammation and central necrosis.

**Fig. 3** Bacillary angiomatosis in a 37-year-old AIDS patient located at the chest. Newly appeared vessel formations can be noted.

**Fig. 4** Detection of anti-\( B. henselae \) antibodies by IFA using cell culture-derived antigen.
B. henselae infections: Diagnostic procedures
Concerning the zoonotic importance of Bartonella infections in cats and dogs, as well as the general relevance of Bartonella infections in humans, obviously, B. henselae represents the most important pathogenic Bartonella species.

A variety of methods for the laboratory identification of B. henselae infections have been employed including histological examination, isolation and culture or molecular and serological approaches. Nevertheless, the detection of the causative agent of CSD by histology or PCR (e.g., via amplification of the 16S-rDNA) requires invasive procedures (biopsy, fine-needle aspiration). Unfortunately, cultivation of the slowly growing and fastidious pathogens from patient specimen is normally not successful. Therefore, the most widely used diagnostic method is the serological testing for B. henselae antibodies. Here, an indirect immunofluorescence assay using whole cell antigen from B. henselae co-cultivated with Vero cells is used (Fig. 4).

The seroprevalence of anti-B. henselae antibodies in cats ranges from 0% in Norway [2] over 35% in Alabama, Maryland and Texas [33] and 71% in Spain [50], up to 93% in North Carolina [37], depending on the region, the method of examination and the type of cats examined (e.g., feral/stray or pet cats).

In dogs the seroprevalence of anti-B. henselae antibodies has been reported between 2% in Brazil [14] and 3% in the UK [1] over 5% in southern Ontario and Quebec [22] and 17% in Spain [51], up to 27% in south-eastern USA [49] and 28% in Italy [40].

The seroprevalence of anti-B. henselae antibodies in humans is between 5-30% and, therefore, much higher compared with the seroprevalence of antibodies against, e.g., Borrelia burgdorferi (causing Lyme disease) and many other pathogens. 13% of cervical lymph node swellings are caused by Bartonella spp. Depending on the region and the method of examination and the type of cats examined (e.g., feral/stray or pet cats).

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Pathogenicity of Bartonella spp.
Two aspects are in the focus of Bartonella research: the ability of the bacteria to induce vessel formation (“neoangiogenesis”) and their ability to cause a long-lasting bacteraemia, both being highly interesting phenomena, especially of interest for developing new strategies of inducing therapeutic vessel growth, possibly influencing angiogenesis research. Strategies of how bacteria cause chronic intraerythrocytic infections are also fascinating as the underlying mechanisms are largely unknown. However, Bartonella research is hampered by the fact that the bacteria are slow growing and no sufficient liquid medium is available. With the recently described “BAPGM”-medium [34] or our new liquid medium [44] new promising tools for liquid cultivation of Bartonella spp. now exist.

Mainly two pathogenicity factors of Bartonella spp. have been investigated in the past: (i) the so-called “VirB/D4 type-4 secretion system” (T4SS), a molecular nanomachine injecting Bartonella effector proteins (Beps) into human host cells and (ii) the so-called “trimeric autotransporter adhesins” which include the Bartonella adhesin A (BadA) of B. henselae [43] and the variably expressed outer membrane proteins (Vomps) of B. quintana [54].

Intraerythrocytic bacteraemia caused by Bartonella spp.
Several Bartonella species cause a long-lasting intraerythrocytic bacteraemia in their mammalian reservoir host. This obviously enables the transmission of the pathogens via blood-sucking arthropods (e.g., fleas, body lice). Furthermore, a persistent bacteraemia with B. henselae in asymptomatic cats (and possibly in dogs) represents an important factor facilitating the spread of microorganism.

Most of the knowledge of intraerythrocytic Bartonella infections was gained using a B. tribocorum rat-infection model which mimics Trench fever [48]. Here, the pathogens are rapidly cleared from the circulating blood after an intravenous infection. Five days later, the bacteria start to be periodically released from their unknown niche into the bloodstream. B. tribocorum persists several weeks in the circulating blood in an immunoprivileged intracellular niche (erythrocyte). This haematotropic infection strategy is probably a specific adaptation of the pathogen to the transmission by blood-sucking arthropod vectors and is shared by many Bartonella species. From all Bartonellae, only B. bacilliformis causes a massive haemolysis of infected erythrocytes often resulting in a fatal haemolytic anaemia [13]. Using human haematopoietic stem cells, it could be demonstrated that Bartonella spp. are able to infect and to persist in erythroid-differentiating stem cells suggesting that haematopoietic stem cells might serve as a potential primary niche in Bartonella infections [35].

Vasculoproliferative diseases caused by Bartonella spp.
B. henselae, B. quintana and B. bacilliformis are able to cause vasculoproliferative disorders. This feature is especially reported from humans; however, less known, also dogs can develop such vasculoproliferations (e.g. peliosis hepatis [29]).

Mechanisms leading to this neoangiogenic lesions are best investigated for B. henselae. Currently, it is speculated that Bartonella species may cause vasculoproliferations by at least three different mechanisms which may act synergistically: (i) triggering the proliferation of endothelial cells directly, (ii) inhibition of endothelial cell apoptosis (via the VirB/D4 T4SS), and (iii) angiogenic reprogramming of infected host cells. This bacterially induced angiogenic gene program is regulated via the activation of “hypoxia-inducible factor (HIF)-1” (which represents the key transcription factor involved in the induction of angiogenesis) and subsequent secretion of angiogenic cytokines (e.g., vascular endothelial growth factor, VEGF), all known to be crucially involved in neoangiogenic processes. Both, HIF-1 activation and VEGF secretion are induced after infection of cultured host cells with B. henselae in vitro and where also demonstrated in patient’s bacillary angiomatosis tissue lesions in vivo.

Based on our own previous work, we favour a “two-step”-pathogenicity model suggesting that host-cell-derived vasculoproliferative factors play a crucial role in the pathogenesis
of neoangiogenesis in Bartonella infections. The resulting proliferation of endothelia might be understood as bacterium-triggered promotion of its own niche: the endothelial cell. These proliferating host cells promote persistence and growth of B. henselae by supplying the bacterium with yet unknown, possibly proteinaceous compounds [27]. It is tempting to speculate that by affecting host-cell metabolism, B. henselae creates its own endothelial habitat [26].

Conclusion

Bartonella spp. are important pathogens in human and veterinary medicine. B. henselae is currently considered to be the most relevant zoonotic pathogenic Bartonella species responsible for CSD, bacillary angiomatosis and other diseases. Besides B. henselae, several other Bartonella species have been isolated from cats, dogs and humans with a variety of clinical manifestations, from symptomless to severe disease. The ability to cause vasculoproliferative disorders and intraerythrocytic bacteraemia are unique features of the genus Bartonella. Obviously, the VirB/D4 T4SS and BadA are key virulence factors of B. henselae responsible for host-cell infection, inhibition of endothelial cell apoptosis and induction of angiogenic gene programming. Elucidating the course of Bartonella infections in animals, arthropods and humans will help to understand and thus possibly prevent such infections in future. Taking into account the current knowledge about Bartonella infections, their zoonotic nature and the transmission risk by arthropods, it can be assumed that vector control strategies reduce the ectoparasite-host interactions thus limiting pathogen transmission.

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Meticillin-resistant staphylococci in companion animals

T. Nuttall(1), N. Williams(2), R. Saunders(1), S. Dawson(2)

Introduction

MRSA (meticillin resistant *Staphylococcus aureus*) and other meticillin resistant (MR) staphylococci are major medical and veterinary health care concerns. MRSA and other meticillin resistant staphylococcal species have been isolated from dogs, cats, rabbits, birds, horses and farm animals. Animals could be at risk of colonisation or infection in veterinary premises and/or act as reservoirs for colonisation or infection of in-contact humans. Colonisation in this context refers to the non-pathogenic temporary (also referred to as ‘contamination’) or persistent (or ‘carriage’) presence of MR staphylococci at mucosal surfaces or on the skin. The aim of this review is to give veterinary clinicians and nurses or technicians an overview of the current state of MR staphylococci in companion animals, and an update on the practical and clinical aspects of controlling these organisms.

MRSA in humans

MRSA is of little concern to healthy people. Approximately 20-30% of people are colonised with meticillin sensitive *S. aureus* (MSSA) but probably less than 1% carry MRSA. The carriage rate in healthcare workers, however, may be 5-10%. Veterinary personnel have also been shown to have higher carriage rates than the general population [1-4].

There has been a dramatic increase in MRSA infections in recent years, especially in patients who have skin or mucosal barrier defects, are immuno-compromised, undergo extensive surgery or are long term in-patients. MRSA clones can be differentiated by several techniques including pulsed field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and antibiotic resistance patterns. In the UK, the majority are hospital acquired (HA) epidemic (E) strains 15 and 16 [5]. EMRSA-15 is resistant to β-lactams, fluoroquinolones and macrolides, whilst EMRSA-16 can be resistant to further antimicrobials. EMRSA-15 is also prevalent elsewhere in Europe. Based on blood culture isolates, the prevalence of MRSA infections varies greatly across Europe,
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Fig. 1 70% alcohol hand rub worn on a nurse’s uniform. All our staff and students wear these for quick and easy hand disinfection between patients. Organic debris must be washed off with a detergent though.

from <1% in Scandinavia and the Netherlands to >40% in southern and western Europe [6].

Community acquired (CA) clones are genetically distinct from HA-MRSA clones [7]. CA-MRSA are usually isolated from individuals without healthcare contact or other risk factors. Infections are rare and sporadic but can be severe and life-threatening. Antibiotic resistance is less marked but CA-MRSA infections are often associated with Panton Valentine Leucocidin (PVL). PVL can result in extensive tissue necrosis and severe disease [8]. The designations HA and CA do not necessarily refer to the source of colonisation or infection: HA-MRSA can be acquired in the community [9] and CA-MRSA clones have been associated with hospital acquired infections [10].

MR staphylococci in animals

MR staphylococci have been isolated from healthy and diseased individuals in many animal species [11-21]. The epidemiology varies between staphylococcal isolates, animal species and geographic location, reflecting differences in staphylococcal species, animal-human interaction, antimicrobial use, and species susceptibility.

MRSA in companion animals

MRSA has been the most frequent MR staphylococcal species isolated from animals in the UK, Ireland and Canada [14-16, 19, 22, 23]. British, Irish and German small animal isolates are mostly identical or closely related to EMRSA-15 [14, 16, 23-25]. EMRSA-16 has also been isolated from dogs in the UK, but is less common [26]. In contrast, canine isolates unrelated to human clones have been occasionally identified in the UK, the Netherlands and Germany [20, 27, 28].

In the US and Canada the predominant human strain, Canadian epidemic MRSA-2, (USA100) is most common in animals [15, 29, 30]. More recently PVL-positive Canadian epidemic MRSA-10 (USA-300) has been isolated from small animals in both Canada and the USA (Weese personal communication). The PVL gene has also been detected in 50% of US MRSA isolates from animals [31].

The majority of isolates from horses, rabbits and other animals in the UK, Ireland, Austria, Germany, Japan, US and Canada, in contrast, are largely restricted to these animal species and are only rarely found in the human population [14, 16, 20, 23, 32, 33]. Equine isolates are predominantly identical or closely related to Canadian MRSA-5 (USA500), which is rare in the general human population but has been isolated from in-contact humans [32, 34].

An MRSA type identified as sequence type (ST) 398 by multi-locus sequence typing has come to prominence because of its association with pig farming, farmers and veterinarians in the Netherlands and Germany [21, 35, 36]. ST398 has also been isolated from horses in Germany and Canada, and neonatal humans in Scotland.

MR S. intermedia/pseudintermedius

MR has also been seen in other staphylococci more commonly associated with animals, particularly S. intermedia [37-39], although it is now thought that most, if not all, canine isolates are actually S. pseudintermedius [40-42]. In a recent Japanese study, 17/18 MR staphylococcal isolates from dogs were S. pseudintermedius [41]. In Germany, 12 identical or closely related S. intermedia strains, resistant to at least five antimicrobial classes including penicillins, cefalosporins and fluoroquinolones, were isolated from skin and ear infections in 11 dogs and one cat [43].

Other MR staphylococci in animals

Other MR staphylococci isolated from animals include the coagulase positive species (CPS) S. schleiferi subsp. coagulans, particularly in the US [37-39], and the coagulase negative staphylococci (CNS) S. schleiferi subsp. schleiferi, S. haemolyticus, S. vitulinus, S. sciuri, S. epidermidis, and S. warneri [44]. CNS are usually regarded as non-pathogenic, commensal species, as they lack many of the virulence factors associated with CPS. CNS, particularly S. schleiferi subsp. schleiferi, have nevertheless been isolated from pyoderma and wound infections in animals [38, 39, 45, 46], although it can be difficult to determine their importance in mixed infections. Commensal CNS will also be exposed to antibacterials, and could act as reservoirs of mobile genetic elements encoding for antimicrobial resistance (see below). Further research on the clinical relevance of CNS and the impact of antibacterials on commensal bacteria is therefore warranted.

Origins and dissemination of MR staphylococci

Several studies have associated identical or very closely related MR staphylococcal isolates with particular veterinary premises in the UK, Denmark, Austria, Germany, Ireland, Japan and Canada [22, 23, 33, 35, 41, 43, 47, 48]. The genetic diversity of MR S. pseudintermedius and intermedia isolates suggests that colonisation can be acquired either in veterinary clinics or
in the community [41, 43], but the similarity of isolates from the staff and patients in particular premises suggests dissemination in veterinary clinics occurs. A US study of 27 veterinary teaching hospitals, however, could not associate specific clones with individual hospitals and concluded that colonisation was probably community acquired [49].

**Antibacterial use and the emergence of MR staphylococci**

It is likely that antibacterials played a role in the emergence of MR staphylococci in animals. One study concluded that the prevalence of MR-CNS in companion animals reflected national and local patterns of antimicrobial use [47]. A significantly higher prevalence of resistance to two or more antibacterials was reported in *S. intermedius* isolates from dogs and domestic pigeons compared to those from wild birds [50]. Prior use of antibacterials is also a risk factor for MRSA infection in humans and horses [18, 51], and recent studies by the authors and others have shown that courses of broad-spectrum antibacterials are a risk factor for MRSA infection in dogs.

**Rational and responsible use of antibacterials**

Measures to minimise the development and spread of antimicrobial resistant bacteria should be adopted [52]. It is beyond the scope of this article to discuss these in detail, but issues to address include:

- Ensuring that the patient has a bacterial infection
- Ensuring that the infection warrants systemic or topical antibacterial therapy
- Selecting the most appropriate drug
- Using an efficacious dose for an adequate treatment period
- Maximising compliance

It can be helpful to divide antibacterials into first, second and third line drugs, although this will depend on the patient, target tissue and type of bacteria. First line drugs include older antibacterials and/or narrow spectrum drugs such as simple penicillins, tetracyclines, and sulfonamides. These are no less potent than other antibacterials in the appropriate circumstances. Second line drugs include newer, broad spectrum products that are more important for humans and animals and/or more prone to resistance (e.g. broad spectrum β-lactamase resistant penicillins, cefalosporins and macrolides). These should be used where culture or good empirical evidence indicates that first line drugs will be ineffective. Third line drugs are those very important to humans and animals (e.g. fluoroquinolones, anti-*Pseudomonas* penicillins, ceftazidine, imipenem etc.). They should only be used where culture indicates they are necessary.

In one study the adoption of antibacterial use guidelines lead to a significant fall in the use of second and third line drugs with first line drugs accounting for approximately 90% of treatment [53].

**The prevalence of MR staphylococci in companion animals**

**Colonisation in healthy animals**

It is likely that the colonisation rate in healthy animals is low. One Slovenian study did not isolate any MR-CPS from 200 healthy dogs [46]. A UK study failed to isolate MRSA from dogs sampled in the community over a two month period [16], and only 1/255 dogs was positive for MRSA in another [54].

**Colonisation and infection in sick animals**

The colonisation rate in non-healthy animals seems to be much higher, although not all reports distinguish colonisation and infection. One UK study found that MRSA was carried by up to 10% of dogs referred to a small animal veterinary hospital without MRSA infections [24], although these had received treatment including antibacterials prior to referral. MRSA was also isolated from approximately 3% of clinical submissions to a UK veterinary diagnostic laboratory [25]. In Ireland, MRSA was recovered from 25 animals (14 dogs, eight horses, one cat, one rabbit and a seal) and from 10 in-contact veterinary personnel [14]. Of 869 submissions from a German small animal hospital over a 20 month period, 3% (approximately 0.05% of the total caseload) were MRSA [20]. Similar proportions of samples were from dogs (3%) and cats (2.7%), with MRSA also isolated from a bird, rabbit, guinea pig, turtle and bat. In the authors’ institution MR-CPS infections comprised 0.04% of all cases and 0.5% of all bacterial infections from 2004-2007. Twelve MR *S. intermedius* isolates identified in one report accounted for 23% of all *S. intermedius* submissions from a German dermatology referral clinic during an 18 month period [43]. MR *S. pseudintermedius*, *S. aureus* and *S. schleiferi coagulans* were isolated from 2.1%, 0.5% and 0.5% respectively of 193 dogs referred to a Canadian Veterinary Teaching Hospital [55].

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**Fig. 2 Fully waterproof and washable keyboard for use in clinical areas.**
A prevalence study at a UK referral hospital isolated MRSA from 6% of staff and 6% of dogs (2/3 had MRSA infections) but no cats [16, 56]. A single day prevalence study at another UK referral hospital found that 18% of staff, 9% of dogs, 10% of environmental samples and again no cats were colonised by MRSA [24]. In Japan, MR-CPS were isolated from 15% of staff, 46% of canine in-patients and 19% of canine out-patients [41]; 2/3 isolates from staff were MRSA, but only one of the isolates from the dogs was MRSA and the remaining 17 were MR S. pseudintermedius.

In contrast, US studies have identified MR staphylococci at much higher frequency. They have been isolated from 15% of healthy cats and 38% of dogs with recurrent pyoderma; MR rates higher frequency. They have been isolated from 15% of healthy In contrast, US studies have identified MR staphylococci at much higher frequency. They have been isolated from 15% of healthy In contrast, US studies have identified MR staphylococci at much higher frequency. They have been isolated from 15% of healthy In contrast, US studies have identified MR staphylococci at much higher frequency. They have been isolated from 15% of healthy...

**Detection of MR staphylococcal infections**

Any Staphylococcus with unusual (e.g. clavulanate potentiated amoxicillin, cefalosporin or fluoroquinolone) antibacterial resistance is suspicious, and resistance to oxacillin and identification of the mecA gene is definitive. It is important to use a reputable laboratory as some techniques overestimate resistance and not using enrichment may miss 8-13% of MR isolates (M. Rich, personal communication). Further genetic typing to identify the strain is important in epidemiology. Swabs should be taken from clinical lesions and implants as appropriate in each case. Swabs should be taken from both the nose and perineum, as single swabs miss 29-56% of dogs colonised with CPS (J. Fazakerley, accepted for publication).

**Treatment**

MR staphylococci are usually resistant to all penicillins, cefalosporins and fluoroquinolones [60]. Most MRSA isolated from animals also appear to have inducible clindamycin resistance [25]. Tetracyclines, trimethoprim-sulfonamides, fucidic acid and mupirocin are often effective. Recently, however, S. pseudintermedius and S.intermedius isolates resistant to five or more classes of antibiotics including -lactams, cefalosporins, fluoroquinolones, macrolides, trimethoprim-sulfonamides, fucidic acid and mupirocin have been reported [41, 43].
Antibiotics should be chosen following bacterial culture and sensitivity tests. These are, however, less reliable in predicting the response to topical antibiotics as direct application can achieve local concentrations in excess of the minimum inhibitory concentration break points established for systemic therapy. For example, of 11 multi-drug resistant Staphylococcus intermedius in one study, seven responded to a combination of systemic and topical antibiotics, and four to topical therapy only [43].

It is most important to identify the underlying cause. Further treatment depends on the nature of the primary problem (e.g., removing implants and or the use of gentamicin impregnated beads, collagen sponges, activated silver dressings etc.) [13]. The majority of animals with MR staphylococcal infections survive, and in about half of the fatal cases, death is attributable to the underlying disease. In the authors’ clinic the mortality rate with MR staphylococcal infections is comparable to that in meticillin sensitive CPS infections.

**Discharged patients and decolonisation**

Patients should be discharged as soon as they are clinically fit. If the animal remains colonised potential risks and precautions must be discussed with the owner. Decolonisation of humans often involves repeated cycles of intranasal fucidic acid or mupirocin and chlorhexidine washes. Treatment clears 91-99% of patients but re-colonisation rates are up to 26%. Experience suggests that dogs require longer courses of treatment of 2-3 weeks. At present, non-antibiotic decolonisation methods are preferred. Hygiene, cleanliness and removal from the source are most important and effective.

**Preventing contamination with MR staphylococci in veterinary clinics**

**Admission**

Admit known or suspected cases directly into a consultation room to avoid contamination and contagion in the waiting room. To minimise contact and contamination procedures should be scheduled for the end of the day, discharging wounds covered with an impermeable dressing and trolleys used to move patients. Surfaces and equipment should be cleaned and disinfected before they are used again.

**Hospitalisation**

- Isolate MRSA patients as far as possible from other patients.
- Use disposable gloves, gowns, hats and mask. Long hair should be tied back and sleeves rolled up to the elbow. Eye protection should be worn if there is a risk of splashing or aerosols. Overshoes, however, may actually increase contamination (presumably from having to balance and handle shoes).
- Strict washing of the hands and forearms after handling the patient.
- Equipment should be used with the affected patient only and then disposed of or disinfected.
- Bathing every 2-3 days with an antibacterial wash may reduce mucosal and cutaneous carriage, but may be not be possible with all individuals or species, increases staff or owner contact and could increase environmental aerosols.

- Before surgery, it may be possible to decontaminate the patient (see above).
- Covering wounds with impermeable dressings and preventing licking (using collars etc.) may help reduce wound infection.

**Routine measures to prevent the spread of MRSA (and other infectious diseases)**

1. Hand washing and disinfection of surfaces and equipment between patients. Alcohol gel pouches on uniforms and kennels are a visual cue and can be quickly used immediately after handling an animal. Remember that alcohol will not be effective in the presence of organic matter - this must be removed by hand washing with a detergent.
2. Avoid materials at hand touch sites that cannot be cleaned - use washable keyboards or keyboard covers etc. Alcohol wipes are effective and easy to use on equipment and keyboards, but soiled surfaces must be cleaned with a detergent.
3. Cover wounds or skin lesions with waterproof dressings.
4. High standards of aseptic technique: minimise theatre staff; sterile gowns, gloves, hats and masks; sterilisation of equipment; and single patient use.
5. High standards of cleaning: clean cages and bedding at least once daily, and thoroughly clean and disinfect between patients. Antimicrobial impregnated materials have not been shown to prevent colonisation in clinical settings. The authors recently found that no difference in bacterial contamination of standard and tolnaftate/triclosan impregnated veterinary bedding material.
6. Segregation of all waste and contaminated material.
7. Ensure that all staff understand and adhere to infection control.

**Monitoring and surveillance**

This is a very controversial and no figures for acceptable microbiological levels in medical or veterinary premises have been established. If surveillance is used, it is essential that it is conducted following advice, is done with a reputable laboratory and that the policy has clear aims.

Fig. 4 MRSA wound infection.
Routine environmental screening could be used to monitor cleanliness – of 82-91% hospital surfaces judged to visually clean only 30-45% were microbiologically clean. MRSA contamination rates have declined where cleaners have been trained to think about microbiological cleanliness. One Canadian study isolated MRSA from 10% of samples (including tables, floors, telephones, keyboards, taps, kennels, stethoscopes and otoscopes) [61]. Surveillance of staff is highly controversial and involves problems with consent, confidentiality, stigmatisation and further action. Screening should therefore be done to identify with consent, confidentiality, stigmatisation and further action. Screening should therefore be done to identify problems in infectious disease control, and not to apportion blame or as a substitute for control measures [62].

Transmission of MRSA between animals and humans

Recent studies suggest that up to 45% of owners of dogs with pyoderma can be colonised by S. intermedius [63] and that concurrent human-animal colonisation occurs in 20% of S. aureus and 67% of S. intermedius positive households [64]. MRSA has also been isolated from up to 17% of veterinary staff and 11% of owners in contact with infected animals, compared to 5% and 0% in contact with MSSA infected animals (Annette Loeffler, personal communication). In another recent case, identical PVL-positive MRSA strains were isolated from three humans and one dog in a household [65]. MRSA colonisation has also been found in 1/1 cats and 16/88 humans in contact with eight dogs and three cats with MRSA infections [29].

MRSA colonization may be an occupational risk for veterinary staff. MRSA has been isolated from the nares of 7% of vets and 12% of nurses sampled at a North American veterinary congress [1] and 10% of vets and nurses sampled at a British congress [2]. At the American meeting, working in large animal practice was significantly associated with colonisation. The pattern of colonisation reflected the strains most commonly seen in animals; Canadian epidemic MRSA-2 was isolated from 11 small animal but only two large animal personnel, whereas Canadian epidemic CMRSA-5 was only isolated from large animal clinicians [1]. In the Netherlands, 4.6% of veterinary staff working with pigs were found to be colonised with porcine-associated MRSA strains that are otherwise rare in the human population [3]. Another study at an equine meeting isolated MRSA from 10% of the participants [4]. Contact with an MRSA patient increased the risk of MRSA colonisation, whereas hand washing between patients and farms was protective. Work conducted by the authors found a prevalence of 8% in veterinary personnel attending a British equine congress. Approximately half the strains were typical of those seen common in small companion animals, with the other halve typical of equine associated strains, which are less common in the human population.

MRSA colonised animals can be reservoirs for re-colonisation of in-contact humans [65-69]. True zoonotic infections, however, are rare and sporadic, and associated with accepted risk factors for opportunistic infection. There is a report, however, describing skin infection in three and nasal colonisation in 10 veterinary staff treating a foal with Canadian epidemic MRSA-5 [70]. Transmission occurred despite barrier nursing precautions and resulted in infection in healthy individuals without risk factors for opportunistic infection.

References

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