



Review Article

Necropsy findings as a post mortem diagnostic technique for transmissible diseases

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Summary

Zoonoses are considered as diseases transmissible between human and animal and can cause severe reactions in humans, leading to high death. To date, significant effort has been made in the prevention and treatment of zoonotic diseases. However, due to the infectious nature of zoonotic diseases, their complete eradication presents a challenge. As zoonotic infections result in a wide range of diseases and death, necropsy is an important procedure for its diagnostic investigations. This article highlights four of the most important zoonoses, including leishmaniasis, rabies, anthrax, and glanders. Leishmaniasis can affect various organs of the body and induces lesions and ulceration. Rabies can be transmitted by dog bites and the nervous system is its main target. Anthrax has three primary forms (cutaneous, alimentary tract, and inhalational) with a high incidence of significant pathological lesions seen at necropsy. Sores on the nasal mucosa, nodules on skin of the abdomen and lower arm can be seen in glanders. The necropsy findings of the aforementioned zoonotic diseases are discussed in the current review to provide scientific guidelines for their diagnosis and exerting preventive measures to control them.

Keywords: Necropsy findings, Anthrax, Leishmaniasis, Rabies, Glanders

Introduction

The diseases that are transmissible from living animals to humans are known as zoonoses (Hubálek, 2003; Allen et al., 2017). The word is from Greek: zoon "animal" and nosos "sickness". There are controversies towards selecting zoonoses. First, diseases transmissible from animal to human were known as anthroozoonoses, and diseases transmissible from human to animals were called zooanthroponoses. However, these two terms caused confusion among scientists who were working in this field, and finally, zoonoses was

adopted as the common term for describing diseases transferred from vertebrate animals and human (Bell et al., 1988; Joint and Organization, 1959). The attention towards zoonotic diseases is not due to their outbreak, since a small number of these diseases can cause an extensive outbreak. However, zoonoses can result in significant mortality and strategies should be performed in limiting the outbreak of these infections. Furthermore, it has been reported that many of zoonoses such as hemorrhagic fevers can be transmitted to health care providers in hospitals. The zoonoses can be classified according to the

ecosystem in synanthropic and exoanthropic (Pavlovsky, 1966; Woodring et al., 1996).

The post-mortem investigation is performed as an important way in identifying the cause of death. Patho-morphological changes are reported in necropsy. Besides, necropsy is beneficial in providing background about animal health in a fallen stock or in a diseased animal. The necropsy is a vital mainstay in the field of veterinary medicine and outbreak of diseases can be determined in a certain population of animals, some unwanted conditions may occur and in this case, necropsy reports are of importance. The necropsy procedure is in line with clinical medicine. Necropsy can approve or reject pre-mortem diagnosis. The necropsy findings can aware us of the presence of diseases capable of transmitting to other animals or even humans. Therefore, cautions can be performed for those that are at risk. The diseases that are emerging in veterinary medicine, can be determined by necropsy (Küker et al., 2018).

In the present review, our aim is to provide guidance on procedural aspects of the necropsy on the major and important zoonoses leishmaniasis, rabies, glanders, and anthrax diseases. In each section we describe briefly basic principles of each communicable disease and their necropsy findings in details, so the disease can be recognized by both clinical and post-mortem examination.

Zoonotic diseases and precautions

A variety of dangerous zoonotic diseases are present with the capacity of transmitting to humans from animals. The people should be aware of signs of a diseased animal, the process of transmission, and preventive measures to inhibit infection. If a potential and dangerous exposure occurs during a necropsy, the person should consult with a medical doctor. As necropsy is a dangerous procedure, protective tools including goggles, latex gloves, masks, hats, shockproof shoes, and clothing should be used. The veterinarian should consider using equipment that is beneficial in this process. The advantage of using such protective tools are reducing any potential risks of injury or infection

in individuals performing necropsy (Law et al., 2012).

Leishmaniasis

An overview

Leishmaniasis is caused by members of the leishmania family and can generate diseases with protean manifestations (De Brito et al., 2020; Kaye et al., 2020; Gurel et al., 2020). Leishmania is a vector-borne parasitic poverty; and malnutrition, poor housing conditions and migration are considered as potential risk factors for its development. The major reservoir of this disease is wild rodents, whereas canines, rats, and dogs are not the main reservoirs. The incidence rate of leishmaniasis has undergone an increase in Syria, Turkey, and Jordan due to the presence of war (Mock et al., 2012; Desjeux, 2004; Isenring et al., 2018; Uzun et al., 2018; Bailey et al., 2017; Alawieh et al., 2014; Du et al., 2016). Furthermore, the migration of humans around the world has significantly enhanced the incidence rate of leishmaniasis (Hodiamont et al., 2014; Showler and Boggild, 2015; David and Craft, 2009). As a complicated disease, leishmania is divided into two categories including visceral leishmania (VL) and cutaneous leishmania (CL) (Kaye et al., 2020). The female phlebotomine sandflies are responsible for the transmission of the leishmania parasite to the host. The parasite changes when it is either in the host or flies. When leishmania is in the fly's gut, these parasites demonstrate a distinct figure as flagellated promastigote. However, when leishmania comes into the mammalian body, attacks macrophages, leading to lose of their flagellum and changing into intracellular amastigote. There is growing evidence demonstrating that in sand fly, promastigote form of leishmania can undergo differentiation to produce metacyclic forms that are completely infective (Sacks, 1989; Dostálová and Volf, 2012; Serafim et al., 2018). Noteworthy, heterogeneity has been also observed in the host body, when parasites are in intracellular amastigote form (Greig et al., 2015; Mandell and Beverley, 2017;

Burchmore and Barrett, 2001). A variety of drugs and vaccines are applied in the treatment of CL and VL, and also, there are diagnostic tools for this complex disease (Torres-Guerrero et al., 2017). However, we still have a long way to complete eradication of this infectious disease and consequently, our understanding of necropsy findings should be improved in providing its post-mortem diagnosis. This aspect is discussed in the next section.

Necropsy findings of leishmaniasis

The necropsy findings of leishmaniasis are different, but some of them can be considered as potential markers for leishmaniasis diagnosis after death. In VL, major organs of the body are affected. It has been reported that these parasites can induce lesions in organs including liver, spleen, skin, and lymph nodes. These lesions are attributed to complex inflammatory responses mediated by parasites. The predominant immune cells are macrophages and it seems that these inflammatory reactions can result in systemic necrotizing vasculitis (Márquez et al., 2012; Baneth et al., 2008; Pumarola et al., 1991). Therefore, inflammation-mediated lesions can be observed in organs of animals infected with leishmania. Noteworthy, the nervous system is one of the

potential targets of these parasites. In CL, histopathological lesions are observed in the central nervous system (Lima et al., 2003). This is known as cerebral leishmaniasis (Garcia-Alonso et al., 1996). Furthermore, it has been revealed that amastigotes of leishmania can result in the generation of myelitis and mild meningoencephalitis (Márquez et al., 2013). The common histopathological findings are when *Leishmania* internalizes in host macrophages and causes inflammation. Amastigotes can proliferate in the reticuloendothelial system of important organs of the body such as liver, spleen, and lymph node. The emergence of this action in necropsy is the enlargement of the aforementioned organs (Schantz et al., 2005). *Leishmania* can also cause skin and mucosal lesions such as alopecia and ulceration that can be detected in postmortem examination (Greene, 1984).

Overall, the following signs can be considered as necropsy findings of leishmaniasis:

- Enlargement of lymph node, spleen, and liver,
- Lesion and ulcerations in organs,
- Presence of nodules in skin
- Broncho-pneumonia and enlargement of bronchial lymph nodes (Figure 1).



Fig. 1. Cutaneous lesions in a dog infected with visceral leishmaniasis (Moheballi et al., 2010).

Differential diagnosis of cutaneous leishmaniasis

In most cases with other skin diseases including tropical wounds, infected insect bites, leprosy, lupus vulgaris, blastomycosis, mycobacteriosis, and skin cancer can be confused and the aforementioned hints should be considered for distinguishing them.

Rabies

An overview

Rabies is a negative-stranded RNA genome belonging to the Rhabdoviridae family and *Lyssavirus* genus (Gold et al., 2020). Rabies is a viral zoonosis causing high death around the world (Pantha et al., 2020). Dog is known as the major source of rabies virus and is capable of infecting mammals. This virus can be transmitted to humans and domestic animals from carnivores and bats. Up to 95% of rabies in humans is due to dog bites (Ma et al., 2020). Saliva is the most important way for the transmission of rabies from dog to human (Leung et al., 2007). This virus primarily affects the central nervous system causing encephalitis. The fatality of rabies is near to 100% (Fooks et al., 2014). With respect to the development of vaccines, the incidence rate of rabies has undergone a decrease (Briggs, 2012). As domestic dogs are responsible for rabies transmission, their vaccination is of importance (Lembo et al., 2010). As rabies is a virus, there is no absolute treatment for this life-threatening disease and preventive measures should be performed in its eradication. In the next section, necropsy findings of rabies are described.

Necropsy findings of rabies

The nervous system (brain) is the major target of rabies. Swelling of the brain, spinal cord, and nerve roots are considered as the clinical signs of rabies. Overall, there are two major clinical signs in animals including angry form (irritating) and paralyzed form (silent). With respect to paralysis in swallowing, foams are observed in mouth. Then, paralysis extends to limbs, until the death of animal

due to inability of the respiratory system. In fact, following paralysis of limbs, paralysis of the respiratory system leads to death. The diagnosis of this disease is based on biopsies from brain tissue. Synovitis and lymphadenopathy are the symptoms that can be observed after death. Edema in different regions of the body including submandibular, cervical, and vocal cord can be observed that are potential markers of rabies. Blood in bronchi, bronchioles, stomach, and intestine is observed. Furthermore, consolidation of the antero-ventral lung lobe occurs.

Differential diagnosis

This disease should be distinguished with lead poisoning, listeriosis, pseudorabies, and encephalomalacia, and for this purpose, the aforementioned hints can be applied.

Anthrax

An overview

Bacillus anthracis is a gram-positive and spore-forming bacterium inducing a zoonotic disease, known as anthrax or Charbon's disease (Tournier and Rougeaux, 2020; Kondakova et al., 2019). A wide variety of mammals, particularly cattle, sheep, goats, and humans are affected by this disease. Other species such as elephants, camels, and deer are also susceptible. The disease is not serious in birds and ostriches are more sensitive compared to birds. There are three distinct kinds of anthrax including A) cutaneous form, B) gastrointestinal form, and C) pneumonic form. The development of these three forms depends on the way of exposure; for instance, inhalation results in the development of pneumonic form, which is the most severe form due to its systemic nature and rapid extension. The dangerous aspect of anthracis is that each spore of this bacterium is considered a weapon. The spores of this bacterium possess a high survival rate and can survive extreme conditions such as temperature or chemical exposures. They can easily diffuse in the environment and among species (Setlow, 2006;

Nicholson et al., 2000). Due to a high threat resulted from *Bacillus anthracis*, the center for disease prevention and control has been categorized in place A, as a factor that should be in priority (Rotz et al., 2002). Herbivores are major targets of this bacterium and less frequency is observed in carnivores (Hueffer et al., 2020). Different unknown factors are responsible for the development of anthrax as a complicated disorder; however, it has been shown that among ruminants, reindeer and cattle are susceptible to this infection (Quinn et al., 2011). As it was mentioned, anthracis can produce spores tolerant to stress conditions. For survival, *Bacillus anthracis* requires oxygen, and after animal death, it generates spore as its inactive form, until appropriate conditions are provided (Cherkasskiy, 1999). Therefore, even in dead animals, cautions should be performed to prevent its spread. In the next section, necropsy signs that can be used for post-mortem detection of this disease are discussed.

Necropsy findings of anthrax

It has been reported that hemorrhage and fibrosis of the kidney result from anthrax (Ross, 1955,1957; Zaucha et al., 1998). There are some possibilities for the development of these lesions in kidney, but the underlying mechanism has not yet been understood. It seems that inflammation, exotoxins, cardiogenic or septic shock may participate in renal fibrosis and hemorrhage (Ross, 1955). The important aspect is the emergence of these lesions on kidney that can be observed in the kidney of the affected animals. The lung is another target of anthrax and signs such as edema, congestion, and alveolar histiocytosis could be seen (Twenhafel, 2010). There have been efforts in developing models for evaluating necropsy findings of anthrax. For instance, in rabbits, *Bacillus anthrax* can stimulate lymph node hemorrhage, adrenal gland hemorrhage, splenomegaly, and lung edema (Zaucha et al., 1998). Anthrax can also negatively affect the brain. The thick dura matter and meningitis are observed in necropsy (Twenhafel, 2010). However, anthrax negatively affects various organs. Necrosis, hemorrhage, and congestion in the mediastinum,

gastrointestinal tract, and urogenital organs are also observed. Enlargement of spleen occurs and sides of this organ acquire round shape. Pericardial effusion and widened mediastinum are other necropsy findings of this infection (Twenhafel, 2010).

The aforementioned statements demonstrate that necropsy findings are beneficial in the diagnosis of this disease. However, the most important ones are bloody discharges from the muzzle and anus, and dark tarry blood that does not clot. Enlarged spleen and sludge can also be seen in necropsy. Although we put much emphasis on the role of necropsy as a gold standard for the diagnosis of this disease, the autopsy of anthrax cases is not suggested due to the risk of contamination. If it is essential to perform a necropsy, high precaution should be applied. Furthermore, a simple blood smear seems to be completely advantageous for its diagnosis.

Differential diagnosis

Anthrax should be differentiated in cattle and sheep with clostridial infections, bloating and lightning strikes, bacillary hemoglobinuria, anaplasmosis, and acute lead poisoning, and clover poisoning.

Glanders

Basics

Glanders is considered a bacterial disease with high fatality. Glanders negatively affects equine populations and its control is of importance. It is a zoonosis disease, being common between humans and animals. A bacterium known as *Pseudomonas mallei* is responsible for glanders disease that is a non-encapsulated and non-hexagonal gram-negative bacterium. New references use *Burkholderia mallei* instead of *Pseudomonas mallei*. Humans demonstrate high susceptibility to this disease and can cause high death. Among animals, horses, mules, and donkeys are species susceptible to glanders. The infection by the bacterium is complicated. In the first phase, the bacterium infects the lung causing lesions (abscess-like), and then, the disease extends to the bronchioles and infects the upper respiratory tract. The organism can be removed through the mouth

and nose. Using contaminated drinking fountains is a major source for the spread of disease. It has been reported that the skin secretions of horses are also contagious and can spread disease (Nikvand et al., 2018; Tapia et al., 2019; Khan et al., 2013; Van Zandt et al., 2013).

Necropsy findings of glanders

A variety of signs can be observed in necropsy that are of importance for the diagnosis of glanders. Sores on the nasal mucosa, nodules on the skin of the abdomen and lower arm can be seen in carcass. Besides, enlargement of submandibular lymph nodes occurs. There is a cutaneous form of glanders disease that can be identified with the emergence of subcutaneous nodules with purulent discharge and dark-brown colored. The discharge of lymph nodes occurs and lymph nodes are severely involved in this disease. The middle part of the rabbit joint is the best place for observing skin lesions.

In respect to the risk of contamination of a person performing necropsy, carcasses of animals affected

with glanders should not be examined. All tissues demonstrate traces of petechiae hemorrhages. Bronchial lymph nodes are getting enlarged with a severe bronchial pneumonia.

As it was mentioned earlier, the respiratory system is involved in the glanders. Lesions can be observed on the upper respiratory tract in the nasal mucosa and to a lesser extent in the laryngeal mucosa, trachea, and bronchi. Ulcerations can be observed in the forms of nodules, scars, and star-shaped lesions.

On the surface of the skin and under it, there are nodules and sores that can be seen in necropsy. Liver, spleen, lung, and kidney are other organs that may be affected by nodules with gray color. The nodules are round in shape with low size (1 cm in diameter) with a center that is classified. Furthermore, lesions demonstrate regions of inflammation. The lymph nodes of the region are large, bloody, with the presence of purulent; and fibrous foci (Figure 2).



Fig. 2. The presence of lymph node enlargement (left) and injury in mucosal membrane of the nose in an animal infected with glanders (Nikvand et al., 2018).

Differential diagnosis

Glanders disease should be differentiated from strangely and ulcerative lymphangitis.

Conclusion and remarks

Post-mortem investigation is performed as an important way in identifying the cause of death. Besides, necropsy is a vital mainstay in the field of veterinary medicine, and outbreak of diseases can

be determined in a certain population of animals. The necropsy findings can aware us of the presence of diseases capable of transmitting to other animals or even humans. Therefore, cautions can be given for those that are at risk. In the present review, necropsy findings of four major zoonoses were discussed. These signs can be utilized for post-mortem diagnosis of the aforementioned diseases. As they possess high infectious nature, their

control is an increasing challenge and these signs can thus be used for detection of such diseases in a certain region to exert preventive measures for inhibiting its further spread in that region. Leishmania has two forms of CL and VL and can generate lesions and ulcerations in different organs of the body such as kidney, liver, skin, and so on. Swelling of the brain can result from rabies. Meningitis, splenomegaly, lung edema, and hemorrhage in organs are observed in anthrax. These signs can be utilized for their diagnosis and distinction with other diseases. Cautions should be considered while performing a necropsy since there are spores that can induce infection while examining.

Abbreviations

VL, visceral Leishmania; CL, cutaneous Leishmania.

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Conflict of interest statement

The authors declare no conflict of interest.

Ethical approval

Not applicable

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