***Review Article***

# LABORATORY ANIMAL DISEASES AND THEIR MONITORING TOOLS


## ABSTRACT

Microbial diseases pose significant challenges in experimental research, impacting both animal welfare and research outcomes. The review covers a wide range of microbial pathogens, including bacteria, viruses, fungi, and parasites, that commonly affect laboratory animals. It explores the etiology, pathogenesis, and clinical manifestations associated with these pathogens, highlighting their influence on experimental results. Extensively discussed about the monitoring assays employed for microbial disease detection in experimental animals. Traditional and modern techniques are considered, including microbiological culturing, serological assays, molecular diagnostics, histopathology, and advanced imaging methods. Regular health monitoring programs for laboratory animals, emphasizing the significance of early detection and intervention to prevent disease outbreaks and maintain animal welfare. Emerging trends and technologies in microbial disease monitoring, such as next-generation sequencing, metagenomics, and high-throughput screening, are also explored. This review aims to provide researchers, laboratory animal professionals, and regulatory authorities with a comprehensive resource for understanding microbial diseases in experimental animals and selecting appropriate monitoring assay. For the Scientists and Veterinarians this overall review gives a glimpse for implementing effective monitoring strategies, identifying and managing microbial diseases, ensuring the well-being of laboratory animals. The synthesis of current knowledge and best practices will enhance the scientific rigor and reproducibility of experimental studies involving animals. In conclusion, this review emphasizes the critical importance of microbial disease monitoring in experimental animals. Robust and accurate monitoring assays enable researchers to effectively detect and manage microbial diseases, safeguarding animal health and ensuring reliable research outcomes.



*Keywords: Bacteria, Fungi, Viruses, Next generation sequencing, ELISA, PCR*


## 1. INTRODUCTION

Microbial disease monitoring in animals plays a critical role in safeguarding animal health, ensuring food safety, and protecting human populations from zoonotic infections. Animals can harbor a wide range of microbes, including bacteria, viruses, fungi, and parasites, which can cause diseases with significant economic and public health implications. Monitoring and managing microbial diseases in animals are essential for several reasons (Varela et al., 2022).

Maintaining animal health is crucial for the well-being of livestock, companion animals, and wildlife populations. Microbial diseases can have detrimental effects on animal welfare, leading to reduced productivity, impaired reproduction, increased mortality rates, and

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compromised quality of life (Butterworth and weeks. 2009). By monitoring and early detection of microbial diseases, veterinarians and animal health professionals can implement timely interventions to prevent outbreaks, provide appropriate treatments, and minimize the impact on animal populations.

Microbial disease monitoring in animals is closely linked to food safety. Many pathogens can be transmitted to humans through contaminated animal products such as meat, milk, and eggs. Monitoring animal populations for microbial diseases helps identify potential sources of contamination in the food production chain (Heredia and Garcia, 2028). By implementing appropriate control measures, such as vaccination programs, biosecurity protocols, and proper hygiene practices, the risk of transmitting foodborne pathogens to humans can be significantly reduced.

Animals can serve as reservoirs or vectors for zoonotic diseases, which are infections that can be transmitted between animals and humans. Diseases such as avian influenza, rabies, and Lyme disease are examples of zoonotic diseases that can have serious consequences for human health. Monitoring and controlling microbial diseases in animal populations help identify and manage potential sources of zoonotic infections, minimizing the risk of transmission to humans through close contact, consumption of animal products, or exposure to animal environments (Rahman et al., 2020).

Microbial disease monitoring provides valuable epidemiological data and surveillance information. By monitoring the prevalence, distribution, and characteristics of microbial diseases in animals, researchers and public health officials can gain insights into disease trends, identify emerging pathogens, and assess the effectiveness of control measures. This information is crucial for developing targeted prevention and control strategies, allocating resources effectively, and mitigating the impact of microbial diseases on animal and human populations.

Microbial disease monitoring in animals is of paramount importance for protecting animal health, ensuring food safety, preventing zoonotic infections, and maintaining public health. It enables early detection, prompt intervention, and effective management of microbial diseases, contributing to the overall well-being of animals and the safety of human populations. Continuous surveillance and monitoring efforts are essential to mitigate the risks associated with microbial diseases and to foster sustainable and resilient animal health systems.

## 1. TYPES OF MICROBES AFFECTING ANIMALS

### 1.1 Bacteria

Bacterial pathogens can cause a wide range of diseases in animals (Figure 1). Examples include *Salmonella*, which can cause gastrointestinal infections in animals, and *Brucella*, which causes brucellosis in livestock (Table 1). Other notable bacteria include *Clostridium*, *Mycobacterium*, and *Escherichia coli* (E. coli) (Thrall et al., 2016; Molyneux et al., 2011).

**Figure 1: Types of Microbes affecting animals.**

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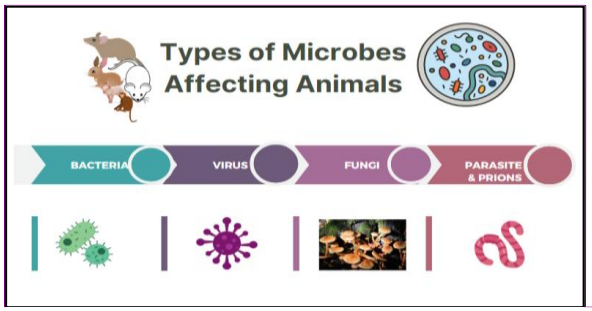


**Table 1: List of Bacterial Pathogens and their infection with symptoms**

| S.No | Bacterial pathogens | Infections and symptoms |
|---|---|---|
| 1 | *Escherichia coli* | Gastrointestinal infections, urinary tract infections, septicemia, and pneumonia |
| 2 | *Salmonella* spp | Salmonellosis, a gastrointestinal infection, in animals, leading to symptoms such as diarrhea, fever, and dehydration |
| 3 | *Staphylococcus aureus* | Skin and Soft tissue infections, respiratory tract infections, and systemic infections |
| 4 | *Streptococcus* spp | Respiratory infections |
| 5 | *Pseudomonas aeruginosa* | Opportunistic infections in animals, particularly respiratory infections, urinary tract infections, and wound infections |
| 6 | *Mycobacterium* spp | *Mycobacterium tuberculosis* or *Mycobacterium avium* |
| 7 | *Bordetella bronchiseptica* | Respiratory tract infections, including bronchitis and pneumonia |
| 8 | *Clostridium* spp | Botulism, tetanus, and enterotoxemia |
| 9 | *Pasteurellamultocida* | Respiratory infections, including pneumonia and upper respiratory tract infections |
| 10 | *Campylobacter jejuni* | Gastrointestinal infections, diarrhea |
| 11 | *Actinobacillus*spp | Respiratory tract infections and septicemia |
| 12 | *Brucella* spp | Brucellosis |

### 1.2 Bacterial pathogens cause diseases in animals through various mechanisms.

Adhesion and Colonization: Bacterial pathogens possess adhesins, which are molecules that enable them to attach to specific receptors on host cells. Adhesion allows the bacteria to establish colonization and initiate infection.

Invasion: Some bacterial pathogens possess invasive factors that enable them to penetrate and invade host tissues. These factors can include enzymes that break down host barriers, such as collagenases or hyaluronidases. By invading host tissues, bacteria can evade the host immune response and establish a deeper infection. For instance, *Streptococcus pyogenes* uses hyaluronidase to invade deeper layers of skin and cause cellulitis or necrotizing fasciitis (Zachary. 2017).

Toxin Production: Bacterial pathogens often produce toxins that can damage host cells and tissues, leading to disease. Toxins can be released into the surrounding environment or directly injected into host cells. Examples of bacterial toxins include Shiga toxins produced by Shigella and certain strains of Escherichia coli, which cause severe gastrointestinal

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symptoms such as bloody diarrhea and hemolytic uremic syndrome (Melton and Shiga. 2014).

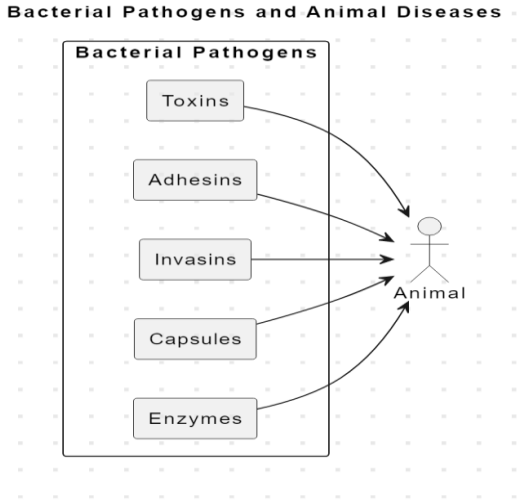
Inflammation and Immune Response: Bacterial pathogens can trigger an inflammatory response in the host, leading to tissue damage and disease symptoms. This response can involve the release of pro-inflammatory cytokines, recruitment of immune cells, and activation of the complement system (Chen et al., 2017). Inflammatory diseases caused by bacterial pathogens include pneumonia caused by Bordetella bronchiseptica and septicemia caused by Salmonella (Zheng et al., 2020).

Evasion of Host Defenses: Bacterial pathogens have evolved various mechanisms to evade or subvert the host immune system. They can modify their surface structures, such as lipopolysaccharides or capsules, to evade recognition by the immune system. Some bacteria can also secrete proteins that interfere with host immune signaling or phagocytosis. Examples include Yersinia pestis, which evades phagocytosis, and Mycobacterium tuberculosis, which can survive within macrophages (Wren, 2000; Finlay & McFadden, 2006).

Nutrient Acquisition: Bacterial pathogens require nutrients to grow and proliferate within the host. They can employ strategies to acquire nutrients from the host, such as siderophore production to scavenge iron or utilization of host metabolites. This competition for nutrients can cause damage to host tissues and contribute to disease progression (Murdoch & Skaar., 2022) (Figure 2).

The different bacterial pathogens employ various combinations of these mechanisms to cause disease in animals. The specific virulence factors and mechanisms vary depending on the bacterial species and the target tissues or organs involved in the infection. Understanding these mechanisms is crucial for developing effective strategies for diagnosis, treatment, and prevention of bacterial diseases in animals (Ewers et al., 2004).

**Figure 2:Mechanism of Bacterial infections**




## 1.3 Viruses

Viral infections are prevalent in animals and can lead to severe diseases. Well-known examples include the rabies virus, which affects mammals and can be transmitted to humans, and the foot-and-mouth disease virus, which affects cloven-hoofed animals such as cattle and pigs. Influenza viruses, such as avian influenza and swine influenza, are other examples (Table 2).

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**Table 2: List of Viral Pathogens and their infection with symptoms**

| S.No | Viral pathogens | Infections and symptoms |
|---|---|---|
| 1 | Murine Norovirus | Gastrointestinal infections leading to diarrhea, weight loss, and mortality |
| 2 | Mouse Hepatitis Virus (MHV) | Hepatitis, enteritis, and respiratory diseases |
| 3 | Lymphocytic Choriomeningitis Virus (LCMV) | Meningitis, encephalitis, and systemic infections |
| 4 | Sendai Virus | Respiratory tract infections and pneumonia |
| 5 | Mouse Parvovirus (MPV) | Reproductive disorders, including fetal death and resorption |
| 6 | Theiler's Murine Encephalomyelitis Virus (TMEV | Encephalomyelitis and demyelination |
| 7 | Vesicular Stomatitis Virus (VSV) | Causes vesicular lesions and systemic infections |
| 8 | Friend Leukemia Virus (FLV) | Leukemia and other hematological disorders |
| 9 | Simian Immunodeficiency Virus (SIV) | Immunodeficiency and AIDS |
| 10 | Feline Immunodeficiency Virus (FIV) | Immunodeficiency and AIDS |
| 11 | Canine Parvovirus (CPV) | Gastrointestinal infections |
| 12 | Avian Influenza Virus (H5N1, H7N9) | Severe respiratory illness and high mortality |
| 13 | Rabies Virus | Fatal neurologic disease |
| 14 | Herpes Simplex Virus (HSV) | Cause oral and genital lesions and other manifestations |

## 1.4 *Viral pathogens cause diseases in animals through various mechanisms.*

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Attachment and Entry: Viral pathogens have specific proteins on their surface, such as viral attachment proteins or spike proteins that allow them to attach to host cells. Once attached, the virus enters the host cell through receptor-mediated endocytosis or membrane fusion. This initial attachment and entry process are crucial for establishing infection.

Replication and Spread: Once inside the host cell, viral pathogens use the host cellular machinery to replicate their genetic material and produce viral proteins. Viruses can replicate within the infected cell and then spread to neighbouring cells or disseminate to other tissues through various means. This replication and spread lead to the widespread infection of host tissues and organs.

Cytopathic Effects: Viral replication can cause damage to host cells through various mechanisms. Some viruses directly kill the infected cells, leading to tissue destruction and inflammation. Others can induce changes in host cell metabolism or disrupt cellular processes, resulting in cell dysfunction and damage. Cytopathic effects caused by viral infection contribute to the development of disease symptoms.

Immune Evasion: Viral pathogens have evolved mechanisms to evade or counteract the host immune response. They can inhibit the production or action of interferons, which are important antiviral molecules produced by the host immune system. Viruses can also produce proteins that interfere with the recognition and killing of infected cells by immune cells, allowing them to evade immune surveillance and establish persistent infections (Maginnis, 2018; Dimitrov, 2004).

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Immunopathology: The host immune response to viral infection can sometimes cause damage to host tissues, leading to immunopathology. Excessive or dysregulated immune responses can result in inflammation, tissue injury, and disease symptoms. Immunopathology is particularly prominent in certain viral infections, such as viral hepatitis or viral encephalitis.

Latency and Reactivation: Some viral pathogens have the ability to establish latency, where they can remain dormant within host cells without causing active infection. Latent viruses can periodically reactivate, leading to recurrent episodes of disease. Herpesviruses, such as herpes simplex virus or varicella-zoster virus, are examples of viruses that can establish latency and reactivate (Rouse & Sehrawat, 2010).

The specific mechanisms employed by viral pathogens can vary depending on the virus family, host species, and target tissues. Understanding these mechanisms is crucial for the development of antiviral therapies, vaccines, and diagnostic strategies to combat viral diseases in animals.

**1.5 Fungi:**Fungal infections, although less common, can affect animals, particularly those with compromised immune systems. Ringworm, caused by various fungal species, can be transmitted between animals and humans. Another example is aspergillosis, caused by Aspergillus fungi, which can affect birds and mammals (Table 3).

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**Table 3: List of Fungal Pathogens and their infection with symptoms**

| S.No | Fungal pathogens | Infections and symptoms |
|---|---|---|
| 1 | *Candida* spp | Oral thrush, cutaneous infections, and systemic candidiasis |
| 2 | *Aspergillus*spp | Respiratory tract infections |
| 3 | *Cryptococcus neoformans* | Respiratory tract and central nervous system |
| 4 | *Trichophyton* spp. and*Microsporum*spp | Dermatophytosis and ringworm |
| 5 | *Histoplasma capsulatum* | Affects the lungs |
| 6 | *Blastomyces dermatitidis* | Affects the lungs, skin, and other organs |
| 7 | *Coccidioides*spp | Respiratory infection |
| 8 | *Pneumocystis jirovecii* | Pneumocystis pneumonia, respiratory infection |
| 9 | *Sporothrixschenckii* | Nodular skin lesions |
| 10 | *Rhizopus*spp | Nasal passages |

***1.6 Fungal pathogens can cause diseases in animals through various mechanisms.***

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Adhesion and Colonization: Fungal pathogens possess adhesion factors that enable them to attach to host tissues and establish colonization. These adhesion factors can include specific proteins or carbohydrates on the fungal cell surface that interact with host receptors. Adhesion allows the fungi to initiate infection and establish a foothold in the host (De Groot et al., 2013; Guy et al., 2008).

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Invasion and Tissue Penetration: Fungal pathogens can invade host tissues by directly penetrating host cells or by breaching epithelial barriers. Some fungi

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produce specialized structures, such as hyphae or invasive filaments that can penetrate host tissues. Fungal enzymes, such as proteases or phospholipases, can also contribute to tissue invasion by degrading host cell membranes and extracellular matrix components.

Toxin Production: Certain fungal pathogens produce toxins that can damage host tissues and contribute to disease. These toxins can disrupt cellular processes, induce inflammation, and cause tissue necrosis. For example, certain species of Aspergillus produce aflatoxins that can damage the liver, while some species of Candida can produce candidalysin, which contributes to tissue damage and immune activation.

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Immune Response and Immunopathology: Fungal infections trigger immune responses in the host, involving both innate and adaptive immune mechanisms. Immune cells such as neutrophils and macrophages are recruited to the site of infection to eliminate fungal pathogens. However, an excessive or dysregulated immune response can lead to immunopathology and tissue damage. Inflammatory reactions caused by the immune response can contribute to disease symptoms.

Host Immune Suppression: Some fungal pathogens have mechanisms to evade or suppress the host immune response. They can produce substances that inhibit the function of immune cells or interfere with immune signalling pathways. By suppressing the immune response, fungal pathogens can establish persistent or chronic infections (Kumari et al., 2021; Garcia et al., 2020).

Nutrient Exploitation: Fungal pathogens obtain nutrients for growth and survival from the host. They can produce enzymes, such as proteases or lipases that break down host tissues and proteins into simpler forms that can be utilized by the fungus. Nutrient exploitation can lead to tissue damage and compromise host physiological processes.

### 1.7 Parasites

Various parasites, including protozoa, helminths (worms), and arthropods, can cause diseases in animals. For instance, the protozoan parasite *Trypanosoma brucei* causes African trypanosomiasis, also known as sleeping sickness, in animals and humans. Tick-borne diseases, such as Lyme disease and babesiosis, are caused by bacteria and protozoa transmitted by ticks (Table 4).

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**Table 4: List of Parasites and their infection with symptoms**

| S.No | Parasites | Infections and symptoms |
|---|---|---|
| 1 | Fleas (*Ctenocephalides*spp) | Infest the fur and skin of mammals |
| 2 | Ticks (*Ixodes* spp., *Rhipicephalus* spp., *Dermacentor* spp.) | Attach to the skin of animals and feed on their blood |
| 3 | Mites (*Sarcoptes* spp., *Demodex* spp., *Cheyletiella* spp.) | Cause skin infestations and various health issues |
| 4 | Lice (*Trichodectes* spp., *Felicola* spp., *Haematopinus*spp.) | Infest the fur or feathers of animals, causing itching, hair loss, and skin irritation |
| 5 | Roundworms (*Toxocara* spp., *Ascaris* spp.) | Intestinal parasites (digestive problems and nutrient deficiencies) |
| 6 | Hookworms (*Ancylostoma* spp., *Uncinaria*spp.) | Infect the intestines of animals, causing anemia and gastrointestinal issues |
| 7 | Tapeworms (*Dipylidium* spp., *Taenia* | Weight loss, malnutrition, and other digestive |

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| | spp.) | problems |
| 8 | Flukes (*Fasciola* spp., *Schistosoma* spp.) | Affect various organs of animals, including the liver and lungs |
| 9 | Heartworms (*Dirofilariaimmitis*) | Severe heart and lung damage |
| 10 | Coccidia (*Eimeria* spp., *Cryptosporidium* spp.) | Infect the intestinal tract of animals, leading to diarrhea and dehydration |

Parasitic pathogens can cause diseases in animals through various mechanisms. Here are some common mechanisms by which parasite pathogens can cause disease:
Invasion and Tissue Migration: Parasitic pathogens have mechanisms to invade and penetrate host tissues. They can use specialized structures, such as hooks, suckers, or spines, to attach to host cells or tissues. Once attached, parasites can invade and migrate through various host tissues, causing damage and disruption.
Nutrient Deprivation: Parasitic pathogens obtain nutrients from the host by feeding on host tissues, fluids, or blood. They can directly consume host cells or compete with the host for essential nutrients. Nutrient deprivation can lead to host malnutrition, anemia, or other metabolic disorders (Walker &Zunt., 2005; Valigurova&Florent et al., 2021).
Mechanical Damage: Parasites can physically damage host tissues and organs. For example, certain parasitic worms, such as hookworms or lungworms, can cause tissue damage through their feeding activities or by creating tunnels within the host tissues.
Immune Evasion: Parasitic pathogens have evolved strategies to evade or modulate the host immune response. They can modify their surface antigens to evade immune recognition or produce molecules that suppress or modulate the host immune system. By evading the immune response, parasites can establish chronic or persistent infections.
Immunopathology: The host immune response to parasitic infections can sometimes lead to immunopathology, causing tissue damage and inflammation. The immune response against parasites can involve the release of pro-inflammatory cytokines, recruitment of immune cells, and formation of granulomas or inflammatory lesions (McSorley&Maizels, 2012; Allen & Sutherland, 2014).
Reproductive Strategies: Many parasite pathogens have complex life cycles that involve multiple stages and hosts. They have evolved reproductive strategies to ensure their survival and transmission. For example, some parasites produce large numbers of eggs or larvae that can cause tissue damage or inflammation as they migrate through host tissues.
Toxin Production: Certain parasite pathogens produce toxins that can contribute to disease. For instance, some protozoan parasites, such as Plasmodium species that cause malaria, release toxins that can damage red blood cells or induce host immune responses.

## 1.8 *Prions*

Prions are unconventional infectious agents composed of misfolded proteins. They are associated with transmissible spongiform encephalopathies (TSEs) that affect the nervous system. Examples include bovine spongiform encephalopathy (BSE), commonly known as mad cow disease, and scrapie in sheep. Prions are unique infectious agents that cause diseases known as prion diseases or transmissible spongiform encephalopathies (TSEs) in animals (CDC, 2021). Prions are composed primarily of an abnormal form of a cellular protein called prion protein (PrP) (Table 5).

**Table 5: List of Prions and their infection with symptoms**

| S.No | Prions | Infections and symptoms |
|---|---|---|
| 1 | Bovine spongiform encephalopathy | Neurological symptoms, weight loss, and ultimately, death |
| 2 | Scrapie | Behavioral changes, itching, and neurodegeneration |
| 3 | Chronic wasting disease | Weight loss, behavioral changes, and neurological symptoms |

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| 4 | Transmissible mink encephalopathy | progressive neurodegeneration |
| 5 | Feline spongiform encephalopathy | Neurological symptoms and ultimately results in death |
| 6 | Exotic ungulate encephalopathy | Affecting captive exotic ungulates |

### *1.9 Mechanism of prion pathogenesis involves the following steps*

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Abnormal Conformation: Prion diseases occur when the normal cellular prion protein undergoes a conformational change and adopts an abnormal. This abnormal form of the protein is highly resistant to degradation and has a tendency to aggregate (Kovas & Budka., 2008; Harris, 1999).
Prion Conversion and Propagation: prion protein can act as a template and induce the conversion of normal prion protein into the abnormal conformation. This conversion leads to the accumulation and aggregation of prion protein in the brain and other tissues. The aggregated prion protein is thought to be the infectious form of the prion and is responsible for transmitting the disease.
Neurotoxicity and Tissue Damage: The accumulation of aggregated prion protein in the brain leads to the formation of insoluble protein deposits, known as amyloid plaques. These plaques disrupt normal cellular processes and cause neurodegeneration. The precise mechanisms by which prion protein induces neurotoxicity are still under investigation but are believed to involve disruption of cellular homeostasis, induction of oxidative stress, and activation of inflammatory responses.
Spread within the Host: Prions can spread from infected tissues to other tissues and organs within the host. They can be transmitted through ingestion of contaminated tissues, direct contact with infected tissues, or even through aerosol transmission in some cases. Once introduced into a new host, prion protein can initiate the conversion of normal prion protein, perpetuating the disease process.
It's important to note that prion diseases are unique among infectious diseases because they do not involve the replication of genetic material, as in the case of viruses or bacteria. Instead, prion diseases are characterized by the conformational change and accumulation of misfolded proteins, leading to progressive neurodegeneration and the characteristic clinical signs associated with prion diseases (Corsaro et al., 2012; Poggiolini et al., 2013; Zheng &Gengfu, 2013).
Examples of prion diseases in animals include bovine spongiform encephalopathy (BSE or "mad cow disease") in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk, and transmissible mink encephalopathy (TME) in mink.
Understanding the types of microbes involved in animal diseases is crucial for prevention, diagnosis, and treatment. Veterinary medicine focuses on identifying and managing these infectious agents to protect animal health and prevent the transmission of diseases to humans. Control measures, such as vaccination, antimicrobial treatment, and parasite prevention, play a vital role in mitigating the impact of these microbial-related animal diseases.

## 2. Why microbial contamination checking is necessary?

Experimental integrity: Microbial contamination can significantly affect the outcome and reliability of animal model experiments. Microbes can induce inflammatory responses, alter immune functions, or interfere with the efficacy of drugs or treatments being tested. By ensuring animals are free from microbial contamination, researchers can obtain more accurate and reproducible results (Turner et al., 2011; Herati & Wherry, 2018).
Consistency: In scientific research, consistency is crucial. Introducing variables such as microbial infections can lead to inconsistent or confounding results. By starting with animals free from microbial contamination, researchers can better control and standardize

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experimental conditions, improving the reliability and comparability of their findings (Richa & Dominik et al., 2021).

Animal welfare: Microbial infections can cause significant discomfort and health issues in animals. By screening animals for microbial contamination, researchers can identify and address any existing infections, ensuring the animals are healthy and minimizing potential distress or suffering (Turner et al., 2011).

Human safety: Some microbes carried by animals can pose risks to human handlers and researchers. Certain bacteria, viruses, or parasites can be zoonotic, meaning they can be transmitted from animals to humans, potentially causing infections or diseases. Screening animals for microbial contamination helps protect human handlers and researchers from potential health hazards.

Regulatory compliance: Many research institutions and regulatory bodies have guidelines and standards in place for animal research, including requirements to minimize microbial contamination. By conducting appropriate screening and ensuring animals are free from microbial infections, researchers can meet these regulatory standards and maintain ethical and legal compliance (Figure 3).

**Figure 3: Importance of Contamination check in Animals**

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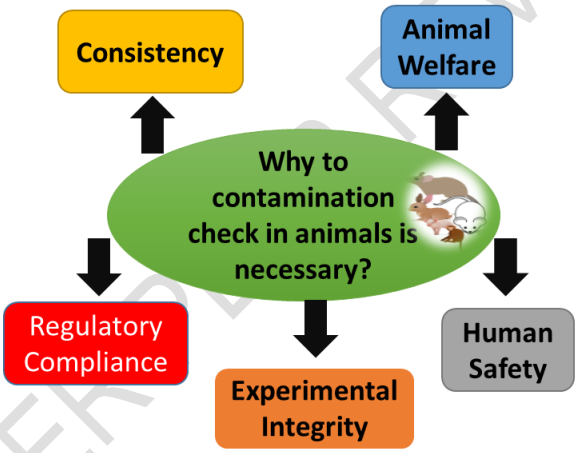


## 3. Disease monitoring methods

There are several types of microbial disease monitoring tests used to detect and diagnose microbial infections in animals. These tests can vary depending on the specific microbe being targeted and the desired level of sensitivity. Here are some common types of microbial disease monitoring tests:

### *3.1 Microbiological Culture*

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Traditional method involves isolating and growing microorganisms from a sample, such as blood, tissue, or body fluids, on specific culture media. The growth of bacteria, fungi, or parasites can be visually identified and further characterized using biochemical tests. Culture-based methods allow for the identification and antimicrobial susceptibility testing of specific pathogens. Microbiological culture tests are commonly used in animal disease monitoring to isolate and identify microbial pathogens (Bommet et al., 2019). Different culture techniques can be employed based on the type of microbe being targeted. Here are some types of microbiological culture tests used for animal disease monitoring:

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Bacterial Culture: Bacterial culture involves the isolation and growth of bacteria on specific culture media. Selective media can be used to promote the growth of specific bacteria while inhibiting others. Differential media can differentiate between different bacterial species based on their metabolic characteristics or the appearance of colonies. Commonly used culture media include blood agar, MacConkey agar, and Mannitol salt agar.

Fungal Culture: Fungal culture is used to identify and isolate pathogenic fungi causing infections in animals. Various types of agar media, such as Sabouraud agar, are used to support the growth of fungi. Fungal cultures often require longer incubation times compared to bacterial cultures due to the slower growth rate of fungi (Bommet et al., 2019)

Parasitic Culture: Parasitic culture involves the cultivation of parasitic organisms, such as protozoa and helminths, from clinical samples. For example, stool cultures can be used to identify parasitic organisms causing gastrointestinal infections in animals. Specialized media and techniques, such as agar slants or liquid media, may be used for the cultivation of specific parasites (Ahmed, 2014; Garcia et al., 2017).

Viral Culture: Viral culture is more challenging and time-consuming compared to other culture methods due to the requirement of host cells for viral replication. Animal cells, such as primary cell cultures or established cell lines, are inoculated with clinical samples suspected of viral infection. The presence of viral growth is detected through specific cytopathic effects or by using immunofluorescence or molecular assays (Dolskiy et al., 2020; Leland & Ginocchio, 2007).

Mycoplasma Culture: Mycoplasmas are a group of bacteria that lack a cell wall, making them challenging to culture using traditional methods. Specialized media, such as Mycoplasma-specific agar or broth, are used to isolate and cultivate mycoplasma organisms. Additional techniques, such as PCR or serological tests, are often employed for the detection and identification of mycoplasma infections (Kashyap&Sanrkar, 2010).

Culture tests may require specific growth conditions, temperature, and atmospheric conditions to support the growth of microorganisms. Additionally, the use of appropriate transport media and proper handling of clinical samples are critical to ensure the viability and accuracy of the culture results.

### *3.2 Polymerase Chain Reaction (PCR)*

PCR is a molecular technique used to amplify and detect specific DNA or RNA sequences of microbial pathogens. It enables rapid and sensitive detection of pathogens from various sample types. PCR-based tests can be designed to detect a broad range of microbes, including bacteria, viruses, and parasites, providing valuable diagnostic information. Molecular PCR (Polymerase Chain Reaction) tests are widely used in animal disease monitoring for their sensitivity, specificity, and ability to detect and identify various microbial pathogens. Here are some types of molecular PCR tests commonly employed for animal disease monitoring:

Pathogen-specific PCR: These PCR tests target specific DNA or RNA sequences unique to a particular pathogen of interest. Primers are designed to bind to conserved regions of the pathogen's genome, allowing for the amplification and detection of the target sequence. Pathogen-specific PCR tests can be designed for a wide range of microorganisms, including bacteria, viruses, fungi, and parasites (Yang & Rothman, 2004).

Multiplex PCR: Multiplex PCR enables the simultaneous detection and differentiation of multiple pathogens in a single reaction. It uses multiple sets of primers that amplify specific target sequences from different pathogens. By incorporating different fluorophores or detection methods, each pathogen's amplified product can be identified and distinguished. Multiplex PCR is valuable for screening and surveillance programs where multiple pathogens need to be detected efficiently.

Real-time PCR (qPCR): Real-time PCR monitors the amplification of target DNA or RNA in real-time using fluorescent probes or intercalating dyes. It allows for quantitative detection, providing information on the initial amount of the target pathogen in the sample. Real-time

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PCR is highly sensitive and offers rapid results, making it suitable for early diagnosis, monitoring disease progression, and assessing treatment efficacy.

Reverse Transcription PCR (RT-PCR): RT-PCR is used to detect and quantify RNA viruses by first converting viral RNA into complementary DNA (cDNA) using reverse transcriptase. The cDNA is then amplified using PCR. RT-PCR is commonly used for detecting RNA viruses, such as influenza viruses, retroviruses, and coronaviruses.

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Nested PCR: Nested PCR involves two rounds of PCR amplification. In the first round, outer primers amplify a larger region of the target DNA or RNA. Then, a portion of the first-round product is used as a template for a second round of amplification using inner primers targeting a smaller, specific region within the first amplification product (Green & Sambrook, 2019). Nested PCR increases sensitivity and specificity, particularly when low levels of the pathogen are present

These molecular PCR tests enable rapid and accurate detection, identification, and quantification of microbial pathogens in animal disease monitoring. They play a crucial role in early diagnosis, surveillance, and control measures, contributing to effective disease management and prevention in animal populations.

**Comment [n35]:** He did not mention the downside of each technique

### *3.3 Serological Assays*

**Comment [n36]:** Write correctly and without italics

Serological tests detect the presence of specific antibodies produced by an animal's immune system in response to a microbial infection. These tests can indicate a current or past infection. Serological assays, such as enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent antibody (IFA) tests, are commonly used for the detection of viral or bacterial infections. Serological tests are commonly used in animal disease monitoring to detect the presence of specific antibodies produced by an animal's immune system in response to a microbial infection. These tests provide valuable information about past or current infections and are particularly useful for detecting viral or bacterial pathogens (Gong et al., 2021; Pote et al., 2018). Here are some types of serological tests used for animal disease monitoring:

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA is a widely used serological test that detects and quantifies specific antibodies in an animal's blood or other body fluids. The test involves immobilizing the target antigen on a solid surface (e.g., microplate) and then adding the animal's serum or plasma sample. If the animal has been exposed to the antigen, antibodies present in the sample will bind to the immobilized antigen. The binding is then detected using an enzyme-conjugated secondary antibody, which produces a color change or fluorescence signal. ELISA can be performed as direct, indirect, or competitive formats, depending on the specific application.

Indirect Fluorescent Antibody Test (IFA): IFA is a serological technique that uses fluorescently labeled antibodies to detect the presence of specific antibodies in an animal's serum. The test involves incubating the animal's serum with a substrate that contains fixed, antigen-coated slides or cells. If the animal has been exposed to the antigen, specific antibodies in the serum will bind to the antigens on the slide or cells. The bound antibodies are then visualized using a fluorescence microscope. IFA is commonly used for the diagnosis of viral infections in animals, such as influenza, rabies, or herpesvirus (Rudd et al., 2013; He Q et al., 2005).

Serum Neutralization Test (SNT): SNT measures the ability of antibodies in an animal's serum to neutralize the infectivity of a specific virus. The test involves serially diluting the animal's serum and incubating it with a standardized amount of the virus. The mixture is then added to susceptible cells, and the cells are examined for viral cytopathic effects. The highest serum dilution that completely inhibits viral replication is considered the neutralizing titer, reflecting the presence of specific neutralizing antibodies (Manenti et al., 2020)

Hemagglutination Inhibition (HI) Assay: HI assay is commonly used for the detection of antibodies against viruses that possess hemagglutinin (HA) antigens, such as influenza viruses. The test involves mixing the animal's serum with virus particles and then adding red

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blood cells (Kaufmann et al., 2017). If specific antibodies are present in the serum, they will inhibit the agglutination (clumping) of red blood cells induced by the virus. The HI titer is determined by the highest serum dilution that prevents hemagglutination.

Western Blot: Western blot is a technique used to detect and identify specific antibodies against individual proteins of a microbial pathogen. It involves separating the pathogen's proteins using electrophoresis, transferring them onto a membrane, and then incubating the membrane with the animal's serum. If specific antibodies are present in the serum, they will bind to the corresponding proteins on the membrane (Mahmood & Yang, 2012). The bound antibodies are detected using labeled secondary antibodies, and the presence of specific bands indicates the presence of antibodies against specific pathogen proteins.

Serological tests provide valuable information about an animal's immune response to microbial infections and are essential tools for surveillance, diagnosis, and monitoring of animal diseases. It contributes to understand the disease prevalence, assessing vaccination effectiveness, and implementing control measures in animal populations.

### *3.4 Antigen Detection*

Comment [n37]: Write correctly and without italics

Antigen detection tests directly identify microbial components, such as proteins or cell surface markers, in a sample. Rapid diagnostic tests, such as lateral flow assays, are commonly used for the detection of specific viral or bacterial antigens in clinical samples.

Antigen detection tests are commonly used in animal disease monitoring to directly detect the presence of microbial antigens in clinical samples. These tests are particularly useful for rapid diagnosis and surveillance of infectious diseases. Here are some types of antigen detection tests used in animal disease monitoring:

Immunochromatographic Assays: Immunochromatographic assays, commonly known as lateral flow assays or rapid tests, are simple and rapid tests that provide qualitative or semi-quantitative results. These tests typically consist of a strip with specific antibodies immobilized in a line (Kczula&Gallotta, 2016). When a sample containing the target antigen is applied to the strip, it migrates along the strip and binds to the immobilized antibodies. This binding forms a visible line, indicating the presence of the target antigen.

Immunohistochemistry (IHC): IHC is a technique used to detect antigens in tissue samples. It involves labeling specific antibodies with a visible or fluorescent tag. The labeled antibodies bind to the target antigens in the tissue sections, allowing for their visualization under a microscope. IHC is particularly useful for localizing and identifying specific antigens within tissues.

Rapid Antigen Tests: Rapid antigen tests are point-of-care tests designed to detect specific antigens directly in clinical samples. These tests often use lateral flow or similar formats and provide rapid results within minutes. They are commonly used for the detection of viral antigens, such as respiratory viruses in animals.

Antigen detection tests provide a rapid and direct means of detecting the presence of specific microbial antigens in clinical samples. They are valuable tools for early diagnosis, surveillance, and monitoring of infectious diseases in animal populations.

### *3.5 Next-Generation Sequencing (NGS)*

Comment [n38]: Write correctly and without italics

NGS technologies allow for the comprehensive analysis of microbial communities in a sample, providing a snapshot of the microbial diversity present. This approach is particularly useful for studying complex microbial ecosystems and identifying novel or emerging pathogens. Next-generation sequencing (NGS) technologies have revolutionized the field of animal disease monitoring by enabling high-throughput sequencing of DNA or RNA samples. NGS allows for the comprehensive analysis of microbial genomes, transcriptomes, and metagenomes, providing valuable insights into the diversity and dynamics of pathogens (Hilt & Ferrier, 2022). Here are some types of NGS tests commonly used for animal disease monitoring:

Comment [n39]: It is written in abbreviation only because it was mentioned previously

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Whole Genome Sequencing (WGS): WGS involves sequencing the complete genome of an organism, including both pathogen and host genomes. It provides a comprehensive view of the genetic composition of the pathogen and can be used for the identification of specific genetic markers, characterization of virulence factors, and tracking the spread of pathogens within animal populations. WGS is particularly useful for studying bacterial pathogens, such as Salmonella, Escherichia coli, or Mycobacterium spp. (Quainoo et al., 2017; Gilchrist et al., 2015; Uelze et al., 2020).

Metagenomic Sequencing: Metagenomic sequencing is used to analyse the collective genetic material of microbial communities present in a sample. It provides a snapshot of the microbial diversity and allows for the identification of known and novel pathogens, including bacteria, viruses, fungi, and parasites (Petrosino et al., 2009). Metagenomic sequencing is useful for studying complex diseases with multiple microbial components, such as enteric diseases or respiratory infections.

Transcriptome Sequencing (RNA-Seq): RNA-Seq is used to analyze the complete set of RNA transcripts in a sample, providing insights into gene expression levels and dynamics. RNA-Seq can be used to identify differentially expressed genes in response to infection, assess host immune responses, and characterize pathogen gene expression patterns during infection (Byron et al., 2016). It is particularly valuable for studying viral infections and host-pathogen interactions.

Targeted Amplicon Sequencing: Targeted amplicon sequencing focuses on specific genomic regions of interest, such as conserved genes or genetic markers. This approach allows for the targeted detection and characterization of specific pathogens or virulence factors. Targeted amplicon sequencing is commonly used for the surveillance and monitoring of specific pathogens, such as avian influenza viruses or bovine respiratory pathogens.

Comparative Genomics: Comparative genomics involves the comparison of genomic sequences from different isolates or strains of a pathogen to identify genetic variations and evolutionary relationships. It can help in understanding the genetic diversity, transmission patterns, and emergence of drug resistance in microbial pathogens. Comparative genomics studies often involve whole-genome sequencing of multiple isolates followed by bioinformatics analyses.

NGS tests provide a wealth of genomic information that can aid in understanding the epidemiology, transmission, and pathogenesis of animal diseases. These tests offer insights into microbial diversity, host-pathogen interactions, and genetic factors influencing disease outcomes. NGS-based approaches are increasingly becoming essential tools in animal disease surveillance, outbreak investigations, and the development of targeted control strategies.

Each of these tests has its advantages and limitations, and their selection depends on factors such as the target microbe, sample type, availability of resources, and desired diagnostic accuracy. Implementing a combination of these testing methods can provide a comprehensive approach to microbial disease monitoring and diagnosis in animals.

## 4. Conclusion

The microbial pathogen detection in laboratory animals plays a crucial role in ensuring the health and welfare of these animals, as well as the reliability and validity of research findings. Laboratory animals, such as mice, rats, rabbits, and non-human primates, are used extensively in biomedical research, drug development, and safety testing.

Accurate detection and monitoring of microbial pathogens in laboratory animals are essential to prevent the introduction and spread of infections, which could compromise research outcomes and pose risks to both animal welfare and human health. Detecting pathogens in laboratory animals involves various techniques, including regular health monitoring, microbiological culturing, molecular diagnostics, serology, and histopathology.

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By implementing robust microbial pathogen detection protocols, researchers and laboratory animal professionals can identify potential infections, assess disease status, and implement appropriate measures for disease control and prevention. Regular health monitoring and screening programs help identify asymptomatic carriers and prevent the introduction of new pathogens into animal colonies, thereby ensuring the reliability and reproducibility of research results.

Furthermore, microbial pathogen detection in laboratory animals enables researchers to study the interactions between pathogens and host immune systems, understand disease mechanisms, develop new treatments and vaccines, and contribute to the advancement of medical knowledge.

Overall, through diligent microbial pathogen detection and surveillance in laboratory animals, researchers can maintain high standards of animal welfare, minimize experimental variability, and enhance the safety and integrity of scientific research involving these animals.

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