ANKARA- 2025

DIAGNOSIS, TREATMENT, AND GENOMIC CONTROL OF ZOONOTIC INFECTIONS

Dr. R. DEVI -Dr. R. SRINIVASAN- J. YESHWANTH
L. DIANA -V. RANJANI -Muhammad ADIL- Farrah DEEBA
Anas Sarwar QURESHI -Gideon Yakusak BENJAMIN
Sirajuddien BIALANGI

ISBN: 979-8-89695-148-3

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AUTHORS

[1]Dr. R. DEVI

[2]Dr. R. SRINIVASAN

[3]J. YESHWANTH

[4]L. DIANA

[5]V. RANJANI

[6]Muhammad ADIL

[7]Farrah DEEBA

[8]Anas Sarwar QURESHI

[9]Gideon Yakusak BENJAMIN

[10]Sirajuddien BIALANGI

[1]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.

[2]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.

[3]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India

[4]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India

[5]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India

[6]University of Agriculture, Department of Clinical Medicine & Surgery, Faculty of Veterinary Science, Faisalabad, Pakistan

[7]University of Agriculture, Department of Clinical Medicine & Surgery, Faculty of Veterinary Science, Faisalabad, Pakistan

[8]Ripah International University, Department of Basic Bioscience, Ripah College of Veterinary Sciences, Lahore, Pakistan.

[9]State University of Medical and Applied Sciences, Department of Applied Biological Sciences (Applied Microbiology and Brewing Unit), Enugu State, Nigeria

[10]Universitas Negeri Gorontalo, Indonesia

DOI: 10.5281/zenodo.16895352

E mail: ubakyayinevi@gmail.com
www.ubakyayinevi.org
It is responsibility of the author to abide by the publishing ethics rules.
UBAK Publishing House – 2025©

ISBN: 979-8-89695-148-3
August / 2025
Ankara / Turkey

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PREFACE

Zoonotic diseases, lying at the intersection of human, animal, and environmental health, continue to present some of the most pressing challenges of our era. The rapid pace of globalization, environmental change, and intensifying human–animal interactions has heightened the risk of zoonotic spillovers, demanding an integrated and multidisciplinary response. Within this context, the present volume, Diagnosis, Treatment, and Genomic Control of Zoonotic Infections, makes a timely and significant contribution to the expanding body of scholarly knowledge in this critical domain.

This book brings together diverse perspectives from researchers and practitioners who explore zoonotic infections from multiple vantage points—ranging from classical epidemiology and clinical diagnosis to genomic innovations and One Health approaches. The inclusion of both well-known zoonoses and emerging threats underscores the dynamic and evolving nature of these infections, as well as the urgency of developing innovative strategies for prevention, surveillance, and treatment. Particularly noteworthy is the discussion of genomic tools such as CRISPR-Cas systems, which exemplify the potential of cutting-edge science to reshape how zoonotic pathogens are detected and controlled.

By weaving together insights on pathology, treatment protocols, and genomic technologies, the volume not only enhances our understanding of zoonotic diseases but also offers practical pathways for their mitigation. It highlights the need for collaborative efforts across medical, veterinary, and environmental sciences, reminding us that no single discipline can adequately confront the complexity of zoonotic threats.

We extend our sincere gratitude to all contributing authors for their rigorous scholarship and to the editorial team for curating such a valuable work. It is our hope that this book will serve as a resource for academics, clinicians, policymakers, and students alike, fostering a deeper understanding of zoonotic

infections and inspiring further research at the crossroads of health, science, and society.

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CHAPTER 1

A SHORT REVIEW ON ZOOTONIC DISEASE-PSITTACOSIS

[1]Dr. R. DEVI
[2]J. YESHWANTH
[3]L. DIANA
[4]V. RANJANI
[5]Dr. R. SRINIVASAN

[1]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.
[2]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.
[3]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.
[4]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.
[5]Bharath Institute of Higher Education and Research, Faculty of Pharmacy, Chennai, Tamil Nadu, India.

INTRODUCTION

A zoonotic bacterial infectious disease, psittacosis is brought on by the obligatory intracellular bacterium Chlamydia psittaci. By coming into touch with sick birds, one can contract psittacosis, sometimes referred to as ornithosis and parrot fever (figure 1), which can present with a variety of symptoms. Birds are epidemiology's main source [1]. Although the Psittaciformes (parakeets, parrots, lories, cockatoos, and budgerigars) and Galliformes (chickens, turkeys, and pheasants) orders of birds are well-known, this disease process can affect any species of bird and has been reported in 467 species across 30 orders [1].

It is believed that all of the Category B agents produce low rates of mortality and moderate rates of morbidity, are relatively easy to disseminate, and require special enhancements to CDC's diagnostic skills and disease surveillance [2]. Notably, the mortality rate from psittacosis reached 50% in the 20th century [3]. A worldwide psittacosis pandemic that struck in 1929–1930 is estimated to have impacted 800 people. As a result, the United States implemented a quarantine on imported parrots for more than 40 years as a preventative measure. If an efficient antibiotic treatment is not provided, the impact potential of this disease may recur [3].

Figure 1: Psittacosis, (Parrot fever)

1. ETIOLOGY

Birds and mammals are both home to the gram-negative, required intracellular bacterium C. psittaci. It can be recognized and epidemiologically

researched using genotype-specific real-time PCR, which has several genotypes. A variety of genotypes that are associated with specific animal hosts can induce psittacosis [1]. Ten genotypes have so far been found based on the sequencing of the ompA gene. Because of the lack of knowledge about psittacosis, it is challenging to understand pathologic serotypes and their pathogenicity [2]. In the United States, for example, the Centers for Disease Control and Prevention (CDC) learned of only 58 human cases of psittacosis between 2006 and 2012. As of the publishing of the CDC Weekly Morbidity and Mortality report in September 2014, only two of these cases had been confirmed by culture. The other individuals were only diagnosed by serologic tests. The main risk element for human psittacosis appears to be contact with birds, as was previously determined [1]. Additionally, psittacosis can be indirectly contracted from environmental sources such as sick birds' feces, urine, and other secretions [3]. These organisms are most frequently isolated from the following bird species: budgies, parrots, cockatiels, and parakeets. Poultry farmers have occasionally seen outbreaks of psittacosis due to poultry birds. Psittacosis has been reported to be spread via chickens, ducks, and turkeys.

2. EPIDEMOLOGY

Although it can afflict persons of any age or gender, psittacosis seems to be more common between the ages of 35 and 55[4]. The first psittacosis outbreak was connected to pet finches and parrots in 1879. Pandemics in 1929 and 1930 followed. Psittacosis is still considered a rare zoonotic illness. Medical professionals and the general public are therefore less familiar with this sickness entity. An examination of prevalence and incidence data suggests that both the requirement for specialist testing and psittacosis are likely underdiagnosed [5]. The Centers for Disease Control and Prevention (CDC) believe that fewer than ten cases of psittacosis are confirmed annually in the United States, and that most states consider it a reportable condition. This is likely due to underdiagnosis as well as underreporting. It is believed that those who work in the poultry industry, are exposed to pet shops, veterinary clinics, and bird exhibitions, are more prone to contract the disease. Between 1999 and 2006, the US had an estimated 0.01 cases of psittacosis per 100,000 people

[6][7]. In the past, less than 5% of hospitalized pneumonia cases are caused by psittacosis. Psittacosis has been reported worldwide. Between 0 and 6.7% of community-acquired pneumonia (CAP) cases worldwide were caused by C. psittaci, according to a meta-analysis research [8]. A meta-analysis of studies from multiple countries found that C. psittaci was responsible for 1% of all hospitalized CAPs. However, the reported incidence was far lower on an annual basis, indicating that the virus was not being properly recognized. However, although psittacosis is generally rare, outbreaks have been shown. Veterinary clinics, pet shops, and chicken farms are typically linked to these epidemics [9][10]. Psittacosis incidence is likely to appear to rise as a result of improved diagnostic methods and approaches. When using integrated genomic approaches to identify pathogens in cases of severe community-acquired pneumonia in 2022, Chinese researchers discovered that Chlamydia psittaci was the cause of the illness in 6.8% of patients (15 out of 222) [11] (Figure 2).

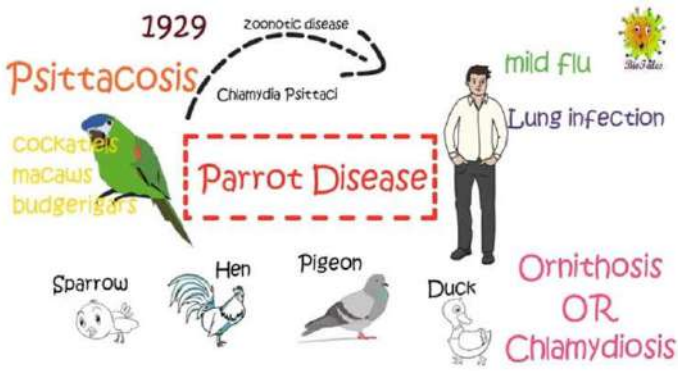

Figure 2: Incidence of psittacosis

3. PATHOPHYSIOLOGY

Gram-negative obligate intracellular bacteria C. psittaci can take on two distinct forms during its life cycle. An outward infectious elementary body and a bigger intracellular metabolically active reticulate body make up the organism. The infectious elementary body attaches itself to its cell membrane receptor and is endocytosed inside the eukaryotic cell upon contact, preventing the host immune system from reacting. It develops into the metabolically active reticulate body as the endocytosed elementary body gets bigger [12][13]. With

the help of the ATP in the host cell, these reticulate entities can binary fission to produce new ones [14].

Reverse endocytosis and cell lysis can liberate these inclusion reticulate structures, which subsequently reorganize into elementary and intermediate bodies [15]. A prolonged and undetectable infection is thought to be made possible by this process. These freed elemental entities then travel hematogenous to various organ systems, infect new host cells, and perpetuate the disease cycle. Recent studies using a bovine model suggest that, although the exact pathogenesis is still unknown, the cells of the alveolar epithelium are initially infected upon inoculation of C. psittaci. The infected host releases chemokines, especially the pro-inflammatory cytokine interleukin-8, which triggers a complex host response and a large neutrophil inflow. Chemokines mediate this acute-phase reaction, which activates reactive oxygen species and an inflammatory cascade to attract and accumulate phagocytes and immune cells from the circulation to the infection site. It is believed that C. psittaci spreads hematogenously by tissue injury and the disintegration of alveolar-capillary membranes [15]. The localized infection and the subsequent inflammatory cascade also create a relative barrier for oxygen transport within the alveoli, leading to alveolar hypoventilation, reduction in lung compliance, and hypoxemia [15].

4. PSITTACOSIS IN BIRDS AND ANIMALS

Ornithosis or avian chlamydiosis are the terms used to describe the illness that occurs when birds contract C. psittaci. Some species of Chlamydiae are known to infect birds, including C. psittaci, C. avium, C. gallinacea, C. buteonis, C. ibidis, and C. abortus [16]. All of these species can cause the disease in birds. It is likely that avian sickness is underreported globally due to the lack of distinctive symptoms and difficulties in obtaining diagnostic tests [17]. The US National Association of State Public-Health Veterinarians states that to prevent disease, psittacine birds who were not purchased from breeding colonies free of disease should be fed feed containing 1% chlortetracycline (CTC) for 45 days [18]. It is not possible to use water as a delivery system for antibiotics. Importers are encouraged to continue treatment for an extra 15 days, but all imported psittacine birds must be fed CTC for the whole 30-day

quarantine. To prevent reinfection, fresh and untreated birds were separated. The ability of commercial pet bird breeding operations to host and spread C. psittaci was most recently shown in a large-scale breeding business with 1000 birds in Washington state [19]. Although psittacosis in non-human mammals is poorly understood, several wild, domesticated, or farmed animals have been connected to human illness. A disease's human spectrum is typically the same, even though its clinical presentation can vary greatly. Sometimes the animal had no symptoms, and other times C. psittaci was isolated from a sick animal. It is known that the bacterium can cause placentitis in sheep, cattle, and horses. It has also been connected to cow syndrome, which results in abrupt drops in milk production, fever, and upper respiratory tract infections. The virus has also been known to cause comparable symptoms in dogs and cats, such as conjunctivitis, respiratory tract involvement, reproductive issues, and other organ systems that seem to be unrelated [20].

5. PSITTACOSIS IN HUMAN

Despite being quite frequent in birds, psittacosis can also infect humans. People are more likely to be exposed to the germs in a wide range of activities. They include those who work in veterinary clinics, poultry farms, pet businesses, and as bird keepers. Even though they are the main cause of psittacosis in humans, the other Chlamydiae that are frequently seen in birds may potentially pose a threat to humans. Bird-borne strains of C. abortus may be zoonotic [21], and the identification of this species in a group of poultry farmers raises the possibility that C. gallinacea is transmitted from birds to people. Seventy percent of incidents are attributed to those who work with caged birds [22]. Some vulnerable groups may have severe sickness or even die without treatment, but those who do are typically just mildly ill or even asymptomatic [23]. Breathing in dust infected with bacteria from dried bird droppings or secretions that contain C. psittaci is the usual way that humans contract the illness. The pathogen can also spread through bird bites. Additional sources of transmission exist. It was initially demonstrated in 1930 that aerosols might spread in a laboratory environment without biosafety precautions [24]. Throughout the birthing season, sheep, cattle, and goats infected with C. psittaci have been neglected due to a lack of specialized testing. According to recent

reports, humans can contract C. psittaci from infected equine placental material [25][26][27]. In 2014, veterinary staff and students in New South Wales, Australia, who had handled placentas or given birth to a foal that later died, were discovered to have five cases of psittacosis (three likely, two suspected) [28]. Bird contact did not correlate with illness [29]. It is possible that the illness was acquired through "spill-over" from an infected native Australian parrot, as evidenced by the identification of a C. psittaci-6BC-like strain in equine tissue. There may be additional unknown psittacosis exposure channels present, as suggested by this recently revealed disease transmission mechanism (Figure 3) [30].

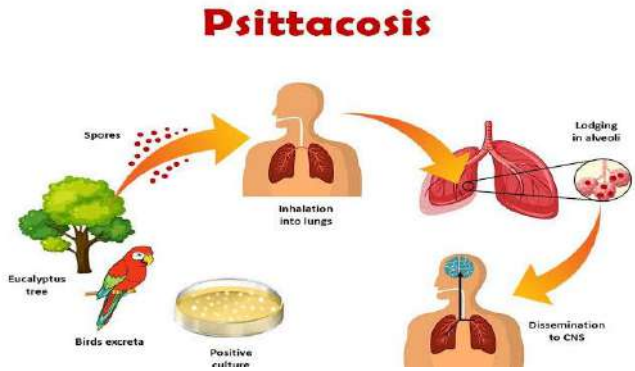

Figure 3: Psittacosis in humans

6. EVALUATION

In laboratory testing, psittacosis is identified by a left shift or toxic granulation with a normal white cell count. Though rare, leukocytosis can occur. Commonly raised are other indicators of acute inflammation, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)[35][36][37]. In addition to hyponatremia, creatine levels are typically high. Additionally, aspartate and alanine aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and ALT—all markers of liver function—show varying elevations. Even though it is mentioned in case reports, homolytic anemia is not common in psittacosis [30]. The abnormalities observed on chest imaging are typically lobar infiltrates. It is important to remember that psittacosis cannot be ruled out by a routine chest X-ray [31][32]. On a chest

computed tomography (CT) scan, unilateral lung involvement is frequently observed, and single lobe involvement was present in over 50% of patients. The removal of CSF fluid typically results in higher protein levels without a discernible increase in white blood cell counts [33][34].

7. DIAGNOSIS

Legionnaires disease, bacterial pneumonia, brucellosis, chlamydia pneumonia, fungal pneumonia, infectious endocarditis, TB, tularaemia, typhoid fever, Mycoplasma pneumonia, Q fever, and viral pneumonias are some of the atypical pneumonias that are frequently linked to psittacosis [37]. Given the clinical appearance and a history of zoonotic contact, the following diagnostic procedures may be useful in reducing the number of possible differential diagnoses [39]:

- Both lobular and lobar pneumonia can be seen on a chest radiograph;
- Liver function tests may show a small elevation;
- There could be an increased level of the erythrocyte sedimentation rate (ESR);
- and a urinalysis may reveal mild proteinuria (<3500 mg/d)

There are now 15 genotypes of C. psittaci known to exist [40]. Clinical manifestations of infection with distinct strains of C. psittaci can vary. Each patient exhibited distinct clinical symptoms, according to a case study of patients infected with C. psittaci strains SZ18-2 and SZ15[41]. With mNGS, such bacterial strain variations can also be quickly identified.

8. TREATMENT

Nowadays, the death rate from psittacosis is around 20% if treatment is not received and as low as 1% if prompt action is taken. It is noteworthy that in a 1930 outbreak in London, the fatality rate from psittacosis was 50% [40]. The serious consequences of this illness could recur if proper and efficient antimicrobial treatment is not received. For 10–14 days, and even up to 21 days, doxycycline and tetracycline are effective treatments for human psittacosis. Erythromycin and azithromycin are frequently used for infants under the age of eight and pregnant women for whom tetracycline is contraindicated. In comparison to tetracyclines and macrolides, fluoroquinolones are less effective

against C. psittaci infections [41]. After 24 to 48 hours, symptoms start to go away with treatment. There have been instances of relapse. Patients who are very sick need to receive doxycycline hyclate intravenously. A major factor in the decline in psittacosis cases has been the development of effective antimicrobial treatments. Nevertheless, chlamydia infections have not responded well to quinolone treatment. The ability of antimicrobial stimuli to cause a brief halt in the replication cycle and the onset of persistence—a viable but non-cultivable state—is noteworthy. It is uncertain how chlamydia persistence is regulated [42].

CONCLUSION

A zoonotic disease caused by Chlamydia psittaci, psittacosis, commonly known as parrot fever, is mostly transmitted from birds to humans. Fever, headaches, and respiratory issues are flu-like symptoms that can develop into serious pneumonia if treatment is not received. Antibiotic treatment, typically with doxycycline, and early diagnosis are essential for effective care. The risk of transmission can be reduced by implementing preventive measures, such as keeping the birds clean, monitoring their health, and handling them with protective equipment. Initiatives for veterinarian control and public awareness campaigns are essential to halting epidemics and safeguarding the health of both people and birds. The potential for human-to-human transmission of psittacosis exists, and the CDC Classifies C. psittaci as a Category B agent is underestimated. The manifestation of psittacosis can be varied, and it is often clinically overlooked. Ignorance of the complexities and difficulties associated with laboratory confirmation and diagnostic testing may make it more difficult to identify psittacosis when it is present. The pathogenicity of C. psittaci is influenced by both its life cycle and its capacity to elude the immune system. Even when there is a clinical index of suspicion for psittacosis, several jurisdictions and nations do not require the reporting of this infection. This could lead to a lack of awareness among public health experts regarding disease clusters that could otherwise trigger a more rapid public health response.

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CHAPTER 2

GENOMIC INNOVATIONS IN ONE HEALTH: THE ROLE OF CRISPR-CAS9 IN CONTROLLING ZOONOTIC INFECTIONS

[1]Muhammad ADIL
[2]Farrah DEEBA
[3]Anas Sarwar QURESHI

[1]University of Agriculture, Department of Clinical Medicine & Surgery, Faculty of Veterinary Science, Faisalabad, Pakistan.
[2]University of Agriculture, Department of Clinical Medicine & Surgery, Faculty of Veterinary Science, , Faisalabad, Pakistan.
[3]Ripah International University, Department of Basic Bioscience, Ripah College of Veterinary Sciences, Lahore, Pakistan.

INTRODUCTION

One Health Concept

The concept of One Health originated in the late 19th and early 20th centuries, initially termed "One Medicine," later evolving into "One World," and ultimately recognized as "One Health" (Atlas, 2012). This framework was shaped by the contributions of Rudolf Virchow, often regarded as the "Father of Comparative Medicine, Cellular Pathology, and Veterinary Pathology," and William Osler, acknowledged as the "Father of Modern Medicine." The One Health Global Network defines this approach as follows: *"One Health recognizes the interconnectedness of human, animal, and ecosystem health. It necessitates a coordinated, collaborative, multidisciplinary, and cross-sectoral approach to mitigate and address potential or existing risks at the animal-human-ecosystem interface"* (Mackenzie & Jeggo, 2019).

Significance of One Health

The importance of One Health has significantly increased over the past three decades. It has long been recognized that most novel and emerging infectious diseases are zoonotic, originating primarily from animals, particularly wildlife. These diseases are largely driven by human activities such as ecosystem disruption, land-use changes, agricultural intensification, urbanization, industrialization, deforestation, and international trade. These factors highlight the crucial role of One Health in ensuring the well-being of humans, animals, and the environment, which together form the One Health triad (Atlas, 2012) (Fig. 1).

Figure 1: Diagram of health report topics apportioned from a One Health perspective (Schwind et al., 2017)

The One Health approach focuses on the early detection, timely diagnosis, and effective management of emerging zoonotic diseases, along with their prevention and control. Many diseases have severe social consequences, particularly in low-income, developing, and underdeveloped regions. Furthermore, antimicrobial resistance (AMR) arises due to the misuse or inadequate use of antimicrobials. This leads to resistance in humans, animals, and the environment, with potential transmission between them (Mackenzie & Jeggo, 2019).

1. EMERGING AND ENDEMIC ZOONOTIC DISEASES

Emerging and endemic zoonotic diseases have had severe impacts on human health in the past, and these threats persist (Table 1). Several emerging infectious diseases, including Zika virus, *Ebolavirus*, Crimean-Congo hemorrhagic fever virus, swine influenza virus, SARS-CoV, MERS-CoV, and more recently SARS-CoV-2, have spread globally. In recent years, multiple zoonotic diseases have emerged in both humans and animals. More than 60–70% of human infectious diseases are zoonotic, originating from animals. Similarly, out of five newly emerging human diseases each year, three have an animal origin.

Despite significant research efforts, infectious diseases such as tick-borne illnesses, *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Brucella* spp., coronaviruses, and *Rabies lyssavirus* continue to pose threats to human health and welfare (Atlas, 2012). Targeted research, such as genome editing (discussed later in the chapter), is crucial for addressing One Health-related diseases, as outlined in Table 1.

2. GENOME EDITING TECHNIQUE AND ONE HEALTH

The advent of genome editing technology in the 1970s marked a transformative era in biology, gaining significant momentum by the mid-20th century. Bioengineering techniques, including gene knockout, knock-in, and targeted replacement of genomic sequences, have enabled controlled genetic modifications and improved organism survival (Gaj et al., 2016). Pronuclear microinjection, introduced in the 1980s, became one of the primary methods for generating genetically modified organisms (Gordon et al., 2020). This

technique involves introducing foreign DNA into the nucleus of a fertilized egg, leading to the development of transgenic animals. The first successful microinjection trials for producing transgenic pigs, sheep, and rabbits were conducted in 1985 (Navarro-Serna et al., 2020).

In the late 1990s, Somatic Cell Nuclear Transfer (SCNT) further advanced genome editing by enabling the transfer of a somatic cell nucleus into an enucleated fertilized egg, resulting in a genetically identical organism (Gouveia et al., 2020). DNA double-strand breaks (DSBs) are a crucial mechanism for site-specific genome editing. Four major classes of DNA-binding proteins meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effectors (TALEs), and the RNA-guided Cas9 endonuclease from the type II bacterial CRISPR system—have been engineered for precise genome modifications (Khalil, 2020). These molecular scissors follow a similar genome-editing mechanism, identifying and binding to target sequences before inducing DSBs at the desired loci (West & Gill, 2016).

DSB repair occurs via either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is responsible for 90% of DSB repair, as it operates throughout the cell cycle except during the M phase. Without a repair template, NHEJ directly ligates broken DNA ends, often introducing insertions or deletions (indels), which may cause gene knockout/in or loss/gain of function due to its error-prone nature. In contrast, HDR utilizes a repair template to introduce precise genetic modifications at the target locus and is restricted to the S and G2/M phases of the cell cycle (Petersen et al., 2013; Ranjha, 2018).

Among genome editing techniques, meganucleases exhibit lower cytotoxicity due to their natural presence in cells. However, their limited protein-DNA interaction specificity reduces their efficiency as a genome editing tool (Zaslavskiy et al., 2014), as outlined in Table 2.

Table 1: Summary of Various One Health Related Diseases Caused by Viral, Bacterial, and Parasitic Pathogens

Pathogen Type	Pathogen	Natural Host	Mode of Transmission	Disease Caused	References
Viral Agent	*Lyssaviruses*	Dogs, bats, cats, foxes	Bites, scratches	Rabies	(León et al., 2021)
	West Nile Virus	Birds	*Culex* mosquito bite	West Nile Fever	(Petersen et al., 2013)
	SARS-CoV-2	Bats	Respiratory droplets, saliva, close contact	Respiratory tract infection	(Yang et al., 2020)
	Monkeypox virus	Monkeys, anteaters, hedgehogs, rodents	Bites, scratches, direct contact	Pustular rash	(Pastula & Tyler, 2022)
	Ebola virus	Bats, non-human primates	Direct contact, body fluids	Ebola hemorrhagic fever	(Groseth et al., 2007)
	Zika virus (*Flavivirus*)	Humans, non-human primates	*Aedes* mosquito bite	Guillain-Barré syndrome, microcephaly	(Musso & Gubler, 2016)
	Hantavirus	Rodents	Urine, droppings, saliva	Hantavirus Pulmonary Syndrome (HPS), Hemorrhagic Fever with Renal Syndrome (HFRS)	(Muranyi, 2005)
	Influenza A (H1N1)	Pigs	Aerosols, direct contact	Respiratory illness	(Olsen, 2002)
	Avian Influenza Virus	Domestic poultry, waterfowl	Feces, respiratory secretions	Respiratory infection	(Lee & Saif, 2009)
Bacterial Agent	*Bacillus anthracis*	Sheep, cattle, horses, goats	Direct contact, inhalation, ingestion	Anthrax	(Sidwa et al., 2020)

Pathogen Type	Pathogen	Natural Host	Mode of Transmission	Disease Caused	References
	Borrelia burgdorferi	Mice, chipmunks, raccoons, squirrels, lizards, white-footed mice	Tick bite	Lyme Disease	(Bernard et al., 2019)
	Yersinia pestis	Rodents	Flea bite	Plague	(Prentice & Rahalison, 2007)
	Brucella spp.	Sheep, goats	Raw dairy, undercooked meat	Brucellosis (Meningitis, Endocarditis)	(Karponi et al., 2019)
	Salmonella spp.	Poultry, cattle, dogs, rodents, swine, cats	Food, water, feces	Gastroenteritis, diarrhea, typhoid, enteric fever	(Ajmera & Shabbir, 2022)
Parasitic Agent	*Giardia duodenalis*	Rodents, cattle, sheep	Water, food, surfaces	Giardiasis	(Sprong, 2009)
	Schistosoma spp. (*S. mansoni, S. haematobium, S. japonicum*)	Snails	Water exposure	Schistosomiasis	(Gryseels et al., 2006)
	Toxoplasma gondii	Cats	Undercooked meat, cat feces	Toxoplasmosis	(Remington et al., 2004)
	Trematodes	Cats, dogs, foxes, pigs, rodents	Water, food	Trematodiasis	(Keiser & Utzinger, 2005)
	Ixodid ticks	Cattle, sheep, goats	Tick bite, blood contact	Crimean-Congo Hemorrhagic Fever	(Whitehouse, 2004)
	Trypanosoma brucei	Domestic cattle	*Glossina* (*Tsetse fly*) bite	Trypanosomiasis	(Brun et al., 2010)

2.1 Meganucleases

The meganuclease-based genome editing approach involves precise target site recognition followed by endonuclease-mediated cleavage. A significant advantage of this technique is its inherent low cytotoxicity due to the natural presence of meganucleases in cells. However, its major limitation is the relatively low specificity in meganuclease-protein interactions with target DNA sequences, which restricts its efficiency and broader applicability in genome editing (Zaslavskiy et al., 2014).

Table 2: Delivery Strategies for CRISPR-CAS9 System

Delivery Method	Characteristics	Limitations	References
Plasmid-Based CRISPR-Cas9 Delivery	Cost-effective, stable gene expression, efficient transfection, flexible vector design	High off-target mutations, delayed nuclease activity, risk of insertional mutagenesis	(Chen et al., 2020)
mRNA-Based CRISPR-Cas9 Delivery	Rapid gene editing, transient expression, no risk of genomic integration, reduced off-target effects.	Susceptible to RNase degradation, low half-life, limited intracellular stability	(Humphrey & Kasinski, 2015)
Protein-Based CRISPR-Cas9 Delivery	High editing precision, immediate enzymatic activity, minimal risk of genomic integration	Short-lived activity, high production cost, potential immunogenicity	(Liang et al., 2015)

2.2 Zinc Finger Nucleases (ZFNs)

Zinc finger nucleases (ZFNs) are artificially engineered nucleases that are created by fusing zinc finger (ZF) proteins with restriction endonucleases, enabling precise target site cleavage. This approach offers advantages over meganucleases due to its simplicity and specificity (Ochiai and Yamamoto, 2017). However, the assembly of zinc finger domains to achieve high-affinity binding to extended nucleotide sequences remains a complex task. Although advancements in research have improved this technique, achieving the desired binding specificity of zinc finger proteins (ZFPs) to target sequences continues to be a major challenge (Ely et al., 2021).

2.3 Transcription Activator-Like Effector Nucleases (TALEN)

Transcription activator-like effector nucleases (TALENs) share structural and functional similarities with zinc finger nucleases (ZFNs), as both involve the formation of a DNA-binding protein-endonuclease complex to mediate targeted DNA cleavage. However, instead of zinc finger (ZF) domains, TALENs utilize transcription activator-like (TAL) effectors for DNA recognition and binding (Hensel and Kumlehn, 2019). TALENs exhibit greater target specificity, as they can recognize and cleave single-nucleotide sequences, whereas ZFNs typically require a minimum of three nucleotides for effective binding (Chandrasegaran and Carroll, 2016). Despite this advantage, TALENs share similar limitations with ZFNs, as previously discussed (Juillerat et al., 2014).

2.4 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

The first evidence of CRISPR was documented in 1987 when a unique repetitive DNA sequence was identified in *Escherichia coli* during the study of phosphate metabolism genes. Subsequently, clustered regularly interspaced short palindromic repeats (CRISPRs) were discovered in archaea in 1993, particularly in *Haloferax mediterranei* (Ishino et al., 2018). The CRISPR/Cas system is an ancient adaptive immune mechanism present in certain bacteria and archaea, functioning as a defense system against bacteriophage DNA. The first application of CRISPR for genome editing in mammalian cells was reported in 2013 (Cong et al., 2013; Khurshid et al., 2018).

2.4.1 Structure of CRISPR-Cas9 System

The CRISPR system consists of two RNA molecules: CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). The crRNA comprises spacer sequences and palindromic repeats, while tracrRNA is a distinct entity. Although crRNA provides target specificity for Cas9, it cannot directly bind to the Cas9 protein. To facilitate this interaction, crRNA and tracrRNA are linked to form a single chimeric molecule known as single-guide RNA (sgRNA), which can be synthetically engineered in the laboratory. The sgRNA associates with Cas9, leading to the formation of the Cas complex.

Additionally, the CRISPR system includes spacer DNA segments, ranging from 26 to 72 base pairs, which are homologous to bacteriophage or plasmid DNA. These spacers are interspersed with repeat sequences of similar length (Jiang and Doudna, 2015). Cas9 can be precisely directed to induce double-stranded DNA breaks at specific genomic sites determined by the guide RNA sequence and the presence of a Protospacer Adjacent Motif (PAM). The PAM sequence plays a crucial role in preventing self-targeting of the CRISPR locus. A commonly utilized PAM sequence, 5′-NGG-3′, originates from *Streptococcus pyogenes* Cas9 (SpCas9) (Cong et al., 2013).

2.4.2 How Does CRISPR-Cas System Work?

The CRISPR-Cas immune response occurs in three phases: adaptation, expression, and interference. During the adaptation phase, a specialized Cas protein complex recognizes a Protospacer Adjacent Motif (PAM) sequence in foreign DNA, excises a protospacer, and integrates it into the CRISPR array by duplicating the repeat sequence at the 5′ end, converting it into a spacer. In the expression phase, the CRISPR array is transcribed into precursor CRISPR RNA (pre-crRNA), which is processed into mature crRNA containing the spacer sequence and flanking repeat fragments. During the interference phase, the mature crRNA guides the Cas nuclease complex to recognize and bind to a complementary protospacer sequence within the invading viral or plasmid genome, leading to targeted DNA cleavage and degradation, thereby neutralizing the foreign genetic material (Lander et al., 2016).

2.4.3 Delivery System For CRISPR Cas9

In the type II CRISPR-Cas system, the crRNA effector complex consists of a single multi-domain protein, Cas9. However, the presence of the *cas9* gene alone is not sufficient for classification as a CRISPR-associated gene; therefore, *cas1* and *cas2* are used as additional markers for identifying the type II system. Cas9 possesses two nuclease domains, HNH and RuvC-like, each responsible for cleaving one strand of the target DNA (Krzysztof, 2014). The CRISPR-Cas9 protein has a molecular weight of approximately 160 kDa, and its association with single-guide RNA (sgRNA) results in a ribonucleoprotein (RNP) complex with an overall negative charge due to the long phosphate backbone of the

sgRNA. This net negative charge presents challenges for CRISPR-Cas9 delivery. Both the delivery vehicle (CRISPR-Cas9) and the cargo (Cas nuclease and guide RNA) play a critical role in effective genome editing. Delivery methods include DNA-, mRNA-, or protein-based approaches (Jinek et al., 2014).

2.4.4 CRISPR Cas-9 and its Application in One Health Related Zoonotic Diseases

The CRISPR-Cas system has numerous applications due to its simplicity and precision. It is widely used in disease diagnosis and treatment, including One Health-related conditions, as follows:

- **Bacterial Diseases:**

Anthrax: Anthrax, caused by *Bacillus anthracis*, is a lethal zoonotic disease with high mortality, primarily due to its spore-mediated transmission and virulence plasmids, pXO1 and pXO2 (Sidwa et al., 2020). CRISPR-Cas technology has been employed to disrupt these plasmids using a guide RNA-directed Cas9 nuclease, significantly reducing *B. anthracis* virulence and enhancing bacterial elimination (Wang et al., 2021). Additionally, CRISPR activation (CRISPRa) studies on anthrax toxin receptor 2 (ANTXR2) revealed evolutionary differences in its expression between humans and non-human primates, highlighting human adaptation to anthrax (Choate et al., 2021). Beyond disease control, CRISPR-Cas applications also facilitate the identification of early ecological changes.

Brucellosis: The genus *Brucella* is responsible for causing brucellosis, with *B. melitensis* being the most prevalent species, primarily affecting ruminants and leading to reproductive disorders such as abortion and infertility. In humans, brucellosis manifests as severe clinical conditions, including arthritis, endocarditis, meningitis, infertility, and pregnancy loss. Treatment remains challenging, and no highly effective vaccine is currently available (Karponi et al., 2019). In a study, ovine macrophages infected with *B. melitensis* were used to model host-pathogen interactions *in vitro*. Lentiviral vectors delivering the CRISPR-Cas9 system were employed to target the *Brucella* RNA polymerase A (*rpoA*) gene, significantly reducing bacterial load per cell (Karponi et al., 2019). Furthermore, Xu et al. (2022) developed a CRISPR-Cas12a-based detection system combined with recombinase polymerase

amplification (RPA) to identify various *Brucella* species in infected blood and milk. This platform, utilizing CRISPR-Cas12a-RPA fluorescent and electrochemical biosensors, demonstrated high sensitivity, capable of detecting as few as 2–3 copies of plasmid DNA, thereby enabling early and accurate diagnosis of brucellosis.

- **Lyme Disease:** Lyme disease is a tick-borne zoonotic infection caused by the spirochete *Borrelia burgdorferi* and transmitted through the bite of *Ixodes* genus ticks (Bernard et al., 2019). The primary reservoir of *Borrelia* is the white-footed mouse, which facilitates transmission to humans via black-legged tick bites. If left untreated, the disease can progress to early-stage arthritis, neurological complications, and cardiac disorders (Norris, 2018). Progress in gene editing for ticks has been limited due to the presence of a waxy coating on tick eggs, which hinders the effective injection of CRISPR components into embryos at appropriate developmental stages without compromising egg integrity (Sharma et al., 2022). Consequently, researchers have shifted focus from vector-targeted approaches to host-based interventions using CRISPR genome editing. By introducing antibody-encoding resistance alleles into the genomes of white-footed mice, the goal was to confer heritable immunity, thereby disrupting the transmission cycle of *B. burgdorferi* across tick populations. To achieve this, genetically engineered mice were introduced into wild populations to breed naturally, allowing for the spread of immunized offspring without requiring a gene drive system (Buchthal et al., 2019).
- **Viral Diseases**

Rabies: Rabies is an acute, progressive encephalitis caused by a *lyssavirus*, a bullet-shaped, single-stranded, negative-sense RNA virus. Bats serve as the primary reservoir hosts, while most mammalian species, including humans, are susceptible to infection. Transmission occurs primarily through direct contact with virus-laden saliva via animal bites or transdermal inoculation (León et al., 2021). Current rabies control strategies rely on mass vaccination and post-exposure prophylaxis (PEP). However, gene therapy and gene-editing technologies offer potential future solutions for treatment and viral control. The CRISPR-Cas9 system, in combination with induced pluripotent

stem cells (iPSCs), can facilitate gene corrections *in vitro*, with gene delivery tools enabling the introduction of edited genes into target organs. Additionally, CRISPR-Cas9-based gene therapy, combined with the microhomology-mediated end-joining (MMEJ) method, has demonstrated potential for eliminating rabies virus within infected neuronal cells. This approach could offer therapeutic intervention even after the onset of clinical symptoms (Nelwan, 2018).

Coronavirus Disease 19 (COVID-19): SARS-CoV-2, a member of the *Coronaviridae* family within the *Nidovirales* order, primarily targets the respiratory system but also exhibits tropism for multiple organ systems. The COVID-19 pandemic led to widespread morbidity and mortality, with zoonotic transmission attributed to bats (Yang et al., 2020). To dissect the molecular mechanisms underlying SARS-CoV-2 infection, researchers have developed a host-pathogen protein interaction network, identifying key proteins implicated in viral pathogenesis. Functional validation through high-throughput CRISPR-based gene knockout screening has significantly accelerated the discovery of novel therapeutic targets for infectious diseases. Moreover, CRISPR technology has facilitated the repurposing of existing drugs for emerging pathogens. Notably, CRISPR-mediated knockout of fatty acid synthase (FASN), a crucial enzyme in lipid metabolism, demonstrated its role in SARS-CoV-2 replication, highlighting FASN inhibitors as potential antiviral candidates (Gordon et al., 2020).

Human Papillomavirus (HPV): Human papillomavirus (*HPV*), a small DNA virus, is a well-established etiological agent of cervical and other malignancies. Oncogenesis is primarily driven by the viral oncogenes *E6* and *E7*, which have been identified as critical therapeutic targets for CRISPR-based genome editing. Several studies have demonstrated that CRISPR-Cas9-mediated disruption of *E6* and *E7* leads to reduced oncogenic protein expression, apoptosis of infected cells, and inhibition of tumor growth (Zhen and Li, 2017). Preclinical investigations utilized stealth liposome-mediated delivery of CRISPR-Cas9 components to target *E7* in *HPV16*-induced tumors in murine models. This approach effectively eradicated tumors without inducing hepatotoxicity or splenic damage. Encouraging preclinical outcomes

have facilitated the progression of CRISPR-based therapeutics into clinical trials for *HPV*-associated malignancies (Jubair et al., 2021).

Human Immunodeficiency Virus (HIV): Acquired Immune Deficiency Syndrome (*AIDS*) remains a significant global health challenge, caused by *Human Immunodeficiency Virus* (*HIV*), which progressively impairs the host immune system (Bowers et al., 2014). The latent nature of *HIV* poses a major obstacle to eradication, even with highly active antiretroviral therapy (*HAART*). To address this limitation, CRISPR-Cas9-based therapeutic strategies have been explored, yielding promising results in *HIV* treatment (Xiao et al., 2019). Experimental studies have employed CRISPR-Cas9 with guide RNA targeting conserved sites within the *HIV-1 LTR-U3* region, demonstrating successful inactivation of viral gene expression and inhibition of viral replication in multiple latently infected cell lines, including T cells, pro-monocytic cells, and microglial cells. Furthermore, minimal genotoxicity and off-target effects were reported (Hu et al., 2014; Lebbink et al., 2017). Recent advancements involve the integration of CRISPR-Cas9 with *Staphylococcus aureus gRNA* in lentiviral vectors, effectively excising latent *HIV-1* provirus and suppressing proviral reactivation (Wang et al., 2008).

West Nile Virus: *West Nile virus* (*WNV*), a member of the *Flaviviridae* family, is an enveloped, single-stranded RNA virus primarily transmitted to humans through mosquito vectors (Petersen et al., 2013). *WNV* infection leads to severe neurological complications, characterized by extensive neuronal cell death. Using a CRISPR-Cas9-based screening approach, seven genes (*EMC2, EMC3, SEL1L, DERL2, UBE2G2, UBE2J1*, and *HRD1*) were identified as key mediators of *WNV*-induced cell death. Disruption of these genes conferred protection against neuronal cell death in three different cell lines. Notably, despite gene knockout, *WNV* replication remained unaffected, indicating that these genes play a crucial role in host cell death pathways rather than in viral replication (Ma et al., 2015).

- **Parasitic Diseases**

Malaria: Malaria, caused by *Plasmodium* parasites and transmitted by *Anopheles gambiae* mosquitoes, remains a significant public health challenge. To combat mosquito-borne diseases, various gene drive systems have been developed utilizing genome-editing techniques. The CRISPR system has been

employed to manipulate the sex-determining gene, promoting male-biased inheritance. This modification, propagated through a gene drive, effectively eliminated infected female mosquitoes from the population. Within 7 to 11 generations, the modified gene achieved 100% prevalence, leading to a gradual decline in egg production and ultimately causing population collapse under laboratory conditions (Hammond et al., 2016). Additionally, researchers targeted the fibrinogen-related protein 1 (FREP1) gene, demonstrating that CRISPR-mediated FREP1 knockout resulted in delayed pre-adult development, reduced blood meal consumption, and lower egg production with decreased viability (Dong et al., 2018).

- **Toxoplasmosis and Chagas Disease**

Toxoplasma gondii is an intracellular protozoan and the causative agent of toxoplasmosis, a zoonotic disease that is often asymptomatic in humans. However, in neonates, children, and immunocompromised individuals, primary infection or reactivation can result in severe disease. Transmission occurs through vertical transfer, organ transplantation, and ingestion of tissue cysts, with improperly cooked meat, contaminated food, and water serving as major sources of infection (Saadatnia and Golkar, 2012). Similarly, Chagas disease, caused by *Trypanosoma cruzi* and transmitted by reduviid bugs, poses significant health risks (Brun et al., 2010).

Advancements in genome-editing techniques have facilitated the study of these parasitic pathogens. CRISPR-Cas9 high-throughput analysis was employed to systematically knock out or down all nuclear protein-coding genes in *T. gondii* using a guide RNA library and constitutive Cas9 expression (Nødvig et al., 2015). In *T. cruzi*, CRISPR-Cas9 was utilized to silence the GP72 gene, responsible for flagellar attachment (Lander et al., 2017). Additionally, CRISPR-Cas9-mediated knockout of the *P21* gene resulted in a loss of its expression, leading to cell cycle arrest at the G1 phase, thereby slowing epimastigote growth and division (Teixeira et al., 2022).

CONCLUSION

Genome editing, particularly the CRISPR-Cas system, offers vast potential for biomedical advancements. However, extensive research is still required to effectively eliminate zoonotic diseases and address challenges

within the One Health framework. CRISPR-based diagnostics and therapeutics are promising but require further refinement. Currently, genome-editing efforts are primarily focused on the prevention and treatment of infectious diseases caused by zoonotic bacteria and viruses. Ensuring safety, efficacy, and minimal off-target effects remains a critical goal in the development of CRISPR-based therapies.

A key lesson from the COVID-19 pandemic is the necessity of proactive measures against zoonotic, emerging, and re-emerging viral infections. Given the high mutability of viruses, conventional treatment approaches often become less effective, necessitating a shift toward advanced genome-editing technologies like CRISPR to address these challenges. The development of novel drugs, vaccines, diagnostic tools, and therapeutic interventions utilizing CRISPR technology holds significant promise in mitigating the impact of zoonotic infectious diseases.

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CHAPTER 3

EMERGING ZOONOTIC DISEASES

[1]Gideon Yakusak BENJAMIN

[1]State University of Medical and Applied Sciences, Department of Applied Biological Sciences (Applied Microbiology and Brewing Unit), Enugu State, Nigeria.

INTRODUCTION

The term "Zoonosis" (zoonoses, plural) is derived from the Greek word "Zoon", which means animal, and "nosos", which means illness, was coined at the end of the nineteenth century by Rudolph Virchow to designate human diseases caused by animals. The term 'zoonosis' is also considered to be shorter and more convenient than 'anthropozoonosis' (animals to humans) and 'zooanthroponosis' (humans to animals), which are based on the prevailing direction of transmission between humans and other vertebrates. The World Health Organization (WHO) defines zoonosis as any disease or infection that is naturally transmissible from vertebrate animals to humans or from humans to animals (WHO, 2020). They are therefore diseases and infections that are naturally transmitted between vertebrate animals and man.

Zoonoses is a great public health concern and a direct human health hazard that may even lead to death. While humans have coexisted with wild animals for millennia, it is believed that mounting anthropogenic activity during these recent decades, such as land-use change and human population growth, has led to increased interactions between humans and wild vertebrates, resulting in an increased risk of disease spillover to human populations (Plowright *et al*. 2021; Gagne *et al*. 2022). The greatest risk for zoonotic disease' transmission occurs at the human-animal interface through direct or indirect human exposure to animals, livestock products (e.g., meat, milk, eggs and derived processed products) and/or their environments, including natural, cultivated, built (i.e., abattoirs) and commercial environments (i.e., wet markets).

As our world grows progressively interdependent and the populations of people, domestic animals, wildlife, and animal products also increase and expand globally, we can expect more interactions among these groups and certainly the era of emerging and reemerging zoonoses will also expand and grow proportionately (Tomley and Shirley, 2009). As the human–animal interfaces intensify and accelerate, there is a growing concern with the emergence and reemergence of more zoonoses and animal-associated diseases, including leptospirosis, leishmaniasis, Q fever, toxoplasmosis, anaplasmosis, food-borne trematodes, ehrlichia, bartonella, Chagas disease, and toxocariasis. Although most of these diseases can be considered in the category of neglected

diseases and are increasingly associated with slums and periurban locations, some of these diseases are also found in developed countries because of the relocation of human populations, global travel, and the movement of food and animal products as part of the rapidly expanding global food system.

1. CLASSIFICATION OF ZOONOSES

Based on etiology, zoonoses are classified into bacterial zoonoses (such as anthrax, salmonellosis, tuberculosis, Lyme disease, brucellosis, and plague), viral zoonoses (such as rabies, acquired immune deficiency syndrome- AIDS, Ebola, and avian influenza), parasitic zoonoses (such as trichinosis, toxoplasmosis, trematodosis, giardiasis, malaria, and echinococcosis), fungal zoonoses (such as ring worm), rickettsial zoonoses (Q-fever), chlamydial zoonoses (psittacosis), mycoplasma zoonoses (*Mycoplasma pneumoniae* infection), protozoal zoonoses, and diseases caused by acellular non-viral pathogenic agents (such as transmissible spongiform encephalopathies and mad cow disease) (Chomel, 2009; Rahman, 2020).

Other classifications of zoonoses may include a classification based on the categories of people at risk or relating to the type of human activity, such as occupational zoonoses (which occur when people are infected during their professional activity; e.g., brucellosis in farmers, veterinarians, or slaughterhouse employees, Lyme disease in foresters, rabies in wildlife trappers or taxidermists), zoonoses associated with recreational activities (e.g., plague, hantavirus infection, Lyme disease, tularemia, or parasitic larva migrans), domestic zoonoses (diseases acquired from pets), or accidental zoonoses (some very rare and peculiar circumstances of infection, as well as foodborne outbreaks) (Chomel, 2009)..

Another aspect of zoonoses classification concerns their clinical manifestations and their diagnosis. Clinical diagnosis of zoonoses is not always easy, especially if the symptoms are different in animals and humans, or if clinical signs are present only in humans. If clinical signs are observed in animals and humans, zoonoses are designated as phanerozoonoses. If symptoms are similar in both animals and humans, they are considered isosymptomatic (rabies and tuberculosis), whereas they are anisosymptomatic if the symptoms are different in humans and animals (anthrax, brucellosis,

psittacosis, and Rift Valley fever). In some instances, subclinical infection is observed in animals and clinical illness in humans, or vice versa. In such cases, these zoonoses are designated as cryptozoonoses (Chomel, 2009).

When considering alternatives for control measures, it is the primary epidemiological classification based on the zoonosis maintenance cycle that is of major importance. This classification divides the zoonoses into four categories:

- Direct zoonoses (orthozoonoses) are transmitted from an infected to a susceptible vertebrate host by direct contact, by contact with a fomite, or by a mechanical vector. Direct zoonoses may be perpetuated in nature by a single vertebrate species, such as dogs or foxes for rabies or cattle, small ruminants or swine for brucellosis.
- Cyclozoonoses require more than one vertebrate species, but no invertebrate host, in order to complete the developmental cycle of the agent. Examples are human taeniases or pentastomid infections. Most of the comparatively few cyclozoonoses are cestodiases.
- Pherozoonoses (also called metazoonoses) are zoonoses that require both vertebrates and invertebrates for the completion of their infectious cycle.
- In pherozoonoses, the infectious agent multiplies (propagative or cyclopropagative transmission) or merely develops (developmental transmission) in the invertebrate; there is always an extrinsic incubation period in the invertebrate host before transmission to a vertebratehost. Examples are arbovirus infections, plague, Lyme borreliosis, or rickettsial infections.
- Saprozoonoses have both a vertebrate host and an inanimate developmental site or reservoir. The developmental reservoir is considered nonanimal, such as organic matter, including food, soil, and plants. In this group of zoonoses, direct infection is usually rare or absent. Examples are histoplasmosis, *Erysipelothrix* infection, or listeriosis (Chomel, 2009).

2. EMERGING AND RE-EMERGING ZOONOSES

Emerging zoonoses are zoonotic diseases caused either by apparently new agents or by previously known microorganisms, appearing in places or in

species in which the disease was previously unknown. New animal diseases with an unknown host spectrum are also included in this definition. Natural animal reservoirs represent a more frequent source of new agents of human disease than the sudden appearance of a completely new agent (Meslin, 1992). Therefore, while emerging zoonoses are new or re-spreading diseases, reemerging zoonoses are diseases that have returned after a decline.

Emerging and re-emerging diseases have significant impacts, not only on public health, but also on socio-economic issues around the globe (Bao *et al*., 2017). Among 175 reported emerging diseases, 132 diseases are considered to be emerging zoonotic diseases. Another report estimated that about 60.3% of the emerging diseases can be categorized under zoonoses. Among them, 71.8% originated from wildlife (Jones *et al*., 2008; Rahman *et al*., 2020).

Factors explaining the emergence of a zoonotic or potentially zoonotic disease are usually complex, involving mechanisms at the molecular level, such as genetic drift and shift, and modification of the immunological status of individuals and populations. Social and ecological conditions influencing population growth and movement, food habits, the environment and many other factors may play a more important role than changes at the molecular level. Bacterial enteric diseases due to *Salmonella enteritidis* and *Escherichia coli* O157:H7 are examples of diseases associated with changing farming practices, trade and consumer habits (Meslin, 1992).

The spectrum of infectious diseases is changing rapidly in conjunction with dramatic societal and environmental changes. Exponential human population growth with expanding poverty and urban migration is occurring worldwide, international travel and trade is increasing. Exponential human population growth with expanding poverty and urban migration is occurring worldwide, international travel and trade is increasing, and technology is rapidly changing – all of which affect the risk of exposure to infectious agents. Disease emergence often follows ecological changes caused by human activities such as agriculture or agricultural change, migration, urbanization, deforestation, or dam building. Of these new diseases, surprisingly, most of the emergent viruses and many of the emergent bacteria are zoonotic (Baker *et al*., 2022).

Examples of major emerging zoonoses include avian influenza, bovine spongiform encephalopathy (BSE), feline cowpox, rotavirus infection, norovirus infection, ebola virus diseaese, hantavirus infection, west nile fever, canine leptospirosis, MRSA infection, cat scratch disease, severe fever with thrombocytopenia syndrome (SFTS), middle east respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and the most recent coronavirus disease 2019 (COVID-19) (Wang *et al*., 2020). On the other hand, rabies, brucellosis, Japanese encephalitis, tuberculosis (*M. bovis*), and *Schistosoma japonicum* infection are considered to be re-emerging zoonoses in many parts of the world (Rahman *et al*., 2020).

3. ZOONOTIC TRANSMISSION

Zoonotic transmission may be direct such as with rabies or indirect through either vectors such as ticks, mosquitoes or other insects (trypanosomiasis) or through food, water, or soil (helminths) (King, 2011). Zoonotic transmission involves the interaction of a pathogen and at least two host species: (a) a natural reservoir, infected with the pathogen and often asymptomatic (shedding the pathogen), (b) a recipient host, presenting the disease (infected with the pathogen from a different host), and (c) an intermediate host, that may or may not be present, acting as a bridge or mixing vessel (vertebrate or invertebrate vector). Pathogens can be transmitted to the recipient host (humans) directly from the natural reservoir, from the intermediate vertebrate or invertebrate host, or from the environment, resulting in transmission to humans without spread ("dead-end spillover"), or in adaptation for human-to-human transmission (Ellwanger and Chies, 2020). Although these events are relatively rare, in the last century, outbreaks of emerging and re-emerging viral zoonoses have increased in frequency and magnitude with significant human and animal health impacts, as well as incalculable and far-reaching economic consequences, as a result of the intensification of the animal-human interface, driven primarily by anthropogenic factors (Baker *et al*., 2022). Table 1 lists some major zoonotic diseases with their etiological agents and animal host.

Table 1: Major Zoonotic Diseases, their Etiological Agents and Hosts (Rahman, 2020)

Disease	Etiology	Animal Host
Bubonic plague	*Yersinia pestis*	Rock squirrels, wood rats, mice,rabbits, ground squirrels, chipmunks
Leprosy	*Mycobacterium leprae*	Monkeys, rats, mice, and cats
Tularemia	*Francisella tularensis*	Rabbits, squirrels, muskrats, deer, sheep, bull snakes, wild rodents, beavers, cats, and dogs
Bordetellosis	*Bordetella bronchiseptica*	Cats and dogs
Enterohemorrhagic *Escherichia coli* infections	*E coli* O157:H7	Cattle, sheep, pigs, deer, dogs, and poultry
Salmonellosis	*Salmonella enterica, Salmonella bongor*	Domestic animals, birds, and dogs
Rabies	Rabies virus, Genus—*Lyssavirus* Family—*Rhabdoviridae*	Cattle, horses, cats, dogs, bats, monkeys, wolves, skunks, rabbits, and coyotes
Newcastle disease	Paramyxovirus,	Poultry and wild birds
Avian influenza	Influenza A virus	Ducks, chickens, turkeys, dogs, cats, pigs, whales, horses, seals, and wild birds
Dengue fever	Dengue virus	Monkeys and dogs
Zika fever	Zika virus	Apes and monkeys
Rift Valley fever	Rift Valley fever virus	Buffaloes, camels, cattle, goats, and sheep
Ebola virus disease (Ebola Hemorrhagic Fever)	Ebola virus Genus—*Ebolavirus* Family—*Flaviviridae*	Monkeys, gorillas, chimpanzees, apes, and wild antelopes
Marburg viral hemorrhagic fever	Marburg virus	Fruit bats and monkeys
Chikungunya fever	Chikungunya virus	Monkeys, birds, and rodents

Zika fever	Zika virus	Apes and monkeys
Severe acute respiratory syndrome (SARS)	SARS coronavirus (SARS-CoV)	Bats, dogs, cats, ferrets, minks, tigers, and lions
Trichinellosis	*Trichinella* spp.	Pigs, dogs, cats, rats, and other wild species
Cryptosporidiosis	*Cryptosporidium parvum*	Cattle, sheep, pigs, goats, horses, and deer
Coccidioidomycosis	*Coccidioides immitis*, *Coccidioides posadasii*	Dogs, horses, pigs, and ruminants
Toxocariasis	*Toxocara canis*, *Toxocara cati*	Dogs and cats
Toxoplasmosis	*Toxoplasma gondii*	Pigs, sheep, goats, poultry, and rabbits
Psittacosis	*Chlamydia psittaci*	Parrots, parakeets, lories, cockatoos, cattle, sheep, and goats
Mad Cow Disease, also known as BSE (Bovine spongiform encephalopathy). In human known as Creutzfeldt–Jakob disease (CJD)	Prion protein	Cattle, sheep, goats, mink, deer, and elks
Tinea/ringworm infection	*Microsporum* spp., *Trichophyton* spp.	All animals like cattle, sheep, goats, cats, and dogs
Aspergillosis	*Aspergillus* spp.	All domestic animals and birds
Lyme disease	*Borrelia burgdorferi*	Cats, dogs, and horses
Bordetellosis	*Bordetella bronchiseptica*	Cats and dogs

4. MEDICAL AND ECONOMIC IMPORTANCE OF ZOONOSES

Zoonoses are important to Public Health because of their number, their frequency, and their severity in relation to human health. There are more than 250 zoonoses according to the WHO Zoonoses Expert Committee. There are very few vertebrates that are not involved with one or more zoonoses. Human

infection most often occurs when infection persists in animals, such as rabies, brucellosis, or tuberculosis. Zoonoses frequency varies for each disease and depends on the geographical distribution of reservoirs, agents, and population density, as well as efficiency of controlled measures. Some zoonoses are ubiquitous, such as salmonellosis and leptospirosis. (Chomel, 2009).

Zoonoses are a tremendous economic burden to humans due to the loss of diseased animals and agricultural production, cost of prevention, and treatment, debilitation of and productivity losses to humans. It is quite difficult to evaluate such costs precisely, but some estimates have been published that illustrate the economic impact of zoonotic diseases. Economic losses resulting from foodborne parasitic zoonoses are difficult to assess, as underlined by Murrell. In Mexico, for example, porcine cysticercosis was reported to be responsible for a loss of more than one-half of the national investment in swine production and for more than $17 million annually in hospitalization and treatment costs for humans with neurocysticercosis. In Africa, losses of $1–2 billion per year due to bovine cysticercosis have been reported (Chomel, 2009).

5. NEGLECTED ZOONOSES

A neglected zoonotic disease (NZD) is a zoonosis that is commonly associated with poverty and impacts the lives and livelihoods of millions of poor livestock keepers or those living in periurban slums primarily in developing countries. Neglected zoonotic diseases are a subset of the neglected tropical diseases. The term “neglected” highlights that the diseases affect mainly poor and marginalized populations in low-resource settings (WHO, 2015). Some NZDs are part of existing lists of neglected tropical diseases (NTDs) or comprise their own list but all share similar characteristics and attributes (King, 2011).

A key characteristic of NZDs is that they are closely associated with poverty and they disproportionately affect neglected populations. Poor people are more at risk of contracting many zoonoses. For example, anthrax, bovine tuberculosis, and brucellosis are primarily occupational diseases, and small livestock producers worldwide are at risk and more frequently acquire these infections from their animals (King, 2011).

The poor are also more vulnerable to diseases associated with consumption of livestock products and are at risk for zoonotic diseases such as cysticercosis and other parasitic and food-borne illnesses. In addition, vectors, water, and the environment can also be sources of NZDs. Once infected, it is the poor that are least likely to get proper medical care. The impact of NZDs is also worse in poor households where a dual burden is borne because both people and their animals are involved. Thus, NZDs not only make members of families ill, but also at the same time, limit the productivity of their livestock and poultry and, thus, take away the funds that would be used for emergencies, their family's well-being, and funds used to cope with these illnesses (King, 2011).

As the human–animal interfaces intensify and accelerate, there is a growing concern with the emergence and reemergence of more zoonoses and animal-associated diseases, including leptospirosis, leishmaniasis, Q fever, toxoplasmosis, anaplasmosis, food-borne trematodes, ehrlichia, bartonella, Chagas disease, and toxocariasis. Although most of these diseases can be considered in the category of neglected diseases and are increasingly associated with slums and periurban locations, some of these diseases are also found in developed countries because of the relocation of human populations, global travel, and the movement of food and animal products as part of the rapidly expanding global food system (King, 2011).

Many zoonotic diseases are endemic in the developing world, which negatively impacts the health conditions and livelihoods of poor people. Because of their endemic nature, they tend to be under-reported and have been largely neglected by many funding agencies compared to emerging and re-emerging zoonoses and thus have been named as neglected zoonoses (Maudlin *et al*., 2009). Most developed countries have been successful in the control and elimination of neglected zoonotic diseases (WHO, 2011).

Mainly, tropical countries are more vulnerable for neglected diseases, which is why these diseases have been sometimes known as neglected tropical diseases. Since the neglected zoonotic diseases have lower priority in the health systems in many countries, they have silently triggered significant morbidity among rural people. The basic features of neglected zoonotic diseases are shown in Figure 1.

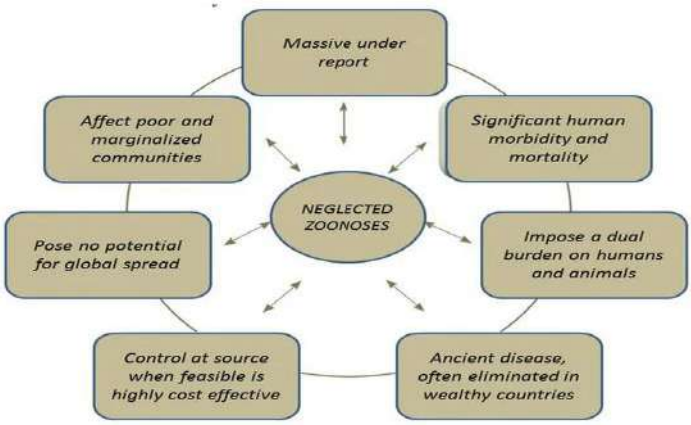

Figure 1: Basic features of neglected zoonotic diseases (Rahman *et al*., 2020).

6. CHALLENGES OF MANAGING ZOONOTIC INFECTIONS

According to the world health organization (WHO, 2025), the challenges of managing zoonotic infections can be summarized as follows:

- **Organizational:**

(i) Poor level of awareness among policy and decision-makers about the serious nature of zoonotic diseases; (ii) Insufficient information on the burden , trend and risks of zoonotic diseases; (iii) Inadequate resources and skilled manpower for control of zoonotic diseases; (iv) Presence of other competitive health priorities often taking precedence; (v) Lack of transparency of the countries to report emergence or occurrence of zoonotic disease for fear of repercussions; (vi) Weakness or absence of collaboration and cooperation between the public health, veterinary, agriculture and wildlife sectors; (vii) Inadequate collaboration and partnerships to harness resources to support the prevention and control programme of zoonotic diseases (viii) Absence of cross-talk within the health sector between the surveillance, clinical services and laboratory services departments. (ix) Breakdown or weakness of health infrastructures especially in countries with complex emergencies;

- **Diagnosis and detection:**

(i) Lack of integration of human and veterinary sector for exchange of epidemiological and laboratory surveillance data of the human and health sectors; (ii) Weak disease surveillance system and inadequate diagnostic capacities to detect zoonotic infections; (iii) Difficulties in international transfer of samples for logistic and economic reasons. (iv) Difficulties in onducting field investigation in remote areas where most of the emerging zoonotic outbreaks occur. (v) Weak cross-border collaboration, surveillance and information exchange between the countries (vi) Inadequate community engagement in the zoonotic control programme

- **Control and interruption of transmission:**

(i) Insufficient capacities of countries to plan, mobilize and implement appropriate control measures. (ii) High probability of nosocomial transmission of some of the newly emerging zoonoses in health-care settings; (iii) Poor application of strict barrier nursing and other appropriate infection control measures in health-care facilities. (iv) Lack of information on high-risk behaviours, including cultural and social factors, that are associated with risk of transmission of emerging zoonoses in the community; (v) Inappropriate or inadequate vector control operations. (vi) Lack or insufficient evidence on some of the public health control measures.

Antimicrobial resistance is a complicating factor in the control and prevention of zoonoses. The use of antibiotics in animals raised for food is widespread and increases the potential for drug-resistant strains of zoonotic pathogens capable of spreading quickly in animal and human (WHO, 2020).

7. PREVENTION AND CONTROL OF ZOONOSES

The unpredictable emergence of zoonoses, their potential to cause severe diseases in humans and animals, and the frequent absence of effective vaccines and antiviral treatments, make their containment difficult. Therefore, the ability to predict and prevent future outbreaks depends on recognizing, understanding, and mitigating this complex and multifactorial process, which involves the interaction of animals, environment, pathogens, and humans, creating a favorable environment for interspecies transmission. However, to effectively

achieve these actions, collaboration and transdisciplinary partnerships are required (Villarroel *et al*., 2023).

Prevention methods for zoonotic diseases differ for each pathogen; however, several practices are recognized as effective in reducing risk at the community and personal levels. Safe and appropriate guidelines for animal care in the agricultural sector help to reduce the potential for foodborne zoonotic disease outbreaks through foods such as meat, eggs, dairy or even some vegetables. Standards for clean drinking water and waste removal, as well as protections for surface water in the natural environment, are also important and effective. Education campaigns to promote handwashing after contact with animals and other behavioural adjustments can reduce community spread of zoonotic diseases when they occur (WHO, 2020).

7.1 One Health Approach for Zoonoses Control

One Health is defined as the collaborative effort of multiple disciplines—working locally, nationally, and globally—to attain optimal health for people, animals, and the environment (AVMA One Health Task Force, 2009). It is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The one health concept encourages collaborations among wildlife biologists, veterinarians, physicians, agriculturists, ecologists, microbiologists, epidemiologists, and biomedical engineers to ensure favorable health for animals, humans, and our environment (One Health, 2020).

The premise for strategic framework for control of zoonotic infections should lie on the concept of "One Health" approach which is a common coordination mechanism, joint planning, joint implementation, community participation, capacity building and joint monitoring and evaluation framework between the animal health and human health sector. According to Pieracci *et al*., (2016). the recommendations provided by one health approach to prevent and control zoonoses are: (1) developing "Zoonotic Disease Unit" for betterment of the human and animal health agencies; (2) developing national strategy for "Zoonotic Disease Unit"; (3) engaging leadership among multi-

sectoral researchers and relevant personnel to prioritize zoonotic disease research; (4) adopting veterinary public health policies with collaborators from other countries; and (5) reviewing the zoonotic diseases on a regular basis (2–5 years) to address the emerging and re-emerging diseases through regular surveillance, epidemiological implementations, and laboratory diagnosis.

In brief, the one health concept plays a significant role to address emerging and re-emerging zoonoses; to control the effect of zoonotic diseases among humans, animals, and environmental components; and to make the world free from threats of zoonotic diseases.

CONCLUSION

Many human infectious diseases seem to originate from animals. These pathogens cause diseases in animals and also pose a serious threat to humans. The majority of the human infectious diseases have animal origins. These pathogens do not only cause diseases in animals, but they also pose a serious threat for human health. Altered food habit, climate change, and environmentally unfriendly human operations have in many cases influence the emergence and reemergence of many zoonotic diseases because of the increased contact between humans and wild animals. The recent COVID-19 pandemic exemplifies the devastating impact of zoonosis on the human population. Because of the strong interrelatedness among animals, humans, and environment; research focusing on the one health approach need to be prioritized to identify critical intervention steps in the transmission of pathogens. Robust active surveillance targeting all components of the one health approach needs to be implemented to enable early and accurate detection of zoonoses, so that effective control measures could be taken.

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CHAPTER 4

RABIES IN INDONESIA

[1]Sirajuddien BIALANGI

[1]Universitas Negeri Gorontalo. Indonesia.

INTRODUCTION

Background

Rabies is an acute infectious disease that affects the central nervous system and is commonly known as "mad dog disease." This disease is caused by a virus that can be transmitted through bites or scratches from animals such as dogs, cats, and monkeys. Once clinical symptoms appear in animals or humans, the condition almost always leads to death. Rabies is a fatal disease if a person is infected, but it can be prevented through vaccination. Dogs are the most common source of transmission, with 99% of cases of rabies transmission to humans originating from dogs. This disease poses a significant health risk to humans, which is why dog vaccination and the prevention of bites are crucial steps in rabies control efforts. In the United States, although rabies can be found in various wild animals such as bats, raccoons, skunks, and foxes, rabies transmission from dog bites is relatively rare. Most cases of transmission occur through bat bites. Other animals such as wolves, coyotes, and minks can also be infected, while smaller animals like hamsters, squirrels, rats, and rabbits have not been shown to spread rabies. The World Health Organization (WHO), the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO), and the Global Alliance for Rabies Control (GARC) have collaborated in the global initiative "United Against Rabies" to develop a joint strategy for tackling rabies and achieving the goal of eliminating human deaths from rabies by 2030.

In Indonesia, the first recorded case of rabies occurred in 1884 by Esser in a buffalo, followed by Pening in 1889 in a dog, and Eileris de Zhaan in 1894 in a human. All of these incidents took place in West Java Province, and since then, rabies has spread to other regions in Indonesia (Ministry of Health of the Republic of Indonesia, 2011). Rabies has become a major zoonotic health issue in Indonesia, as the disease has been reported in 18 provinces with a high number of bite cases each year, reaching approximately 16,000 cases. No effective cure or treatment has been found for rabies patients, so the disease almost always leads to death in both humans and animals. This situation creates concern in the community, especially among those at risk of rabies bites (Fahmi U, 2008).

Rabies Situation

Rabies causes a significant number of deaths, especially among humans. The World Health Organization (WHO) estimates that around 50,000 people die from rabies each year worldwide. This number of fatalities is substantial, and it is believed that the actual figure may be even higher. In India, for example, approximately 25,000 people die from rabies annually (Wilde Hendry, et al.). This disease also causes deaths in various other countries, including those in ASEAN, though in relatively smaller numbers. Rabies has spread across the globe, except for the continent of Australia. In much of Africa and Asia, dogs serve as the primary hosts and are responsible for transmitting the virus to humans, causing fatalities. The most vulnerable age group is individuals aged 14-15 years. By 2005, only a few countries were officially considered rabies-free, most of which were small and had advanced societies. These included the United Kingdom, Ireland, Scandinavia, the Netherlands, Belgium, Switzerland, Japan, Australia, and New Zealand. In Asia, rabies-free areas included Hong Kong, Singapore, Japan, Brunei Darussalam, and Bahrain. The United States and Canada have managed to control rabies effectively by reducing cases in both domestic and farm animals. However, fully eradicating rabies in wild animals remains a challenge. In Latin America, cattle frequently contract rabies due to bites from vampire bats, which feed at night. It is important to note and be thankful that this type of bat does not exist in Indonesia.

1. INTRODUCTION TO RABIES DISEASE

1.1 What is Rabies?

Rabies is a viral disease caused by the rabies virus, a type of Rhabdovirus (ssRNA virus; genus Lyssavirus; family Rhabdoviridae). It can affect the central nervous system (CNS) of all warm-blooded animals and humans, with dogs and cats being the most commonly affected animals (Ferry Fong, 2010). According to the guidelines for planning and managing cases of suspected animal bites/rabies in Indonesia, issued by the Subdirectorate of Zoonosis Control, Directorate of Animal-Borne Disease Control, Directorate General of Disease Control and Prevention, Ministry of Health of the Republic of Indonesia (2011), the disease can be outlined as follows:

1.2 Pathogenesis

After the rabies virus enters through a bite wound, it can be detected in the area around the bite for up to two weeks. During this time, it mostly reaches the ends of the posterior nerve fibers without causing any functional changes. The incubation period varies, ranging from 2 weeks to 2 years, but generally lasts between 3 to 8 weeks, depending on the distance the virus needs to travel to reach the brain. Once the virus multiplies in central neurons, it moves toward the peripheral nervous system along the efferent nerve fibers, affecting both voluntary and autonomic nerves. As a result, the virus attacks almost every organ and tissue in the body, multiplying in tissues such as the salivary glands, kidneys, and others.

1.3 Clinical Symptoms

Prodromal Stage: Early symptoms include fever, malaise, nausea, and a sore throat lasting for a few days.

Sensory Stage: The patient experiences pain, a burning sensation, and tingling at the site of the bite. This is followed by anxiety, along with an exaggerated reaction to sensory stimuli. Muscle tone is affected, and there is dysfunction in the sympathetic and autonomic nervous systems, causing symptoms such as excessive sweating, salivation, lacrimation, pupil dilation, and more. During this excitatory stage, the disease reaches its peak, and a distinctive feature is the presence of various phobias, with hydrophobia being one of the hallmark signs. Muscle contractions in the pharynx and respiratory muscles may be triggered by sensory stimuli, such as blowing air on the patient's face, shining light into their eyes, or clapping hands near their ears. This stage may also lead to apnea, cyanosis, convulsions, and tachycardia. The patient's behavior becomes irrational, sometimes maniacal, though they may still show occasional responsiveness. These excitatory symptoms can persist until the patient dies, but as death approaches, muscle weakness often occurs, leading to flaccid paralysis.

Paralytic Stage: Most rabies patients die during the excitatory stage. However, in some cases, there are no excitatory symptoms, and instead, progressive paralysis of the muscles occurs. This is due to spinal cord damage, resulting in paralysis of the respiratory muscles.

1.4 Laboratory Examination

Rabies can lead to death within 3 to 5 days after the onset of symptoms, which often means serological tests cannot be conducted in time, even though the clinical diagnosis is usually clear. In cases with a longer disease progression, such as those with predominant paralysis symptoms that obscure the diagnosis, laboratory tests become very helpful in confirming the diagnosis. The rabies virus can be isolated from saliva, cerebrospinal fluid, and urine of the patient. However, virus isolation may not always be successful from brain tissue or these samples after 1-4 days of illness due to the presence of neutralizing antibodies. The Fluorescent Antibody Test (FAT) can detect the virus antigen in brain tissue, saliva, mucosal scrapings, cerebrospinal fluid, urine, skin, and corneal swabs.

However, FAT can also yield negative results if antibodies have already been formed. Virus isolation is also performed on the same specimens. In unvaccinated cases, neutralizing antibodies will not form until the tenth day of treatment, but after that, the titer will increase rapidly. A quick increase in titer can also be seen between days 6-10 after the onset of clinical symptoms in patients treated with anti-rabies therapy. This immune response characteristic in vaccinated cases can assist in diagnosis. Although clinical signs are typically pathognomonic, Negri bodies, when examined microscopically (Seller's method), may be absent in 10%-20% of cases, particularly in those who had been vaccinated or survived for more than 2 weeks.

1.5 Administration of Anti-Rabies Vaccine (VAR) and Anti-Rabies Serum (SAR)

The administration of treatment can be done using:

- Anti-Rabies Vaccine (VAR)
- Anti-Rabies Vaccine (VAR) and Anti-Rabies Serum (SAR)

Anamnesis:

- Contact/bite/lick
- Incident occurred in an area with rabies risk/infested/free zone
- Was the incident preceded by a provocative action or not?

- The animal that bit the person is missing, fled, and cannot be captured or killed
- The biting animal is dead, but there are doubts about whether it had rabies
- Has the person who was bitten received the anti-rabies vaccine before, and when?
- Has the biting animal received the anti-rabies vaccine, and when?

Physical Examination:

- Identification of the bite wound (local status).
- Low-risk wounds include licking on the skin, scratches, or abrasions (erosions and excoriations), and small wounds around the hands, body, and legs.

High-risk wounds include:

- Licking on intact mucous membranes, such as the mucous membrane of the eyes (conjunctiva), mouth, anus, and external genitalia.
- Licking or wounds above the shoulders (neck, face, and head).
- Bite wounds on fingers and toes (areas with dense nerve endings).
- Bite wounds on the genitalia.
- Deep or wide bite wounds.
- Multiple bite wounds.

  These two categories will determine the indication for administering VAR or VAR & SAR.

Others:

- Findings during animal observation
- Results of specimen examination from the animal
- WHO guidelines

For low-risk wounds, only VAR should be administered, whereas for high-risk wounds, both VAR and SAR should be given. In cases of contact (with saliva or the saliva of a suspected rabid animal or rabies-infected person), but with no wound, no direct contact, or no contact at all, there is no need to administer VAR or SAR. If there is contact with saliva on a harmless skin wound, VAR should be administered, or a combination of VAR and SAR should

be given if the contact occurs on a dangerous wound. Rabies, a deadly disease, has been feared worldwide for centuries. When an animal infected with rabies bites a person, the rabies virus is transmitted through the animal's saliva, traveling through the body to attack the central nervous system. Death typically follows after clinical symptoms appear. The disease can progress rapidly and be fatal within a few days. To date, rabies has not been found to spread from person to person. Theoretically, transmission could occur if a rabies-infected person bites a healthy individual, but no such cases of human-to-human transmission have been reported.

2. PREVENTION AND DIAGNOSIS OF RABIES

Prevention can be achieved by vaccinating pets, such as dogs, and capturing stray dogs to be kept in shelters where they are vaccinated against rabies. The United States has successfully reduced the number of rabies cases by vaccinating domestic animals. People at the highest risk of exposure are veterinarians, travelers visiting areas at risk of rabies, and laboratory workers handling rabies samples. Preventing contact with wild animals, not feeding or handling them, and avoiding the disposal of waste in places where wild animals may gather to search for food are important strategies to prevent rabies transmission and infection with this deadly virus. The diagnosis of rabies is conducted by a doctor. If a person exhibits symptoms, the doctor will perform a physical examination and ask questions to determine if the individual may have been exposed to an animal infected with rabies.

Diagnosis can be confirmed through laboratory tests, including blood tests and cerebrospinal fluid analysis to check for antibodies against the rabies virus. Skin biopsies and saliva tests can also be performed to detect signs of infection. One of the best diagnostic tests involves examining brain tissue from an animal that may be infected with rabies. Both positive and negative results can be obtained from the information provided by the doctor (U.S. Center for Disease Control and Prevention /CDC).

3. RABIES-TRANSMITTING ANIMALS

Rabies is an acute disease that attacks the central nervous system, caused by the rabies virus, which is primarily transmitted through bites from infected

animals, especially dogs, cats, and monkeys. Infected animals have rabies virus present in their saliva, making it a primary medium for the virus. Transmission from an infected animal to another animal or to humans can occur through bites or sometimes through licking by the infected animal. Once the rabies virus enters the body through a bite or lick, it travels along the nerves to the spinal cord and brain of the animal, eventually moving to the saliva through the nervous system and salivary glands. Dogs are the most commonly found animals infected with the rabies virus among rabies-transmitting animals.

According to Ferry Fong and Djap Hadi Susanto, rabies in dogs is still frequently reported in Latin America, Africa, and Asia due to the lack of widespread rabies vaccination among pets. Infected animals can either experience aggressive rabies (furious rabies) or paralytic rabies. In furious rabies, the animal becomes agitated and aggressive, eventually becoming paralyzed and dying. In paralytic rabies, the animal experiences paralysis from the start, either local or total. Although rare, rabies can also be transmitted through inhalation of contaminated air. There have been reports of two cases where explorers inhaled air in caves populated by bats, leading to rabies infection. Symptoms of rabies in animals include:

- ***Furious Rabies:*** For example, a dog may no longer obey its owner's commands, show fear of water, excessively drool, tuck its tail between its hind legs, attack and bite anything it encounters, experience seizures, become paralyzed, and usually die within 4-7 days.
- ***Dumb Rabies:*** The animal hides in dark, cool places, experiences brief seizures that may often go unnoticed, suffers paralysis, drools excessively, and dies quickly.
- ***Asymptomatic Rabies:*** The animal shows no symptoms of illness and may suddenly die.

The management of rabies-transmitting animals can be carried out through:

- Burying or incinerating livestock infected with rabies after the animals have been euthanized, with the prohibition of consuming them.
- Disinfecting animal enclosures, feeding bowls, and other equipment used for caring for animals infected with rabies.
- Quarantining animals suspected of having rabies.

- Culling wild animals in rabies-endemic areas.
- Disinfecting areas suspected of being contaminated with the saliva of infected wild or domestic animals after they have been eliminated.

4. FIRST AID FOR INDIVIDUALS WITH RABIES ANIMAL BITE WOUNDS

4.1 What Should Be Done for Animal Bite Wounds from Rabies-Transmitting Animals?

Panic may arise if a person is bitten by an animal suspected of transmitting rabies, especially if the animal is a dog infected with rabies and showing symptoms of the disease. No one wants to experience such an event. A bite from a rabies-transmitting animal is highly risky for contracting rabies if the animal is rabid. If not treated immediately, this could lead to the person's death. I recall a case from several decades ago when my sibling was bitten by a dog strongly suspected of having rabies. Thankfully, the treatment was prompt. The first step taken was to wash the bite wound, apply chili to the surface of the wound, and then promptly administer Rabies Vaccine (VAR) and Rabies Serum (SAR). According to guidelines from the Indonesian Ministry of Health (2011), any case of an animal bite by a rabies-transmitting animal should be treated immediately. To neutralize or kill the rabies virus entering the wound, the most effective action is to wash the wound with running water and soap or detergent for 10–15 minutes, followed by the application of antiseptics (such as 70% alcohol, Betadine, Savlon, etc.). Even if the wound has already been washed by the patient, the healthcare center (Puskesmas/clinic/hospital) should repeat the wound washing as described above. Bite wounds should not be sutured unless absolutely necessary. If suturing is required (in cases of situational necessity), Rabies Serum (SAR) should be administered according to the appropriate dosage via intramuscular injection. Additionally, consideration should be given to whether tetanus serum/vaccine, antibiotics to prevent infection, and analgesics are required.

4.2 Rabies Treatment in Humans

The treatment for rabies in humans, as outlined by the Subdirectorate for Zoonosis Control, Directorate of Animal-Borne Disease Control, Directorate

General of Disease Control and Environmental Health, Ministry of Health of the Republic of Indonesia (2011), is as follows:

- The patient should be referred to a hospital.
- Before referral, the patient should be given an infusion of Ringer's Lactate, NaCl 0.9%, or another suitable fluid. If necessary, anticonvulsants may be administered. The patient should be securely fixed during transportation, and attention should be paid to any irrational behavior, which may sometimes be manic, along with moments of responsiveness.
- Once at the hospital, the patient should be placed in a treatment room and isolated.
- Medical interventions and symptomatic or supportive medications should be administered, including antibiotics if needed.
- To prevent the potential transmission of rabies, healthcare providers should wear gloves, goggles, and masks while handling rabies cases. It is also advisable to securely fix the patient in their bed during treatment.

5. RABIES ERADICATION POLICY

According to the WHO recommendations for rabies control, the strategy should include 70% dog vaccination coverage and 30% elimination efforts. In Indonesia, the implementation of rabies control follows WHO guidelines, while also considering the local conditions and socio-cultural factors of the target areas. This approach involves vaccinating pet dogs and eliminating stray dogs through measures such as poisoning them, particularly in areas that are either infected or at risk of rabies. The administration of Pasteur treatment is carried out at a designated Rabies Treatment Center (Rabies Center) that meets the following requirements:

- Availability of trained doctors in handling rabies cases
- Availability of trained paramedics in handling rabies
- Functional cold chain system
- Continuous supply of VAR (Rabies Vaccine)

The use of strychnine poison in the implementation of the dog elimination program is commonly practiced, but it is often reported that obtaining this poison is difficult, and it frequently becomes ineffective. The

implementation of an elimination program using strychnine is not ideal due to the poor quality of the poison and the low mortality rate of the targeted animals, or dogs, which may not be effectively killed.

To address this issue, the Veterinary Research Center conducted studies on alternative poisons to replace strychnine by researching the toxicity of 13 different poisonous plants. These plants included lelatang leaves, rubber seeds, kapok seeds, castor seeds, ceremai tree bark, kipahit tree bark, kemalakian seeds, picung seeds, gadung tubers, tobacco leaves, strychnos nux vomica leaves, tuba roots, and tikusan leaves. The research found that among these 13 poisonous plants, the most toxic extracts for killing test animals (mice and dogs) were the extracts from kemalakian seeds (Croton tiglium) and picung seeds (Pangium edule) (Yuningsih, 2004). The lethal dose for dogs of both extracts was 5 ml per 3.5 kg of body weight, administered via forced feeding. The time to death for dogs with picung seed extract was 1.5 hours, while with kemalakian extract, it was 3.5 hours. However, when these extracts were mixed with food, the effective dose was only 0.5-1 ml, which did not cause death but only led to weakness, paralysis, and immobility in the dogs (Yuningsih, 2014). Based on this research, it is evident that the use of poison still remains ineffective as it requires a long duration (1.5 to 3.5 hours) and a significant volume (2 ml). Additionally, administering poison orally to dogs is difficult, further complicating the process.

Ferry Fong and Djap Hadi Susanto (2010) explain and categorize the control or prevention of rabies as follows:

- **Physical Environment:** Rabies can be found in various locations, both in urban and rural areas, where there are rabies-carrying animals such as dogs, cats, bats, rats, and foxes. Rabies is a disease that has a reservoir or breeding ground for the disease (environmental reservoir), which is the rabies-transmitting animals as the natural reservoir. Therefore, to control the spread of rabies, it is necessary to control the spread of these rabies-carrying animals. The steps taken include vaccination of rabies-carrying animals and the elimination of these animals in areas affected by rabies.
- **Non-Physical Environment:** The social and economic factors of a community in an area influence the control of rabies spread in that

region. The success of rabies control efforts is closely linked to the level of awareness, knowledge, and participation of the community. The concern of pet owners, the community, and local governments in areas affected by rabies also plays a crucial role in preventing the spread of rabies. Rabies spread often occurs because animals such as dogs and cats that are infected are allowed to roam freely without supervision. Additionally, vaccination of these animals is often not carried out due to a shortage of rabies vaccines from the government. Social and cultural practices also affect rabies control, because if the large population of dogs and cats is not properly managed (e.g., not vaccinated) and is allowed to roam freely, this increases the risk of rabies transmission in endemic areas.

6. RABIES EDUCATION

Health education plays a crucial role in the prevention and management of dog, cat, and monkey bite cases, as it helps raise public awareness and equips them to handle issues related to pet ownership (such as dogs, cats, monkeys, etc.), as well as actions to take when dealing with bites from these animals and how to respond to individuals who are bitten. The efforts are carried out in an integrated manner between public health education and the Animal Husbandry Department, with involvement from the rabies control division of health promotion in both the District/City and Provincial levels. Community Health Centers (Puskesmas) collaborate with the Health Departments of District/City and Provincial governments to conduct rabies education during various events and in different settings, adjusting to the local situation and conditions.

Education activities can be delivered through various media, such as leaflets, banners, billboards, print/electronic media, radio, public stages, and direct counseling in both formal and informal meetings. Direct counseling is considered the most effective method to improve public knowledge, attitudes, and behaviors regarding the prevention and control of rabies. However, this direct approach has its limitations, such as reaching the entire population that requires education. Despite these challenges, direct education is still the most effective method because it allows for discussions, and any questions regarding rabies-related issues can be addressed and resolved immediately.

CONSLUSION

Rabies is still a major public health concern in Indonesia, primarily due to transmission through dog bites. Although preventive measures such as vaccination and public awareness campaigns exist, their implementation remains inconsistent across regions. The high fatality rate of rabies, once symptoms appear, highlights the critical need for early intervention, proper wound care, and timely administration of vaccines and antiserum.

Effective rabies control requires a comprehensive strategy, including mass dog vaccination, stray animal management, community education, and improved healthcare access. With coordinated efforts between health authorities, veterinary services, and the public, Indonesia can significantly reduce rabies cases and work toward the global target of eliminating dog-mediated human rabies deaths by 2030.

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ISBN: 979-8-89695-148-3