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Original Article

Application of Animal Models to Study Infectious Diseases

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Abstract

Infectious diseases, which are caused by microorganisms such as bacteria, viruses, fungi, or parasites, can be contracted from other people, the environment, animal contact, or insect bites. Infectious diseases are becoming escalating concerns, mainly due to increasing antibiotic resistance. These disorders remain one of the primary causes of human mortality. Due to the lack of human data on new emerging diseases, ethical values, and the lethal risk of these pathogens, animal models are often recommended for experimental research on these diseases. According to the similarities between humans and animals in physiology, genetics, anatomy, availability, ease of handling, and production rate, scientists evaluate different medical problems in animal models before applying their findings to humans. According to the recent advent of the isolation of novel microorganisms, researchers must challenge the infectious ability of microorganisms in the biological system of animal models. An infectious disease animal model attempts to mimic the host-pathogen interaction. Accordingly, a disease model is defined by both the host and pathogen combination. In this review article, we aimed to discuss various animal models established for studying different infectious diseases.

Keywords: Infectious Disease; Animal Models; Microorganisms; Pathogen

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Introduction

Microorganisms such as bacteria, viruses, fungi, and parasites cause infectious diseases, which can be contracted through contact with other people, the environment, animal contact, or insect bites. Infectious diseases are becoming escalating concerns, mainly due to increasing antibiotic resistance. According to the statistics in

a study by Hamer in 2019, the sepsis incidence rate continues to expand and contributes to over 5.3 million deaths annually worldwide (1). Infectious disease remains one of the primary causes of human mortality. A study from 1980 through 2014 includes a report that 5.4% of the overall mortality rate is caused by infectious diseases (2). Although the World Health Statistics between the years 2000 and 2016 show a relative reduction in the rate of the global mortality of infectious and parasitic disease (from 16.4% in 2000 to 9.7% in 2016), pathogenic organisms continue to cause a high prevalence of contagious diseases in human populations (3). While death rates caused by pathogens with drug-resistant strains had not decreased, human vaccine development resulted in a reduction in mortality (2). More than 1400 pathogens, including different species of viruses, bacteria, fungi, protozoa, and helminths, can cause human diseases. Thirteen percent of them were considered emerging and reemerging pathogens that cause Emerging and Re-Emerging Infectious Diseases. Emerging Infectious Diseases are infections that have recently emerged in a population, whereas re-merging infectious Diseases have previously existed but are rapidly increasing in incidence or geographic range. Seventy-three percent of remerging pathogens were considered zoonotic (4). Due to the lack of human data on new emerging diseases, ethical values, and the lethal risk of these pathogens, for experimental infectious disease research, animal models are frequently recommended (5, 6).

Due to the complex relationship between the systemic responses of the host and the microorganisms, using animal models as a biological system is more beneficial than other experimental techniques, including cell culture and isolated organs as a biological model. Using a biological system is essential to study the mystery of host-pathogen interactions, particularly those eventuating in infectious diseases **(Figure 1)**. Animal models, particularly unconventional ones, have been used to study the emergence and progression of infectious diseases over the last two decades. The significant advancements have further bolstered the utilization of animal models in research on infectious diseases.

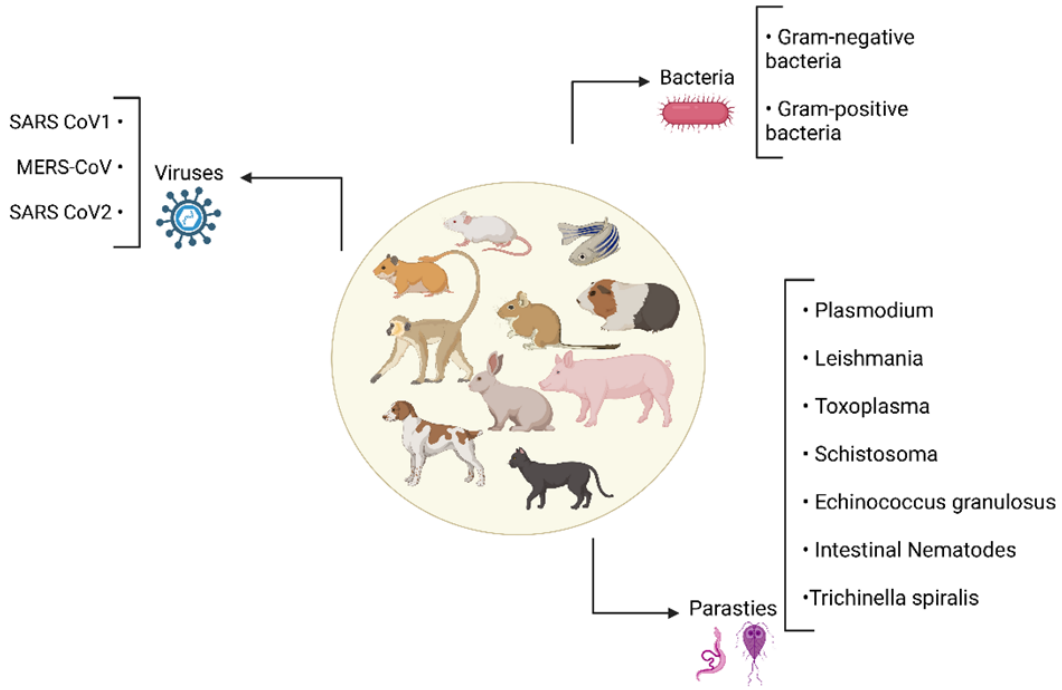

Figure 1. Different animal models as biological systems are used to study host-pathogen interactions, particularly those that occur in infectious diseases. Created with BioRender.com

The systemic interaction between the host and pathogens makes the *in vivo* studies a much more reliable technique in experimental research, especially the experiments that investigate the efficiency of vaccines and different therapies (5, 7-9).

There are several items for a researcher to design an appropriate animal model for a study, which consist of understanding both general features of various models and species of animals and characteristics of infectious diseases (10).

This study aims to review various animal models that can be used as tools to investigate different infectious illnesses.

The significant role of animals in modeling infectious diseases

The burden of infectious diseases on the public health system has significantly decreased over the past century due to advancements in the development of vaccines, antibiotics/antivirals, and infection control techniques. Infectious agents still have a high morbidity and mortality rate in human populations. The rise of drug-resistant organisms, bioterrorism, global trade, and travel rates has increased the rate of infectious diseases, necessitating the development of novel methods to control the spread of pathogens. To combat these threats, constant innovation in the form of new vaccines and therapeutics is imperative. However, the creation of novel infection control methods necessitates monitoring the biology of the target pathogen and the disease pathogenesis, as well as the development of suitable testing methods for the safety and efficiency of novel medications or vaccines [9]. The similarities between humans and animals in physiology, genetics, anatomy, availability, ease of handling, and high production rate encourage scientists to investigate different diseases in animal models before applying their findings to humans (11, 12). An animal model of disease aims to mimic the host-pathogen interaction. Therefore, both the host and pathogen combination define a disease model. Reliance on the survey of the results of animal model experiments depends on understanding the exact relation between the model and human diseases (6).

Animals were used as models of human physiology in observational studies that originated in ancient Greece, 6th BCE. Alcmaeon of Croton used dogs as animal models in a study to determine brain intelligence and sensory integration. Aristotle studied embryogenesis and ontogeny in chicks in the 4th century BCE (12). Scientists pursue using animal models as a research tool to understand human physiology and pathology better (13).

In 1902, William Castle began breeding mice for genetic studies. Seven years later, Clarence Little began breeding mice to eliminate variation, and the use of animals became more experimental than observational (12). Considering the importance of the genetic background of animal models in research, scientists started using rodents and non-rodent animals such as rabbits, dogs, cats, fruit flies, zebrafish, and so on for different experiments. Since 1970, the role of rodents, especially mice, as animal models has increased (12).

Investigators face the challenge of selecting the most informative species for an animal model, which requires careful evaluation of various factors such as financial feasibility, previous experimental outcomes using a specific species, biological characteristics of the species, and available imaging and molecular techniques that can be used with the species (12).

The role of animal models in studying infectious diseases to provide much more valid information started over a century ago (14). Using animal models is vital in infectious diseases for studying the immune response to the infection, biochemical, behavioral, and physiological changes, vaccine development, safety testing, and virulence testing for diagnosis and treatment purposes (14).

There are many objectives to decide on using animal models in infectious disease studies, such as obtaining information that helps define the appropriate dose range, determining the rate of disease onset, and conducting pilot studies before embarking on large-scale experiments (15).

The development of protective and safe medicine and vaccines depends on choosing models of diseases responsive to the agent (6).

Animal models of human bacterial diseases

Bacteria have caused some of human civilization's most lethal diseases and widespread epidemics. Some of these deadly diseases, such as tuberculosis, typhus, plague, diphtheria, typhoid, cholera, dysentery, and pneumonia, have claimed

Table 1. Animal Models of Bacterial Diseases

Pathogenic Bacteria(63)	Disease	Animal Species	Significant Features	Application	Ref.
Gram-negative bacteria					
Escherichia coli, Uropathogenic Escherichia coli (UPEC)	Gastroenteritis, urinary tract infections, neonatal meningitis, UTIs	Mouse(C57BL/6)	Similarities in immune responses to UTI	clarification of UTI pathogenesis, advanced potential treatment, infection prevention strategies	[25]
Salmonella enterica	Gastroenteritis	1-week-old White Leghorn chicks	having very few organisms show no disease or gross evidence of systemic effects	highlight important differences in systemic and intestinal colonization levels	(44)
		Salmonella-resistant CBA/J mice	Resistance to development of systemic disease following infection (they possess a wild type NRAMP1G169 allele (Natural Resistance Associated Macrophage Protein 1))		
Shigella dysenteriae	Bacillary dysentery	Monkey Shigellosis or Dysentery	-similarities of the course and pathology of shigellosis in monkeys to human dysentery -identical progression and appearance in mucosal lesions	To study experimental shigellosis	(51)
		Cynomolgus Monkeys (Macaca fascicularis)	-Occurrence of shigellosis in monkeys naturally - similarities in symptomatology, shedding of the organism and histopathologic findings -has the potential to advance the design of novel Shigella vaccines	for studying pathogenesis, infection-derived immunity, and, likely, vaccine efficacy	(52)
		piglet	Mimic symptoms and manifestations (severe diarrhea, dehydration, anorexia, bacterial colonization, cellular invasion, mucosal inflammatory reaction, and damage to the mucosa specifically) targeting the large bow	providing a useful tool with which to compare vaccine candidates for immunogenicity, reactogenicity, and response to challenge; investigating the role of virulence factors; and testing the efficacy of microbial agents	(53)
Pseudomonas aeruginosa	Opportunistic infections, swimmerï¿½s ear, hot tub itch, cellulitis, pneumonia, UTIs(28), more	Mouse(C57BL/6)	Similarities in immune responses to UTI	clarification of UTI pathogenesis, advanced potential treatment, infection prevention strategies	[25]
Vibrio cholerae	Asiatic cholera	infant (suckling) mouse	thought to be due to the relative immaturity of the immune response	Identification of several important virulence factors such as accessory colonization factors, a hemagglutinin, several metabolic proteins, identification of toxin-coregulated pilus (TCP), virulence of *V. cholerae* (competition assay between mutant and wild type)	(40, 41, 64-70)
Bordetella pertussis	Whooping cough	female Sprague-Dawley rats	low husbandry costs, availability of animals, ease of use	Studying pathogenesis, host response during pertussis, host response during pertussis, vaccine-mediated immunity	(71)
		newborn piglets	anatomical and immunological features of the respiratory tract more similar to humans than rodents, and similar transportation pathway for the secretion of immunoglobulin A (IgA) and IgG (transferred via colostrum and milk to the offspring)	Investigation of the roles of both maternal and mucosal immunity in disease protection against pertussis to design new vaccines for early life protection	(72)
		infant rhesus macaques	Similar clinical spectrum to humans (Low-grade fever, paroxysmal coughing, leukocytosis, a long-lived anti pertussis toxin (PT) antibody response, protection against subsequent challenge, and transmission)	To study pertussis pathogenesis and evaluation of vaccine candidates	(73)

Table 1. continued

Haemophilus influenza	otitis	BALB/c mice	near-complete genetic information for the opportunity for genetic manipulations and advanced molecular biological procedures	to investigate the local and systemic reactions and to compare these reactions with those commonly found in children and rats	(74)
Helicobacter pylori	Gastric and duodenal ulcers	non-human primates, germ-free or barrier-raised piglets, germ-free dogs, and laboratory-raised cats, *H. felis* mouse	oral immunization	susceptibility of the *H. felis*-infected mice to antimicrobial agents is very similar to that found in the H. pylori-infected human.	(42)
Campylobacter jejuni	Gastroenteritis, enteropathy, and diarrhea	Sigirr-/- mice	Sigirr-/- mice development of significant intestinal inflammation in response	Study pathogenesis, host immunity to this enteric pathogen	(55)
		zinc deficient mouse	Similarities in primary clinical manifestation in humans of bloody diarrhea and growth failure, inflammatory histopathology and biomarker expression, and fecal shedding that is sustained over at least 2 weeks	lead to therapeutic treatments and vaccines	(56)
Neisseria gonorrhoeae	Gonorrhea	hCEACAM1 transgenic C57BL/6 mice	Binding to CEACAM receptors (carcinoembryonic antigen-related cellular adhesion molecule) mediate bacterial entry	Establishment of animal model of gonococcal infections	(58)
		estradiol-treated BALB/C mouse model	It's a valuable system determination of the importance of gonococcal factors that mediate evasion of host innate effectors, host gonococcal adaptation to hormonally driven host factors in females' application of Animal Models to Study Infectious Diseases	Examination of bacteria mechanisms to regulate host immune response	(59)
Neisseria meningitidis	Meningococcemia and meningitis	Modified B10.M mice by administration of human holo-transferrin upon bacterial challenge (with meningococci grown under iron restriction to up-regulate the expression of the transferrin receptors)	potential for the investigation of infection systems like human	evaluation of outer membrane vesicle (OMV) vaccine-induced protection by using survival and bacteriemia parameters, and the importance of Transferrin receptors (Tbps) in protection induced by OMV vaccines	(75)
		humanized mouse model grafted human skin	immunocompromised background results in the allogenic transfer of human immune cell populations, capability of defining adhesive properties of Tfp involved in vascular colonization	making new possibilities for the development of novel treatment targets	(61)
		ICR mouse	greatest antibody dose-response range	determining most immunogenic vaccine formulations to enhance clinical examination	(62)
Brucella abortus	Undulant fever	Guinea pigs (Cavia porcellus)	-similarity of immunologic components and reactions to humans (complement and delayed-type hypersensitivity reactions) -capability of infection by different routes of administration (subcutaneous, conjunctival, i.p., intranasal, i.v., vaginal, oral, or cutaneous scarification) and development of systemic disease - highly susceptible to infection -similar disease symptoms such as fever and languidness to human -similarities to human placentation Disad: Differences in the persistent yolk sac and sub placenta	-investigating the efficacy of vaccine candidates, and growth characteristics of Brucella - estimating the pathogenesis of aerosol inoculation	(76)

Table 1. continued

			- large, expensive to house, and having fewer reagents available to analyze immunological events		
		Mice	Ads: availability of reagents and genetic mutants Disad: -less biologically relevant route of administration (i.p., inoculation) than inhalational or oral routes of administration -resistant to Brucella infection and requirement of higher doses (>10 ^{4}) inoculation via aerosols or i.p. to demonstrate systematic disease - Failure to develop a fever in response	Investigation of the pathogenesis of infection, reproductive and osteoarticular disease	
		non-human primates (NHPs) rhesus macaque (Macaca mulatta)	Ad: Similar disease manifestations such as fever, reproductive failure, and colonization of the reticuloendothelial organs Mimicry of a natural route of administration, such as aerosols or ingestion of Brucella-laden milk Disad: limitation of the robustness of the statistical analysis (because of high husbandry and veterinary care expenses)	study of brucellosis	
		rats	low disease susceptibility and transient nature of infection	study of brucellosis	
		rabbits	low disease susceptibility and transient nature of infection	study of brucellosis	
Chlamydia trachomatis	Chlamydia,lymphogranuloma venereum, trachoma	Female Mouse	small size, ease of handling, availability in sufficient amounts, and low cost susceptibility to infection Disad: does not have the capability of developing chronic infections - upper genital tract pathology can barely be produced due to vaginally infection with C. trachomatis -inbred	studying genital chlamydial infections	
		nonhuman primate models (pig-tailed macaque model)	- similar anatomy, physiology, menstrual cycle, and vaginal microflora of the female reproductive tract to human - relatively quiet character and an ideal size - susceptibility to genital tract infection Disad: ethical considerations and practical disadvantages (high costs, adequate facilities, and expertise)	studying genital *C. trachomatis* infections	
		pig	- physiologically and genetically similar to humans -several similar genes to humans expressed in porcine female reproductive tissues -similar immune responses to humans - susceptibility to genital tract infection -appropriate for usage as laboratory animals practically and ethically Disad: more expensive and more complicated than using rodents	for screening vaccine candidates against genital chlamydial infections	
Rickettsia rickettsii	Rickettsiosis: typhus, RMSF	guinea pig (Cavia porcellus)	Ad: longer life span, ease of handling, and more extensive blood volume Similarities in physiology, genetics, and function of human immune systems Disad: larger size and longer gestation period	studying Rocky Mountain spotted fever - demonstration of prophylactic anti-rickettsial antibiotics	(77)
Treponema pallidum	Syphilis	C57BL/6 mice	- the capability of developing a persistent infection Disad: no obvious external lesions	clarification of the pathogenic mechanisms of this bacterium help to design a new research model of *Treponema pallidum*	(78)
Borrelia burgdorferi	Lyme disease	DBA/1 murine	allowing direct comparison of murine models of CIA (Collagen-induced arthritis) and B. burgdorferi infection	analysis of experimental Lyme disease, including arthritis	(79)

Table 1. continued

Gram-positive bacteria					
Staphylococcus aureus	Food poisoning, wound infections, toxic shock syndrome, biomaterial-associated infection (BAI)	A Zebrafish Embryo Model	optical transparency, low cost, and control of an immune system highly	studying BAI progression and host-pathogen/ host-material interactions	(45)
		fluorescent transgenic mice	availability of reagents, reasonable cost, short duration of pregnancy, amenability of the mouse to drug and gene therapy, and immune response and physiology similarities	to study unique immune mechanisms of disease, microbial pathogenesis, vaccines, and therapeutic interventions	(50)
Streptococcus pneumoniae	Pneumonia, otitis media, meningitis	baboons (Papio cynocephalus)	Similarities in clinical characteristics, organ involvement, disease severity, inflammatory response, and progression of the disease	test vaccines and treatments, measure biomarkers to diagnose pneumonia, and predict outcomes	(47)
		swine	mechanical ventilation, and circulatory shock, severe pneumonia can develop in just 8–12 h, using a clinically relevant, penicillin- and macrolide-resistant S. pneumoniae serotype	evaluation of pathogenesis, the effects associated with macrolide resistance, and the development of new diagnostic strategies and antibiotic or complementary therapies	(48)
Bacillus anthracis	Anthrax	silkworm (Bombyx mori) (injection of different cell numbers of B. anthracis Sterne strain 34F2 into silkworm larvae hemolymph and observation of the survival)	Innate immunity is similar to human antimicrobial peptide production (activation of several signaling cascades)	confining the roles of unknown genes in the virulence of B. anthracis, generating novel therapeutic options	(80)
Bacillus cereus	Food poisoning	porcine	anatomical and physiological similarities (including the digestive and the cardiovascular system) to humans the capability of analyzing toxicokinetics for other toxins similar to cereulide (such as mycotoxins)	Decoding the routes of cereulide translocation within the body and the routes of cereulide excretion after oral exposure recognizing the impact of organs and tissues fast and reliable cellulite diagnosis by appropriate clinical specimens	(81)
Clostridium perfringens	Food poisoning, gas gangrene, uterine infections	Rabbit	-potential for ensuring CPE for enterotoxigenic C. perfringens type A is essential to produce enteric disease by utilizing rabbit intestinal loops -susceptibility of rabbit colon to the action of purified CPE (fluid secretion and mucosal damage) - CPE dose- and time-dependent mucosal necrosis in small intestinal loops	-to examine the effect of CPE (*Clostridium perfringens* endotoxin) in the small intestine (significant damage to the mucosa of the jejunum and ileum) - To study the binding of CPE to extraintestinal tissues to define systemic changes in patients with enterotoxigenic *C. perfringens* type A-associated disease	(82)
		Mouse	inoculation CPE into intestinal loops was found to bind and form CH-1-like complexes in the liver and kidney - CPE dose- and time-dependent mucosal necrosis in small intestinal loops	-to study the intestinal and systemic effects of CPE - indication result of death observed in constipated human patients with CPE-positive *C. perfringens* type A infection is due to absorption of CPE from the intestine	
		Rat	CPE produces respiratory difficulty, ECG alterations, an increase of liver enzymes (GPT, GOT, and LDH), hyperkalemia (the result of the cytotoxic action of CPE on hepatocytes), and death	-study *in vivo* effects of CPE	
Clostridium botulinum	Botulism, infant botulism	rabbit	- Capability to simulate a beneficial antitoxin treatment after the beginning of symptoms of botulism - Similarities with human disease pathophysiology and the stereotypical differential	- To indicate the prevention of symptom beginning and the lessening of disease duration by a useful drug - To test other possible	(83)

Table 1. continued

			pattern of antitoxin treatment efficacy	anti-BoNT compounds (in the late stages of the disease) - Development of a novel model of chronic botulism and spontaneous recovery	
Clostridium difficile	Antibiotic-associated diarrhea, pseudomembranous colitis	C57BL/6 mouse	- ability to reduce colonization resistance and lessen infection with a toxigenic strain of *C. difficile*	-analyzing disease pathogenesis, testing new treatments, and clarifying mechanisms of protective immunity, establishing a conventional mouse model of antibiotic-induced CDAD (C. difficile–associated disease) -analyzing critical elements of CDAD, including involvement of the entire colon, pseudomembrane formation, variable severity of disease, regeneration of disease after vancomycin therapy, and the development of resistance to regressive disease	(84)
		Syrian hamsters	demonstration of the possible utility of toxin-deficient strains of C. difficile as a preventive measure against CDI	for understanding multiple aspects of the pathogenesis of CDI	(85)
Corynebacterium diphtheriae	Diphtheria	Caenorhabditis elegans	cost-effective, adaptable model to study C. diphtheriae virulence	Analyzing *C. diphtheriae* virulence factors and pathogenesis	(86)
Mycobacterium tuberculosis	TB (tuberculosis)	Guinea pigs	Two models: the long-term model (monitoring for the disease after infection), short-term model (determining the ability of a vaccine to reduce organism burden)	Study the pathology of the disease, Analyze the capacity of new vaccines	(87)
Mycobacterium leprae	Leprosy	Zebrafish	-Similar optimum growth temperature to *M. leprae* - a facile, genetically susceptible to the disease animal	verification of the role of interaction of innate and adaptive immune loss in leprosy granuloma formation and function	(88)
		Armadillo	-susceptible to high levels of natural infection	-To comprehend pathogenesis and focus on the dynamics of the natural infection among free-ranging armadillos and the essence of these animals as a reservoir for human infection -for modeling new diagnostic techniques for early detection of leprosy	(89)

many lives **(Table 1)** (16). Pneumonia, tuberculosis, and diarrhea were the three leading causes of death at the turn of the twentieth century. Decreasing morbidity and mortality due to infectious diseases during the 20th century led to continuing research into the treatment and control of these infectious microbes (16).

According to the recent advent of the isolation of novel microorganisms, researchers must challenge the infectious ability of microorganisms in the biological system of animal models. A wide range of identification methods have promoted the discovery of novel bacterial species and have resulted in a rapid increase in identified bacterial taxonomy (17, 18). Using an animal model to study the pathogenicity of isolated bacteria will provide valuable information for understanding the dynamics of microorganisms in various aspects of host or environmental sources and their pathogenic properties (19, 20). The convenience

Table 2. Animal Models of Pathogenic Viral Diseases

Pathogenic viruses	Disease	Animal Species		Significant Features	Application	Ref.
(SARS CoV1)	Severe acute respiratory syndrome coronavirus infection	Mouse	Young, 6- to 8-week-old BALB/c mice	-viral replication detected in the lungs and nasal turbinate (peak on day 2) -no clinical signs, aside from reduced weight gain, and have little to no inflammation within the lungs (pneumonitis) -	-To model the epidemiological finding -To develop a mouse model of *SARS-CoV* infection	(91, 94, 98)
			C57BL/6 (B6)	generates reduced weight gain and viral replication in the lungs (peak on day 3)		
			Old BALB/c mice 13–14 months	- show weight loss, hunched posture, dehydration, and ruffled fur on day 3–6 postexposure -demonstration Interstitial pneumonitis, alveolar damage, and death	-recognition age-dependent virulence observed in humans	
			129SvEV mice	-develop bronchiolitis, with peribronchiolar inflammatory infiltrates and interstitial inflammation in adjacent alveolar septae - Viral replication and disease (resolves by day 14 postexposure)	-To develop a mouse model of *SARS-CoV* infection	
			Beige, CD1−/−, and RAG1−/− mice	- similar outcomes to infected BALB/c (viral replication, timing of viral clearance, and a lack of clinical signs)	-To develop a mouse model of *SARS-CoV* infection	
			STAT1 KO mice	severe disease (weight loss, pneumonitis, interstitial pneumonia, and death)	study of pathogenicity, pathology, and evaluation of vaccines	
			transgenic mice	-expression of human ACE2 - 100% mortality - severe lung and brain infection	-identification of the correlation between severity and level of hACE2 expression	
		Syrian golden hamsters (strain LVG)		-virus replication in nasal turbinates and lungs, resulting in pneumonitis - no obvious signs of disease - decrease in nighttime activity -observation of limited mortality - detection of virus in spleen and liver (not observed damage in these tissues) -an excellent model for *SARS-CoV*	suitable for immunoprophylaxis and treatment studies	
		ferrets		-clinical observations, including lethargy, fever, sneezing, and nasal discharge -detection of *SARS-CoV* in pharyngeal swabs, trachea, tracheobronchial lymph nodes, and high titers within the lungs - Mortality around day 4 - most similar to human SARS, albeit with a shorter time course - observation of Severe hepatitis in vaccinated ferrets with antibody enhancement in liver		
		NHP		**General features:** -very mild infection - replication within the lungs and diffuse alveolar damage	-development models of *SARS-CoV* infection	
			rhesus macaques	- little to no disease -only have mild findings upon histopathological analysis		
			cynomolgus macaques (cynos)	- some cynos no illness but others have rash, lethargy, and respiratory signs and pathology		
			African green monkeys (AGMs)	- no overt clinical signs but diffuse alveolar damage and pneumonitis -viral replication in lungs		
***MERS-CoV* virus (genus Betacoronavirus and subgenus Merbecovirus)**	Middle East respiratory syndrome (MERS) CoV infection	BALB/c and B6		-transduced with an adenoviral vector expressing hDPP4 (Ad5-hDPP4) -observation of replication of *MERS-CoV* associated with interstitial pneumonia and viral antigen in the lungs -robust infection with severe respiratory and generalized illness (led to death within days after infection) -High viral titers from different organs - low cost, small size, and availability -capability of genetic manipulation	-evaluation of *MERS-CoV* infection models -to determine the role of immune effectors in the disease	[84, 85]
		Syrian hamsters (Mesocricetus auratus)		Disad: lack of demonstration of clinical signs of disease, weight loss, or changes in body temperature - lack of observation lesions and viral RNA in any examined tissue - lack of overexpression of Mx2 gene expression, an indicator of an innate immune response - lack of viral replication - not seroconvert	- not really good to be MERS model	[84, 85]
		Ferrets (Mustela putorius furo)		- susceptible to several respiratory pathogens - not seroconvert - detection viral RNA in nasal and oropharyngeal swabs up to 2 dpi	-study of respiratory viruses	[84, 85]
		Rabbits(Oryctolagus cuniculus)		- free of clinical signs of disease, and changes in body temperature or weight - No awful pathology at necropsy, microscopically mild to moderate rhinitis, and focal mild to moderate necrosis in nasal turbinates -seroconversion - homology sequence between rabbit and human DPP4	- evaluation *MERS-CoV* infection models	[84, 85]
		Transgenic hDPP4 mice		-detection of viral RNA in brain, heart, spleen, and intestine - Observation of gross lesions (red to dark red discoloration and multifocal consolidation) in lungs	- evaluation *MERS-CoV* infection models	[84, 85]

Table 2. continued

			-overexpression of antiviral cytokines, proinflammatory cytokines and chemokines - susceptible to severe disease -observation of lethality		
		Nonhuman primate Rhesus macaques	- first described MERS animal model - Koch's postulates assuring *MERS-CoV* as the causative agent of MERS -observation of clinical signs, body temperature, reduced appetite, increased respiratory rate, cough, piloerection and hunched posture -detection of viral RNA (in lungs, conjunctiva, nasal mucosa, tonsils, pharynx, trachea, bronchus and mediastinal lymph nodes -Overexpression of proinflammatory processes associated genes -physiological and immunological similarities of NHPs to humans -high costs, limited availability and individual variation	-study pathogenesis of coronavirus infection -to evaluate coronavirus vaccine	(99) [84, 85]
		Dromedary camels (Camelus dromedarius) aged between 2 and 5 years old	-observation of mild clinical signs (rhinorrhea) -positive infection in oropharyngeal and nasal swabs -No virus detection in any other tissue except for lymph nodes -characterization of respiratory tract lesions as mild to moderate acute intraepithelial and submucosal inflammation	- evaluation *MERS-CoV* infection models	[84, 85]
***SARS CoV2* infective disease (COVID19) pandemic**	*COVID-19*	mouse (Mus musculus)	Disad: lack of appropriate receptors to initiate viral infection	-evaluation of vaccines and antiviral agents, and some share features with the human disease -no mouse model recapitulates all aspects of *COVID-19* in humans (unusual features such as the pulmonary vascular disease and hyperinflammatory syndromes)	(96)
		genetically modified mice	-Expression of human ACE2 -highly lethal encephalitis -less severe neurological infection - some of them show severe pneumonia (when brain infection is not severe) -thrombosis and anosmia after infection	-to study vaccination -to study pathogenesis	
		Collaborative Cross model of genetic diversity	-a panel of recombinant inbred mice with expanded susceptibility to viruses that normally do not cause disease in laboratory mice -combination of human immune system and ACE2 expression -virus disease susceptibility	-to study efficacy of vaccines and therapies -identify mechanisms of pathogenesis and genetic loci that determine susceptibility -exploration of an expanded range of *SARS-CoV-2* phenotypes in mice	
		Syrian hamsters (Mesocricetus auratus)	-susceptible to infection with *SARS-CoV-2* -shows some of the demographic differences of *COVID-19* in humans -Additional signs of morbidity (lethargy, ruffled fur and a hunched posture) -aged hamsters and male hamsters develop a more severe disease than young and female hamsters -high levels of virus replication -histopathological evidence of disease -intestine demonstration viral antigen expression (associated with severe epithelial-cell necrosis, damaged and deformed intestinal villi, and increased infiltration of the lamina propria by mononuclear cells) - Expression of chemokines and cytokines in the lungs Disad: lack of research tools in comparison to mice	-show mild-to-moderate disease with progressive weight loss that starts very early after infection -evaluation of therapeutic agents and vaccines -to study transmission -confirmation of YF17D-vectored *SARS-CoV-2* vaccine	
		Ferrets (Mustela putorius furo)	- undetectable or mild clinical manifestation (may include lethargy, nasal discharge, wheezing, oropharyngeal build-up of mucus, sneezing, and loose stools) -infection by small-particle aerosols (similar to disease) -elevated body temperature - Minor alterations in hematological parameters - Shedding of *SARS-CoV-2* virus (in nasal and oropharyngeal swabs) -virus replication (respiratory and gastrointestinal tracts) - Efficient transmission	-useful for transmission studies -to study the efficacy of mucosal vaccines and therapeutic agents	
		Non-human-primate models (rhesus macaques (Macaca mulatta), cynomolgus macaques (Macaca fascicularis) and African green monkeys	- viral replication for 7–14 days (including both viral RNA and infectious virus) in both the upper and lower respiratory tract -mild clinical disease -demonstration of natural protective immunity	- study COVID-19 vaccine -highlighting the importance of age criteria in selecting animal model	

Table 2. continued

	(Chlorocebus aethiops))	-show radiographic abnormalities -aged macaques shed virus from nose and throat for longer periods of time than young adult macaques -Higher viral loads in aged rhesus macaques	
	mink (Neovison vison)	- susceptible to natural infection with SARS-CoV2 -demonstration moderate respiratory signs (including labored breathing, and some mink died as a result of infection) -higher viral in the throat swabs than in the rectal swabs - difficult to handle under laboratory conditions	-to study applications for virus ecology and the evolution of the current pandemic
	Cats (Felis catus)	- highly susceptible to infection with *SARS-CoV-2* -able to transmit the virus to naive cats - virus replication in the upper, lower respiratory tract, and the gastrointestinal tract -observation of interstitial pneumonia, loss of cilia and epithelial necrosis, inflammation in nasal turbinates and trachea - observation of virus antigen in epithelial cells of the nasal turbinates, necrotic debris in the tonsil, submucosal glands of the trachea and enterocytes of the small intestine -virus transmission by droplets - difficult to handle in biosafety level-3 containment, and are not a standard animal model	-to study environmental contamination (cages, beds, food and water bowls, litterboxes and so on) or on transmission efficiency to inform veterinary and public health authorities about the risk of cats as intermediate hosts or virus carriers at the interface between *SARS-CoV-2*, humans and animals
	Dogs (Canis lupus familiaris)	- susceptible to *SARS-CoV-2*, but to a very mild degree	- antibody testing in these species could be a useful tool for epidemiological studies
	pigs (Sus scrofa domesticus)	- not susceptible to infection with SARS-CoV-2 - No clinical signs and no clear evidence of virus replication	-not a suitable model for *COVID-19*
	Fruit bats (Rousettus aegyptiacus)	-natural reservoir of many coronaviruses (including *SARS-CoV* and *SARS-CoV-2*) -efficient replication in the upper respiratory tract -seroconversion - Transmission occurred to one out of three direct-contact animals -lack of observations of clinical signs (but rhinitis could be detected by immunohistology)	-help to model the physiopathology of the virus in its host

and cost of using animal models for bacterial research are appealing. Researchers must consider experimental factors such as animal species, genetics, age, and diet, which may control efficiently in laboratory animals, inoculation route used, bacterial species and strain, bacterial inoculum size, time to first antibacterial treatment, length of study, and study endpoint to aid in translating information from animal models to humans. (21, 22).

Uropathogenic *Escherichia coli* (UPEC) is the leading cause of community-acquired UTIs. UPEC strains have numerous structural (as fimbriae, pili, curli, flagella) and secreted (toxins, iron-acquisition systems) virulence factors that contribute to their ability to cause disease. Also, they can adhere to host epithelial cells in the urinary tract (23). The majority of UTIs begin when UPEC enters the urinary tract via the urinary meatus before ascending the urethra and into the bladder lumen(24). Some pathogens are more frequently associated with severe UTIs which are Uropathogenic Escherichia coli (UPEC), *Staphylococcus saprophyticus*, *Morganella morganii, Providencia stuartii, Pseudomonas aeruginosa, Enterobacter,* and *Serratia sp* (25-28). Urinary tract infections (UTIs) are among humans' most common bacterial infections and a significant burden to healthcare systems. UTIs have major consequences, such as repeated recurrences, pyelonephritis with sepsis, renal damage, and problems induced by chronic or repetitive antimicrobial usage, such as multi-class antibiotic resistance (29). The higher incidence of community-onset UTI in women is attributed to anatomic factors such as shorter distance from the anus to urethral opening and shorter urethral length, and vaginal/perineal microenvironment that may facilitate colonization of the urethra that enable transit of uropathogenic bacteria from a gastrointestinal tract reservoir to the urinary tract (29). Murine UTI models illustrate a powerful method for studying human UTI. Interestingly, nowadays, using a mini-surgical bladder inoculation method in both male and female mice, before this, mouse modeling of UTI had been limited to females due to the complicated access to the male mouse bladder via catheter insertion (29). It is essential

to evaluate the variables such as dissection of the roles of cytokines in innate defense, including CCL2, CCL4, IL-1b, IL-6, IL-8 (mouse equivalent CXCL1/2), IL-9, IL-10, IL-17A, IL-12p40, G-CSF, and TNF-a. The evolution and characterization of murine models of human infection significantly improved our understanding of the pathogenesis of UTI. Several studies that used mouse strain in UTI described the correlation between early cytokine responses and peak bacterial burdens in the bladder and subsequent UPEC clearance. Investigating essential components of the immune responses against UTI in mice and humans, such as TLR4 and CXCR1, confirms that murine models are suitable for studying human infections (28, 30-33).

The human Gastrointestinal (GI) tract is a complex system that begins from the oral cavity, continues through the stomach and intestines, and finally ends at the anus. Practical studies generally use animal models to understand the GI tract processes better (34, 35). In response to several environmental factors, such as diet, genetics, age, gut structures, metabolism, xenobiotics, and pathogens, the crosstalk between the microbiome and the human immune system and feedback loops contributes to the microbiome composition, host physiology and disease susceptibility (36-39). General comparisons of mice (36), pigs (37), and rats to humans were recently conducted to aid in translating information from animal models to clinics. Different species have different anatomical structures and pH levels at various points along the GI tract (41). The human colon has a thicker mucosal layer than mice and rats, which influences the diversity of microbiota colonizing the colon (37). Firmicutes and Bacteroidetes (42) dominate human gut bacteria, as they do the GI tracts of commonly used model animals (43).

The bacterium Vibrio cholera colonizes the human small intestine and causes cholera's life-threatening diarrheal disease. Many different animal models have been used to reproduce human cholera disease. The Suckling mouse is the most commonly used animal model of cholera in order to better understand pathogenesis mechanisms and identify virulence factors produced by bacteria. Due to the relative immaturity of the immune response, infant mice (3–5 days old) are efficiently colonized, while adult mice cannot be colonized by *V. cholera* without eliminating the microbiota (40, 41). Any microbial infection can have suitable animal models to comprehend its pathogenesis better, to test new therapies against it, or to design vaccines. Due to the absence of an eligible model of human *H. pylori* infection, scientists use several methods that consist of utilizing *H. pylori* infection in animals following the result of natural helicobacter infection or involving infection of unnatural animal hosts with some of the non-*H. Pylori* gastric helicobacters. The evolution of animal models of Helicobacter infection delivers several powerful tools to analyze many infection factors, which are utilized to treat and prevent this infection by an effective vaccine. *H. pylori* will only colonize in some hosts, including non-human primates, germ-free or barrier-raised piglets, germ-free dogs, and laboratory-raised cats. The *H. felis* mouse model has been used to develop human vaccines against *H. pylori.* It protects against infection with extensive doses of viable *H. felis* by oral immunization using sonicates for *H. felis*, *H. pylori*, or recombinant H. pylori urease and cholera toxin B subunit as the mucosal adjuvant (42).

Salmonella enterica serotype Typhimurium (*S. typhimurium*) is the leading cause of gastroenteritis and bacteremia throughout the world (43). Sivula et al. used Salmonella enterica serotype Typhimurium ATCC14028 to compare intestinal and systemic colonization in two animals for the first time (chicken and Salmonella resistant mouse models); moreover, they highlight significant dissimilarities in systemic and intestinal colonization levels between chicken and murine serotype Typhimurium infections. Differences in intestinal colonization may affect the presence or absence of increased systemic colonization. Also, there are differences in systemic colonization after oral infection disease and noticeable systemic influences in Salmonella-resistant murine models. Likewise, there are inadequacies in intracellular colonization of the cecal epithelium in both animal models.

Further, they found that *Salmonella Pathogenicity Island-1* (SPI-1) is essential for infection in the murine model and connection with the cecal epithelium in 1-week-old chicks. Finally, they estimated the fecal shedding of serotype Ty-

phimurium ATCC14028 in chicken and murine infection models. It does not accurately reflect intestinal colonization in infected 1-week-old White Leghorn chicken. (44).

Staphylococcus aureus is one of the most prevalent pathogens that can cause biomaterial-associated infection (BAI). In a study by Zhang et al., the potential of zebrafish embryos to study BAI in real-time in vivo has been shown. Zebrafish embryos are highly economical, a live model organism for intravital visualization and research of infection progression and related host responses. Their remarkable features, such as optical transparency, low cost, high reproduction rate, and similar immune response to humans, make them an adaptable *in vivo* tool for studying host-pathogen relations and infection pathogenesis of several bacterial species. They used *S. aureus* RN4220 expressing mCherry fluorescent protein (*S. aureus*-mCherry) and transgenic zebrafish line expressing Kaede green fluorescent protein in the macrophages and blue fluorescent polystyrene microspheres (45).

In a review by Waack et al., nine bacterial species, including two Gram-positive (*Streptococcus pneumonia* and *Staphylococcus aureus*) and seven Gram-negative organisms (*Klebsiella pneumoniae, Stenotrophomonas maltophilia, Haemophilus influenzae, Acinetobacter baumannii, Escherichia coli, Enterobacter cloacae, Enterobacter cloacae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) were used in the IND database. Various animals were used as pneumonia animal models in published literature (guinea pig, rabbit, mice, rat, and pig) and IND studies (mice, rabbit, and rat) (21).

Streptococcus pneumoniae (*S. pneumoniae*) can colonize the human nasopharynx and have the potential to cause diseases, including otitis media, pneumonia, bacteremia, and meningitis (46). Reyes et al. in 2016 demonstrated that S. pneumoniae intrabronchial inoculation in baboons caused clinical features, organ involvement, disease severity, inflammatory response, and disease progression. That is approximately similar to pneumococcal pneumonia in humans (47). For the first time in 2021, Amaro et al. represented a model of pneumococcal pneumonia caused by penicillin- and macrolide-resistant *S. pneumoniae* serotype 19A in pigs that were ventilated mechanically for 72h, with extreme hemodynamic impairment and requiring the usage of vasoactive drugs (48).

Staphylococcus aureus is one of the most common infectious agent-related causes of morbidity and death globally. This pathogen may cause various illnesses, from mild skin infections to deadly pneumonia and sepsis (49). In 2021, Klopfenstein et al., with advancements *in vivo* imaging of fluorescent transgenic mice incorporated with fluorescent/bioluminescent bacteria, provided protocols for studying the immune response to these infections, experimental validation for the molecular basis of microbial pathogenesis, clarification of the utility of factors as antigens for vaccines, and therapeutic interventions for *S. aureus* infection. They applied different types of *S. aureus* infection in models such as localized (method of subcutaneous skin infection by f tape striping infection in the ear), invasive (osteomyelitis model), and systemic infection model (i.v.). One of the primary animal models used to study S. aureus in different diseases is mice (used in pneumonia, bacteremia, meningitis, septic arthritis, neonatal sepsis, osteomyelitis, and subcutaneous and superficial skin infections). Some features of mice as animal models of *S. aureus*, including the availability of reagents, reasonable cost, short duration of pregnancy, amenability of the mouse to drug and gene therapy, and immune response and physiology similarities, make them a good choice for these studies (50).

Due to similarities in the system and pathology of shigellosis between monkeys and human dysentery, such as the formation and progression of mucosal lesions in both hosts, monkey shigellosis is an ideal model for studying numerous aspects of human Shigella infections. Examining this animal model will help us understand acute colitis better because colonic changes in experimental monkey shigellosis are parallel to other types of acute colitis in men (51). Shipley et al. have developed a dedicated challenge infection model with wild-type *S. dysenteriae 1* in cynomolgus macaques that reproducibly generate disease and trigger immune responses. This animal model can enable study protection from reinfection and disease pathogenesis and determine correlations of protective immunity to *S. dysenteriae* type 1 infection due to its genetic similarities and the high

attack rate(100%) (52). Jeong et al. characterized a piglet model for *S.dysenteriae* type 1, with many similar clinical symptoms and gastrointestinal manifestations, providing a valuable tool to compare vaccine candidates for immunogenicity, reactogenicity, and response to challenge; examine the function of virulence factors, and test the effectiveness of microbial agents. Some symptoms and manifestations include severe diarrhea, dehydration, anorexia, cellular invasion, mucosal inflammatory reaction, and damage to the mucosa targeting the large bowel (53).

Acute gastroenteritis caused by the foodborne bacterial infection *Campylobacter jejuni* is frequently characterized by inflammation, stomach discomfort, fever, and diarrhea (54). Stahl et al. provide a novel murine model of *Campylobacter jejuni* gastroenteritis single IgG Interleukin-1 related receptor-deficient (Sigirr -/-)(a repressor of MyD88) mice as an applicable animal model for studying innate immune responses to *C. jejuni's* pathogenicity factors, managing infection of the microorganism, and the system of initiating an inflammatory reaction. The reason for the establishment of a new animal model was colonization resistance against *C. jejuni* in traditional WT mice as an animal model because of the commensal microbiota supplies. This protection has been disrupted through vancomycin treatment, but the WT mice persisted substantially tolerant to the presence of *C. jejuni*, resulting in almost no inflammatory response. On the other hand, Sigirr-/- mice's immune system is established to be dramatically more responsive to *C. jejuni,* maybe by enhancing the sensitivity of TLRs expressed on epithelial cells. (55). Giallourou et al. represented a powerful mouse model to demonstrate reproducible bloody diarrhea or growth failure of *C. jejuni* infection with clinical features similar to malnourished children, with zinc or protein-deficient diet and antibiotic alteration of normal microbiota before infection. This study has indicated that the mouse feeding standard, zinc-deficient diet, or protein-deficient diet affect the amount of colonization, organism shedding in stool, inflammatory biomarkers, and intestinal architecture (56).

Neisseria gonorrhoeae (gonococcus) is the etiologic agent of gonorrhea, a sexually transmitted infection (STI), and colonizes the genital mucosa still it can also colonize the ocular, nasopharyngeal, and anal mucosa (57). Li et al. designed an animal model by expressing the transgene of a eukaryotic vector, pCDPCAM1-GI, for gonorrhea research. CEACAM molecules (on the surface of transfected epithelial cells) bind to gonococcal colony opacity-associated (Opa) proteins, making bacterial entry easier. In general, CEACAM1 is one of the critical factors that can moderate gonococcal infection (58). An experimental mouse model of *Neisseria gonorrhoeae* genital tract infection was developed by Raterman et al. Since they can control the evasion of host innate effectors, host gonococcal adaptation to hormonally driven host factors in females, and analysis of *Neisseria gonorrhoeae* mechanisms to control the host immune response, it has been thought to be a useful system for figuring out the function of gonococcal factors (59).

Neisseria meningitidis (meningococcus) is a gram-negative diplococcus that can cause septicemia and meningitis in susceptible people (60). Melican et al. used *in vivo* models to research the Neisseria meningitidis infection pathogenesis, which was previously constrained by the bacterial specificity for humans. N. meningitidis only adheres to human veins, causing severe vascular damage, inflammation, and occasionally the appearance of a purpuric rash. For prevention and therapy strategies to be as effective as possible, it is crucial to comprehend how infection produces this vascular damage. They used a humanized model for this infection in which human skin containing dermal microvessels is grafted onto immunocompromised mice. N. meningitidis was induced intravenously into this model, explicitly adhered to the human endothelium, and produced a pathology that is similar to what is reported in clinical patients, including vascular damage and purpuric rash development (61). Because humans are the only host for *Neisseria meningitis*, developing a suitable animal model for meningococcal vaccines is difficult. Arunachalam et al. describe the development and optimization of a mouse model to determine whether tetravalent meningococcal polysaccharide (MenACYW-TT) protein conjugate vaccine formulations are the most immunogenic for clinical testing. ICR mice immunized subcutaneously with 0.25 g per serogroup polysaccharide-protein conjugate vaccine candidates on days 0 and 14, with serum samples

obtained on day 28 for immunogenicity evaluation, are the best pre-clinical immunogenicity model with the largest antibody dose-response range (both anti-polysaccharide IgG and bactericidal antibodies) (62).

As mentioned in this part, mammalian animal models are primarily used in bacterial infection animal experiments, and it is challenging due to many reasons, including requiring many animals to be tested because there are many bacterial strains that need to be checked and set up. Also, it is costly, and time-consuming and there is moral conflict from an animal rights point of view (19, 20).

Animal models of human viral diseases

The high prevalence, easier spread of the disease, and straight transmission routes have made viruses a substantial cause of pandemics. According to the study by Bhadoria et.al in 2021, previous pandemics mainly involved respiratory viruses, for example, severe acute respiratory syndrome (SARS CoV-1)(2002-2004), Influenza A H1N1 2009 (Swine Flu) Pandemic (2009-2010), Middle East respiratory syndrome (MERS) CoV infection (2012-present), Western African Ebola virus epidemic (2013-2016), Zika Virus Epidemic (2015-2016), and SARS-CoV-2 infective disease (COVID19) pandemic (2019-present) (Table 2) (90).

Collated data on earlier major viral pandemics in the last two decades can help combat the current pandemics and prepare for future ones (90). Animal models are required to understand the viral disease processes, pathogenesis, host-pathogen interactions, infection parameters (e.g., clinical signs, virus growth, and clinicopathological parameters), cellular and humoral immune responses, and immunologic responses to human viral infections to test vaccines and medical countermeasures (91).

Coronavirus disease (COVID-19) outbreak has been a public disaster and a source of global concern. COVID-19 symptoms include cough, fever, myalgia, fatigue, and signs of a lower respiratory tract infection (92). According to the pivotal role of the ACE2 receptor in COVID-19 pathogenesis, which binds to the SARS-CoV spike protein, there are several animal models for SARS based on this receptor. Many animal models can replicate the SARS-CoV genome, such as rats, dogs, pigs, foxes, cats, mice, ferrets, and monkeys. Although, none of them is appropriate due to the different properties of human disease, including clinical features (pyrexia and respiratory signs), mortality (10%), viral replication, and pathology. The best-characterized models are mice, hamsters, ferrets, and non-human primates (NHP) (Table 2).

One of the six human coronaviruses known to cause respiratory illness in people is MERS-CoV (93). Since the mouse DPP4 receptor differs significantly from human DPP4 (hDPP4) in critical areas of interaction with the MERS-CoV spike protein, mice are not predisposed to MERS-CoV infection. By expressing human DPP4 (hDPP4), mice can overcome their natural lack of sensitivity to MERS-CoV infection. Transgenic hDPP4 mice generate severe and lethal respiratory disease after injection of MERS-CoV. After evaluating the therapeutic and prophylactic effects, transgenic hDPP4 mice are ideal animal models. Homology sequence between rabbit and human DPP4 caused rabbits to be regarded as a promising model for MERS-CoV infection (94, 95).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging respiratory infection caused by introducing of an unknown coronavirus into humans late in 2019 (first discovered in China). Until 18 June 2022, SARS-CoV-2 has infected more than 539M million people and has caused more than 6.32M deaths (96, 97). SARS-CoV-2 infection is characterized initially by mild symptoms, such as fever, cough, dyspnea, and myalgia. These symptoms are caused by the capability of SARS-CoV-2 to replicate efficiently in the upper respiratory tract. Despite resolving the infection by most people, the disease may also be progressed to severe pneumonia. Choosing a suitable animal model is critical for COVID-19 to discover therapeutic agents and vaccines, and other possible medical countermeasures. Investigators can use several small and large animal models to explore essential aspects of COVID-19, including transmission, pathology, and host responses to SARS-CoV-2(96). We mentioned the features of some of the most commonly used animal models of this disease in the Table 2.

This part of the article gives us a perspective of how use of animal models can help us to under-

stand viruses' biology to control viremic pandemics. Thus, it is required to study different aspects of animal models for viremic diseases to generate experimental models that recapitulate the elements of human disease in the best way possible.

Animal models of human parasitic diseases

An efficient way to acquire knowledge on human-infecting parasites is to study different animal models (**Table 3**). Parasitological research frequently studies specific mechanisms, parasite infection factors, host response (e.g., cytokines, antibody response, infectivity, and genetic differences), and mimicry of the natural situation of parasite infection more precisely (e.g., distribution, transmission dynamics). An animal model for parasitic examines will be selected through its mimicry of a human as a host, the interaction of the human host-parasite system, and the examination of immunological, physiological, anatomical, and metabolic similarities and differences (100). Protozoa are single-celled organisms that can grow in humans. These parasites can spread via insect bites, contaminated water and food, and personal contact. The following paragraph will focus on a small fraction of some of the world's most important human infectious diseases that can be zoonotic (101).

Malaria is a significant global health problem affecting young children and pregnant women in poor developing countries. Plasmodium genus is responsible for the disease represented by fever, splenomegaly, hepatomegaly, and anemia. *Plasmodium falciparum* can cause the most lethal form of disease(100, 102). Our understanding of host-pathogen interactions of malaria, immunological responses, and drugs and vaccine formulation has significantly improved due to the use of animal models, particularly mice, and also will allow us to understand better the biology of Plasmodium, as well as the development of new therapeutic strategies. NHP models for malaria are admittedly under-used; they are closer models than mice for human malaria; in particular, NHP models authorize using human pathogens (*Plasmodium vivax, Plasmodium falciparum, Plasmodium malaria*, and *Plasmodium knowlesi*) (103).

Leishmaniasis is a common infectious disease between humans and animals frequently observed in the Mediterranean, tropical, and subtropical regions of the world. According to World Health Organization reports, leishmaniasis has been endemic in more than 100 countries worldwide (104-107). Currently, 12 to 15 million people worldwide suffer from the disease, and one billion are at risk (108). Despite having genetic similarities of experimental models of leishmaniasis in both the parasite and the host, none mimic the outcome of human infection entirely by *Leishmania spp.* Some primary factors contributing to differences between human and animal models are the size and nature of the inocula, the infection route, and the strain of the host or parasite. Different animal models have been used to study immunological aspects of the disease, such as mice, hamsters, domestic dogs, and non-human primates. Several studies have revealed significant differences in the immune mechanisms associated with infections with New World Leishmania species. Additionally, the clinical characteristics and immunological reactions reported in human patients are not mimicked by visceral infection disease in mice. The lack of immunological reagents and the intense immunosuppression of the lymphoproliferative response in hamsters make its use for evaluating vaccine candidates difficult. The hamster model is more appropriate for studying the progressive disease of visceralizing *Leishmania spp*. The possibility of looking the immune response in natural infection has fascinated researchers' interest in using dogs as experimental models. Vaccination of dogs would be a significant step toward reducing human infection disease. Monkeys are another animal models which can be used for testing vaccine candidates. Developing improved experimental models for studying leishmaniasis can help us to recognize possible targets as vaccine candidates (109).

Toxoplasma gondii is an obligate intracellular parasite with a global distribution. Toxoplasmosis is considered an opportunistic parasitic disease. There is a great need to create novel therapies for the acute and latent forms of infection despite the fact that there has been significant advancement in the treatment of human disease. Additional research is needed to determine new drugs using creative high-throughput screening technologies and to enhance experimental models to reflect human disease. Congenital infection in humans and animals can result in severe symptoms in the

offspring, especially in the brain. There is lacking an appropriate animal model for human congenital toxoplasmosis (110, 111). Grochow et al. confirmed and validated the guinea pig as a model for human congenital toxoplasmosis by analyzing the effect of the *T. gondii* infection dose, the duration of infection, and the gestational stage at the time of infection, the survival rate of dams, and the fate of the offspring. *T. gondii* DNA loads in various offspring tissues such as the brain, liver, spleen, heart, lung, and femoral muscles (111). The house mouse served as the main laboratory animal model utilized by Saraf et al. to assess the virulence of the *T. gondii* strain in human infections. Epidemiological data also point to a possible link between mice virulence and the severity of the human toxoplasmosis disease. They measured the pathogenicity of *T. gondii* in mice by looking at the cumulative mortality in animals that had received successive doses of tachyzoites via IP injection (112). Sharif et al. considered the potential of cyst production by *T. gondii,* RH strain, in Wistar rats and BALB/c mouse. They presented an animal model suitable for congenital, cerebral, and ocular toxoplasmosis. Only a few ocular samples were positive. Mouse is the most commonly used animal model for drug studies on cerebral toxoplasmosis. The congenital cerebral toxoplasmosis model yielded the best results in the survey, with pregnant rats infected with the 10^7 parasites and all infants infected (100%). As a result, these infants can be used as a congenital cerebral toxoplasmosis model during the fetal stage and a cerebral toxoplasmosis model one month after birth (113). Cats are the definitive host for *T. gondii* oocysts which can be risk factors for infecting humans. Cornelissen et al. established an experimental challenge model that is necessary to assess the efficacy of a vaccine or drug treatment quantitatively. After an experimental infection, they discovered that cats shed oocysts in a dose- and time-dependent manner. Their *T. gondii* dose-response model in cats can be used to assess various methods for helping cats shed less oocysts (114). Kexin Li et al., in 2021, established a murine model of primary acquired ocular toxoplasmosis (OT) induced via the natural infection route to investigate the immune mediator profiles in the aqueous humor (AH). *T.gondii* infected peroral in C57BL/6 mice. Fluorescein angiography (FA) was performed after observing the ocular fundus. The AH, CSF (cerebrospinal fluid), and serum were collected before infection and at 28 days post-infection, and the immune mediator levels in these samples were determined using a multiplex bead assay. This OT model enables precise ophthalmologic, histopathologic, and immunologic assessments of human OT. The study of AH immune modulators sheds new light on the immunopathogenesis of OT. Furthermore, looking into AH immune modulators could help with the differential diagnosis of uveitis, and these inflammatory molecules could be targets for therapeutic intervention (115). Yoshida et al. in 2020 demonstrated the in vivo control of Toxoplasma by macrophages and emphasized the possibility of the establishment of zebrafish as an animal model to study parasite immunity because parasitophorous vacuole breakage in brain cells and macrophages *in vivo*, suggesting that cell-intrinsic mechanisms may be destroyed by the intracellular niche of tachyzoites (116).

In many nations around the world, schistosomiasis has been a problem for public health for centuries and perhaps longer. In order to eradicate this disease, the World Health Organization is working on it (117). *Schistosoma haematobium* is the etiologic mechanism for urogenital schistosomiasis, a significant source of morbidity and mortality for more than 112 million people worldwide. Infection with S. haematobium causes various immunopathologic sequelae caused by parasite oviposition within the urinary tract, which drives hematuria, inflammation, bladder dysfunction, fibrosis, and vulnerability to urothelial carcinoma (118-120). A novel mouse model developed by Fu et al. could help better in understanding the specific pathophysiological mechanisms underlying the tissue fibrosis, oncogenesis, and epithelial dysfunction linked to urogenital schistosomiasis (121). *S. haematobium* eggs are deposited into the female reproductive tract by adult worms causing Female genital schistosomiasis (FGS), which is the result of pelvic pain, vaginal bleeding, genital disfigurement, and infertility. Co-infection with S. haematobium boosts the risks of contracting sexually transmitted diseases such as HIV. Recent evidence suggests that co-infection with *S. haematobium* grows the risk of contracting sexually transmitted infection diseas-

es such as HIV. L. Richardson et al. established a new mouse model to help enable novel studies of genital schistosomiasis in females (122).WANG et al. discovered a cerebral schistosomiasis model in rabbits to understand better morphological analysis, clinical manifestation observation, and investigations into immunological reactions and pathogenesis of focal inflammatory reactions in neuroschistosomiasis. This model established through the direct injection of schistosome eggs into the rabbit brain. A number of variables must be taken into account in establishing this model, including the antigenic property of eggs, the time of scarification, and the clinical manifestations (123). Watanabe et al. reported that miniature pigs are highly susceptible to the Chinese strain of S. japonicum and can be helpful in establishing animal models for human S. japonicum schistosomiasis. Two miniature pigs of the CLAWN strain (C-1, C-2) were inoculated percutaneously with 200 *Schistosoma japonicum* cercariae of the Chinese strain, and the subsequent infection was monitored parasitologically, pathologically, and serologically. Histological examination of the pancreas, liver, lung, spleen, mesenteric lymph nodes, and small intestine revealed egg deposits associated with inflammatory reactions. This suggests that in the chronic phase of schistosomiasis, decreased fecal egg excretion did not correlate with reduced parasite numbers. This is the first report indicating that the miniature pig could be a model for human *S. japonicum* infection (124).

Echinococcus granulosus is a worldwide zoonotic cestode in the dog's intestine as a definitive host. Hydatid cysts are mostly formed in the liver, lung tissues, and other organs like the brain, eye, and bone by its larval stage, which also infects intermediate hosts. In the experimental models, peritoneal, thoracic, subcutaneous, and cerebral injection of protoscoleces result in hydatid cysts. Radfar et al. launched the first experimental cerebral hydatidosis due to the larval stage of E. granulosus in the rat brain as an animal model by intracranial injection of echinococcal larvae. This model provides an excellent opportunity to study the parasite-host relationship, different transmission ways of infection in the intermediate hosts, and the effect of new drugs (125). *E.granulosus* cestodes are the primary cause of cystic echinococcosis, which is one of the critically neglected chronic parasitic diseases. Kandil et al. established the domestic rabbit as an intermediate host for cystic echinococcosis. They considered the potency of the crude germinal layer and the protoscoleces antigens to protect against the CE. This animal is used to study disease pathogenesis, immunological patterns, and drug efficiency (126).

Numerous appropriate animal models are available for studying human host-parasite relationships. Helminths are another type of human parasite. Helminths are parasitic worms that often root in a person's digestive tract. These parasites eventually pass through a person's stool since they are unable to divide or replicate within a human body. It is possible to research the vertical transmission of parasitic nematodes using animal models. The pig-parasite model can be used to research helminth-bacteria interactions, treatment and control methods, and nutrition-parasite interactions. Although a model is an artificial representation of the real world, and pigs may not always be the best model for some types of research, recent findings indicate that the pig model is crucial for understanding human nematode infections (127). *Trichinella spiralis* (*T. spiralis*) is a food-borne zoonotic parasite worldwide. Ingestion of undercooked or raw meat contaminated with *T. spiralis* larvae can cause infection. Ts-EVs (EVs produced by *T. spiralis*) can simulate developing inflammation in DSS-induced colitis by inhibiting M1 macrophage polarization because of their immunomodulatory ability. Gao et al. 2021 established a mouse model with dextran sulfate sodium (DSS)-induced colitis to study the immunomodulatory properties of EVs produced by *T. spiralis* (128). There are some essential issues in selecting appropriate animal models for nematodes, including the choice of suitable animal hosts for models of human parasite infection, the interaction between animal models and mathematical modeling, the impact of host nutrition and diet, the development of treatment and control strategies, the interaction of parasites with other pathogens (127). As mentioned in this paragraph, understanding of the pathogenesis parasitic diseases was built from the findings of available experimental models. Also, the development of a suitable animal model for the vireic disease is critical for preclinical testing of antiviral drugs and vaccines.

Table 3. Animal Models of Pathogenic Parasitic Diseases

Pathogenic Parasites	Disease	Animal Species	Significant Features	Application	Ref.
Protozoa					
Plasmodium	Malaria	Mice	**Limitation:** Rodent Malaria parasites use to infect mice (Human Plasmodium parasites are unable to infect mice) - Rodent Plasmodium infections in mice display some, but not all, of the main features of the human infection and disease	-study pathogenesis, -discovery of drugs against various stages of the parasite life cycle (except hypnozoites) - models for malarial chemotherapy research - studies on the molecular biology and genetics of drug resistance	**(101, 103)**
		Humanized mouse	-There is no ideal Humanized animal model with a reconstituted human hematopoietic system for studying the blood-stage of the parasite. -There are only transient models such as NOD/SCID/IL2Rγ- (involving perfusion of infected RBCs into immunodeficient mouse strains). - Mice with engrafted human hepatocytes (for transmission and liver-stage studies such as infection with P. falciparum sporozoites, liver-stage schizonts **Drawback:** low to medium throughput, inappropriate for the study of P. vivax, not adapted for the assessment of specific immunity (requires fully competent human immune system)		
		Saimiri sciureus sciureus monkey	- infected with P. falciparum -demonstration of important variant- and strain-specific antigens for activation of acquired immunity to malaria	- Development of vaccines	
		Aotus monkeys	- infected with Plasmodium vivax	-to perform transmission-blocking vaccine trials	
		rhesus monkey (Macaca mulatta)	-Infected with Plasmodium coatneyi	-to perform a model of malaria in pregnancy	
Leishmania	Leishmaniosis	Mouse	**Ads**: genetic differences among inbred mice strains allows scientists to study the effect of genetic diversity on the different phenotype of interest. -the simplicity of keeping, breeding, and reproducing them. **Disads:** Using different parasite species, tissue targets (mice footpad, ear, or base of tail), and doses (105 to 107) of metacyclic promastigotes have caused a wide variety of experiments that do not display the natural infection in human.	-To clarify the cell types, cytokines, signal transduction cascades, and antileishmanial effector mechanisms necessary for controlling parasites and the clinical resolution of disease, resistance to a secondary infection, and vaccine development -understanding of the immunologic mechanisms governing resistance (C57BL/6 strain) and susceptibility (BALB/c strain) to infection	**(109)**
		Hamster (Mesocricetus auratus)	**Ads:** -highly sensitive to infection with visceralizing Leishmania species (L. donovani, L. infantum) - Limited use of hamsters due to the scarcity of reagents (e.g., antibodies, cell markers, and cytokines) to study the role of the immune response in the pathology of the disease **Disads:**- i.v., intracardial, or i.p. routes of infection do not mimic natural transmission by sand fly bite	- best experimental model to study immunological mechanisms involved in the pathogenesis of visceral leishmaniasis (VL)	
		Dog	- main reservoirs of zoonotic visceral leishmaniasis caused by L. infantum in the Mediterranean area, Middle-East, Asian countries and Latin America	- studying the immune response and finding Leishmania antigens involved in protective cellular immunity in canine visceral leishmaniasis - study the epidemiology, pathology, and immunology of canine leishmaniasis and its genetic basis -understanding of the disease -help to develope new diagnostic methods and control measures against the infection (including insecticide-impregnated collars for dogs, new drugs, and second-generation vaccines)	
		Non-Human primate	**Ads:** similarities to humans in anatomy, immunology and physiology **Disads:** expensive laboratory animals that are difficult to obtain and to handle Limited use due to financial and ethical reasons	-to study different aspects of this disease and would accelerate the development of vaccines and testing of new drug candidates	
		Asian rhesus macaques (Macaca mulatta)	**Ads:** -susceptible to Leishmania infection: develop a human-like disease, exhibit antibodies to Leishmania and parasite-specific T-cell mediated immune responses both *in vivo* and in vitro, and can be protected effectively by vaccination - Similar progression and resolution of skin lesions to that observed in humans, confirming the potential for this monkey as a viable surrogate - reproduction of clinical and histopathological features common in L. major-infected humans and	- to study the immune response in human cutaneous leishmaniasis	

Table 3. continued

				in the resistance to secondary infection, indicating the development of an acquired immunity	
			owl monkeys (Aotus trivirgatus), squirrel monkeys (Saimiri sciureus), and marmosets (Callithrix jaccus jaccus)	high susceptibility of owl monkeys to L. donovani infection suggest it may be useful for the study of VL - squirrel monkeys develop a visceral disease with L. donovani but are able to recover from disease and became resistant to reinfection	- potential hosts for studying visceral leishmaniasis
			rhesus macaques	- safety, immunogenicity, and efficacy of a vaccine combining heat-killed L. (L.) amazonensis with human rIL-12 (rhIL-12) and alum (aluminium hydroxide gel) as adjuvants was evaluated in rhesus macaques	cutaneous leishmaniasis candidate vaccines
		Wild Rodent		- Classical laboratory inbred strains of mice **Disads:** Limited genetic polymorphism due to a small pool of ancestors	- for research in immunology and oncology - understanding of the dynamics of infection, especially concerning the ability to control the infection and strengthen parasite populations in a given environment and how the parasites escape from immune response
		Sigmodon hispidus		- high susceptibility of this rodent to human pathogens -low levels of NO production and iNOS expression similar to human macrophages were found in Sigmodon hispidus	- to study of experimental infection has been carried out with this pathogen
		Thrichomys Laurentius (South American caviomorph rodent)		- establishment of the importance of the retention of infection and transmission of Leishmania species due to its monospecific genus	- to confine experimental patterns of *L. infantum* and *L. braziliensis* infections
		Peromyscus yucatanicus		100% of P. yucatanicus inoculated with 102 ("low inoculum") developed a subclinical infection (absence of clinical signs and evidence of parasite´s DNA at the site of inocula), and when immunosuppressed with cyclophosphamide, a reactivation with the appearance of lesions was observed.	- primary reservoir of *L. mexicana*
Toxoplasma	Toxoplasmosis	guinea pig		- similar brain as humans (high degree of maturity at birth) - similarities with humans regarding the placentation - haemomonochorial placenta, in which a single-layer, syncytial chorionic epithelium is in direct contact with the maternal blood - Production of progesterone by the placenta during pregnancy and the sexual cycle characterization by a cyclically recurring estrus	-to study high topicality and clinical signifcance, which address the pathogenesis, diagnosis, therapy and prognosis of congenital toxoplasmosis	**(111)**
		mouse (Mus musculus)		- a potential association between virulence in mice and disease severity in human toxoplasmosis	- for determining the virulence of *T. gondii* strains	**(112)**
		Wistar rat and BALB/c mouse		- infected infants can be used as a congenital cerebral toxoplasmosis model when they are in the fetal stage and can be used as a cerebral toxoplasmosis model one month after birth	- to study an animal model suitable for congenital, cerebral, and ocular toxoplasmosis	**(113)**
		Cats		- shedding of oocysts by cats after experimental infection is dose- and time-dependent	- to quantitatively evaluate the effectiveness of a vaccine or drug treatment	**(114)**
		C57BL/6 mice		Despite anatomic, biochemical, and immunological differences between mice and humans, a murine model of OT can provide critical information about human OT and expedite an exact evaluation of the immunopathogenesis of this disease.	-study ophthalmologic, histopathologic, and immunologic evaluations of human OT - Investigation of AH immune modulators and immunopathogenesis	**(115)**
		zebrafish		-in this animal model macrophages are recruited to the infection site and play a key role in Toxoplasma control	-to study the innate immune response to Toxoplasma *in vivo*	**(116)**
Helminths						
Schistosoma (Schistosoma haematobium)	Schistosomosis	Mouse		Mouse model of S. haematobium urinary tract infection is similar to human urogenital schistosomiasis	To investigate pathophysiological mechanisms of epithelial dysfunction, tissue fibrosis, and oncogenesis associated with urogenital schistosomiasis -to study the basic molecular and cellular immunology of urogenital schistosomiasis and thereby contribute to the development of new diagnostics and therapeutics	**(121)**
		BALB/c mice		-injection of S. haematobium ova appears to trigger vaginal inflammation and scarring infiltration by	To study immune modulation and genitourinary changes that	**(122)**

Table 3. continued

			leukocytes expressing HIV co-receptors, and increased urinary frequency **Disads:** not reproducing the actual disease in which ova migrate from the lumens of host blood vessels to the epithelial surface, Eggs injected below the epithelial surface and did not migrate as seen in natural infection -not finding any vaginal mucosal lesion -The existence of S. haematobium eggs in the vagina did not cause considerable modifications in the overall systemic immune response.	occur with FGS	
Schistosoma japonicum		Rabbit	-first time to test the validity of direct injection of egg suspension into rabbit brain in establishing the NS model **Ads:** - shorter experimental course, compared with percutaneous infection course (5–7 weeks) - This method may help exclude many affecting factors (When the cercariae transform into schistosomula and then adult worms to lay eggs, alternative and complicated immunological reactions may be induced during these stages). -this method can facilitate the infection progress **Disads:** Variations in the antigenic property of eggs due to the difference in the development of embryo or miracidia in the eggs -The time of sacrificing rabbits needs to be carefully selected (based on the rabbits' neurological symptoms and the lifespan of eggs' granulomas)	to understand morphological analysis, clinical manifestation observation, researches into immunological reactions and pathogenesis of focal inflammatory reactions in neuroschistosomiasis (NS)	**(123)**
		miniature pig (CLAWN strain)	- highly susceptible to S. japonicum	showing the miniature pig to be a potential model for human S. japonicum infection	**(124)**
Echinococcus granulosus	Echinococcosis	rat	- suitable animal model for induction of secondary hydatid cysts in brain	first experimental cerebral hydatidosis due to larval stage of *E. granulosus* in the animal model	**(125)**
		domestic rabbit	- can be experimentally infected with active oncospheres or viable protoscoleces - this model might complete the echinococcus life cycle - The germinal layer antigen is a promising vaccine to control CE	evaluation of the immunization efficiency of the crude protoscoleces and germinal layer antigens to be utilized as a vaccine for protection against CE in the developed rabbit model.	**(126)**
Intestinal Nematodes (*Strongyloidosis, Trichuriosis, Ascariosis, Hookworms*)		rodents	- easy to keep and handle -less expensive - reproduce rapidly and in large numbers -best model for: *T. muris*, *Heligmosomoides polygyrus, Trichinella spiralis* -the potential of migration of Larval parasite stages in rodents, e.g., *A. suum* larvae in guinea pigs and mice, and *T. canis* in mice **Limitations:** - host physiology, parasite size constraints and a relatively short host life span - Size limitations (for Ascaris and Toxocara parasites, which are the largest intestinal nematodes, and normally do not complete their life cycle in rodents) - Some mice are prone to trichuriasis, being unable to express protective immunity	- to study specific host–parasite interactions such as immune response, parasite fecundity and survival, and genetic effects - To study valid for detailed immunogenicity	**(127)**
		pigs	- many similarities with humans - the degree of overdispersion of *A. suum* worm burden distributions in continuously exposed pigs are very similar to that of A. lumbricoides in humans	-to study the migration of *A. lumbricoides.* -to understand *A. lumbricoides* population biology	
		primates	- many similarities with humans **Limitation:** - cost - complex logistics -ethical considerations	-to study of all aspects of the population dynamics of a particular infection	
Trichinella spiralis	Tissue Dwelling Nematodes (*Filarioses, Trichinellosis*)	Female Wistar strain rats and C57BL/6 strain mice (6-8 weeks old, male)	- Ts-EVs stopped the overexpression of TNF-α, IFN-γ, IL-17A, and IL-1β observed in the colon of DSS-treated mice.	-To study the immunomodulatory properties of EVs produced by *Trichinella spiralis (T. spiralis)*	**(128)**

Conclusion

More than 1400 pathogens, including viruses, bacteria, fungi, protozoa, and helminths, can cause human infectious diseases, a growing concern due to drug-resistant organisms, bioterrorism, global trade, and travel. Novel methods for preventing pathogen spread, as well as the development of new vaccines and/or therapeutics, should be developed. Animal models have been provided crucial insights into the pathogenesis and treatment of infectious diseases. The careful selection of the most informative species as an animal model remains critical and presents investigators with a unique challenge. It is also necessary to recognize the limitations of animal models and the demand to add other types of studies to

animal experiments to gain more precise results on an infectious disease, such as in vitro studies and clinical trials.

Conflict of interests

There is no conflict of interests.

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