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Mycoplasma spp., Clostridium Piliforme, Streptobacillus Moniliformis, Streptococcus Pneumoniae, Pasteurellaceae spp., Helicobacter spp. infections in Rats

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Abstract
The mouse microbiome, defined as all bacterial species found in or on the mouse, and their interactions among themselves and with their environment, are always present. Non-infective agents or pathogenicity of infective agents can lead to various diseases. The flora of mice in the will become more complex depending on their housing and rearing conditions, meaning that more bacterial species will colonize mice. The same strain may have different flora in different experimental animal breeders. Opportunistic pathogen is often referred to as ''opportunistic'' agent or even simply ''opportunistic''but it is a definition that blends both commensal flora and primary pathogens. Rats, the most commonly used species of experimental animals, are susceptible to a range of bacterial infections that can be enzootic or sporadic but can have high morbidity and motility rates leading to severe disease. When formulating differential diagnoses for sick or diseased animals, it must be recognized that diseases that were once common in animals imported in the 1960s and 1970s are now rare. It is also important to recognize that housing and sanitation conditions can affect exposure to potentially pathogenic bacteria. Animals raised in areas with biosecurity measures in place may be exposed to fewer pathogens than animals raised in mixed or free-range environments. Much of the available literature describing the clinical and epizoological features of bacterial infections of this type is dated and scientists should be encouraged to study the prevalence in rats and publish their results in more detail.
Keywords: *Bacterial disease, infection, rat, mice*

1.Introduction

The mouse microbiome, defined as all bacterial species found in or on the mouse, and their interactions among themselves and with their environment, are always present. Non-infective agents or pathogenicity of infective agents can lead to various diseases. Rats, the most commonly used species of experimental animals, are susceptible to a range of bacterial infections that can be enzootic or sporadic but can have high morbidity and motility rates leading to severe disease. Animals raised in areas where biosecurity measures are emphasized may be exposed to many more potential pathogens than animals raised in mixed or free-range environments. Much of the available literature describing the clinical and epizoological features of bacterial infections of this type is dated and scientists should be encouraged to study the prevalence in rats and publish their results in more detail.

2. Bacterial infections

2.1. Mycoplasma spp. infections

Nocard and Roux isolated some bacteria from cattle with pneumonia in 1898, and in 1929 Novak proposed the name “mycoplasma” for these bacteria, which lack cell walls, although they have divided strands during production and reproduction. Mycoplasmas are reported to be the smallest bacterial organisms without a cell wall and have been associated with various pathological conditions.[1] By analyzing the sequence of the 16S rRNA gene, it is thought that mycoplasma evolved from gram-positive bacteria and clostridia about 600 million years ago, losing redundant parts of its genome. These microorganisms are small, Gram-negative and have no cell walls, but are surrounded by a membrane. These microorganisms usually grow relatively slowly and usually survive in environments where the ambient temperature is about 37-38°C. In laboratory settings, five main mycoplasma species have been identified, namely *M. arthritidis, M. collis, M. muris, M. neurolyticum* and *M. pulmonis*.[2]

M. pulmonis is responsible for one of the most common mycoplasma contaminations in rats, and these contaminations are often a major concern in research laboratories and animal research.[3] *M. pulmonalis* can be isolated from the ovaries, uterus and respiratory systems of rats. This isolation is reported to demonstrate the potential for mycoplasma infections to spread to various body systems.[4]

M. pulmonis infection is quite common in rats and usually affects the middle ear cavity. This middle ear infection leads to a condition called otitis media and can eventually cause twisting of the neck or torticollis. The infection can also cause serious respiratory problems and reproductive tract infections in rats.[5] This disease has a prevalence of 20% to 60% among experimental animals. It can also colonize the trachea and throat, causing pneumonia.[1] Mycoplasma transmission during fetal

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development can occur in two ways. The first is transmission through amniotic fluid and the second is intrauterine infection or transmission during implantation. These situations illustrate the possible routes of spread of mycoplasma infections during fetal development and are important for understanding the risks in the prenatal period.

Mycoplasma neurolyticum was first isolated from the brains of rats in 1938. Later, in 1965, *M. neurolyticum* was isolated from the nasal mucosa and lungs of carrier animals that showed no clinical symptoms. In 1979, Hill studied *M. neurolyticum* in rats and rats and the results showed that *M. neurolyticum* infected 78% of rats and 58% of rats. In 1981, Taley identified the bacterium as a mammalian brain organism that causes nervous disorders under stress.[6]

Mycoplasma collis was first isolated from the nasal cavity and conjunctiva of rats and rats in 1983.[7] This species of mycoplasma grows in anaerobic conditions with an optimum temperature of 35°C and pH=7.8. Some researchers have identified this mycoplasma species as canine mycoplasma, but *M. collis* was first described in rodents.[8]

Mycoplasma muris was identified in 1983 by McGarrity et al. in a study based on the immune response of rats. In this study, it was reported that all rats were pregnant and had tumors. It was reported that the age group of the rats was 3 and 10 months and a new mycoplasma species called Muris emerged based on the morphological similarity with mycoplasma. *M. muris* are small pathogenic bacteria that live in the genital tract of female rats. *M. muris* infection may have detrimental effects on the reproductive health of female rats.[9]

Based on the 16s rRNA gene, Weisburg et al. identified *M. muris* as the ancestor of the pneumonia group consisting of three distinct clusters, *M. pneumonia, M. muris* and *Ureaplasma urealyticum*.[10] Designed specific primers from 16s rRNA for nine mycoplasma species for humans and rodents and evaluated them by PCR assay. In 2017, Zinatizadeh et al. identified this mycoplasma in rats at the Razi Vaccine and Serum Research Institute in Alborz, Iran. A total of 18% of rats in the Animal Breeding Department of the Razi Vaccine and Serum Research Institute were infected with *M. muris* and a new species of *M. muris* was registered in the gene bank using a phylogenetic analysis.[11]

Mycoplasma arthritidis infection is not common and is usually found in large laboratory mice. *M. arthritidis* causes joint arthritis in mice. Some researchers believe that the microorganism enters the body through the mouth and mucous membranes and may be a latent infection.[12] Clinical signs include swelling of the fingers and legs. This species of mycoplasma grows in a neutral pH environment (7.0) at an ideal temperature of 37°C and can grow in the presence or absence of oxygen. The growth of *M. arthritidis* depends on the culture medium. In fiber tissue, it expands as a dense mass in the center and requires sugar, proteins, amino acids, vitamins and nucleic acids for growth.[13]

2.2. Clostridium piliforme infection (Tyzzer's Disease)

Clostridium piliforme is an obligate intracellular bacterium that can stain Gram-negatively and has variable morphology. Most often, it appears as a long, thin bacillus about 8-10 µm long and 0.5 mm wide in the cytoplasm of infected cells, but shorter, thicker, cigar-shaped forms are also occasionally seen. *Cl. piliforme*, which is considered incapable of being grown in artificial media, can be grown in intestinal cell lines, primary chick or mouse liver cells, or in embryonated chicken eggs.[14,15]

Cl. piliforme is the causative agent of Tyzzer's disease. It is spread by spores shed in feces. Can be isolated from different host species.[16] A high molecular weight exotoxin has been associated with pathogenicity in vitro and in vivo. Not all strains produce this toxin, indicating differences in pathogenicity between strains. After ingestion of spores, the bacterium is phagocytosed by intestinal epithelial cells. Inside the cell, the vegetative form escapes the phagosome and begins replication in the cytoplasm. Eventually the cell dies and the bacteria are either released back into the lumen or travel deeper into the intestinal wall where they can sometimes infect smooth muscle cells or access the portal circulation.[17]

Bacteria traveling through the portal vein can infect the liver and/or heart. Infection is usually asymptomatic, with disease occurring primarily in weaned pups and immunocompromised mice, or perhaps in overcrowded environments or concurrent infection with secondary infections. Serologic surveys have either not reported the prevalence of *Cl. piliforme* antibodies or have found seroconversion to be sporadic. However, disease is rare in biosecure laboratory facilities.[18]

Serologic surveillance for *Cl. piliforme* typically uses a whole-cell antigen preparation containing a complex mixture of bacterial proteins. When positive serologic results are obtained, it can be difficult to distinguish a false positive result from a true positive result. Although some clues can be obtained from the score of the positive titer (a high titer is more likely to indicate a true positive), a single positive serology result for *Cl. piliforme* should be followed by additional testing, most often screening of additional serum samples.[18,19]

Two other laboratory methods for the detection of *Cl. piliforme* are polymerase chain reaction (PCR) and histopathologic evaluation. PCR will be positive in fresh tissues if the lesions are due to *Cl. piliforme*. Histopathologic evaluation can be performed on sections cut from paraffin blocks, fecal or environmental samples after the lesions are seen. If fecal screening for *Cl. piliforme* by PCR is to be performed from a group of asymptomatic mice, feces should be collected from mice within 2 weeks of the time of infection, which is thought to be during the weaning period. Mice 4-6 weeks old from a suspected breeding colony are a good age group for screening. Older mice are likely to have cleared the infection and very young mice may not be infected while protected by maternal antibodies. PCR in fecal samples for Cl. piliforme can also be complicated by the occasional presence in feces of substances that can inhibit PCR reactions and the degree of protection provided by the thick wall of the spore, which can make DNA extraction difficult.[19]

Most infected mice present an asymptomatic clinical picture. If disease does occur, it is usually seen in newly weaned rats or rats with genetic or induced immuno-

deficiency. Although weaned rats with Tyzzer's disease have been reported to have a bloated abdomen due to megaloileitis[20], this has not been reported in mice. Mice with Tyzzer's disease may have diarrhea and perianal spotting or may appear weak and unkempt for a short time. Sudden death without prior symptoms may also occur. At necropsy, the ileum, secum and colon may be slightly enlarged and reddened due to hyperemia or mild bleeding. Tyzzer's disease is not usually thought to produce overt ulcerative enteritis. If the infection has spread past the intestinal tract, white or tan foci may be distributed throughout the liver, but vary greatly in number and size (Figure 1). Pale streaks or patches of myocardial necrosis may also be seen in the heart. Giemsastained print smears of liver lesions will often show the characteristic tangle of piliform organisms if the lesions are due to *Cl. piliforme*. Evaluation of such tissue smears is recommended as a useful method for a rapid diagnosis.[21]

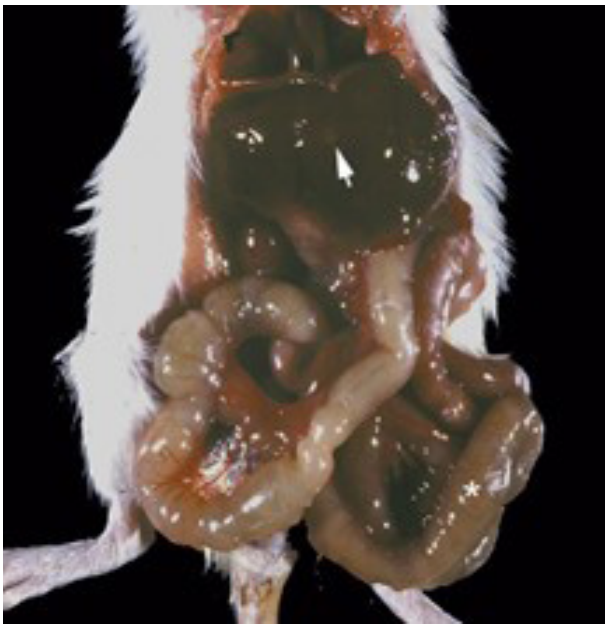

Figure 1. Liver necrosis in Tyzzer disease due to *Cl. piliforme.*[21]

The gold standard for confirmation of *Cl. piliforme* infection has traditionally been the observation of characteristic intracellular bacilli in lesions, but additional support from PCR should be sought when available.[21]

Detection of antibodies to *Cl. piliforme* in mature, non-immunocompromised mice may not always indicate that the mice are actively infected.[22] Subclinical infection, which can occur within approximately the first 2 weeks after infection, can alter cytokine profiles and hemodynamic parameters as well as tumor necrosis factor alpha and interferon gamma for at least several weeks.[23] The major concerns about the presence of *Cl. piliforme* are the clear risk of disease, especially in young mice in breeding colonies, and the difficulty in removing spores from the environment. If a conscious management decision is made that *Cl. piliforme* needs to be eliminated from mice in a research facility, cleaning of all surfaces and equipment and autoclaving of all materials or through disinfection with a high-level disinfectant capable of killing clostridial spores will increase the probability of success.[24,25]

2.3. Streptobacillus moniliformis infection

The causative agent is taxonomically positioned within the Class *Fusobacteria*, Order *Fusobacteriales*, Family *Leptotrichiaceae*, with the genus and species *S. moniliformis*. Despite its monospecific status for almost 90 years, the genus is now recognized to harbor an additional number of species based on genomic analysis, including *Streptobacillus hongkongensis* and *Streptobacillus felis.*[26]

Streptobacillus moniliformis has been indirectly associated with various aspects of respiratory diseases in laboratory rats. Other features of the organism include the pathogenic properties of *S. moniliformis* in rat bite and Haverhill fevers in humans.[15] The Norway rat is considered the leading reservoir host for *S. moniliformis* and is carried asymptomatically by most wild Rattus norvegicus and quite commonly by domestic and fancy rats. *S. moniliformis* has been eradicated mainly from laboratory rats by the gnotobiotic process, but is still encountered serologically or by PCR during diagnostic screening of conventional rat enterprises. Although a streptothrix-like organism was identified in the blood of some human patients with recurrent fever after a rat bite, the organism was not isolated and characterized in pure culture until 1914.[27] *S. moniliformis* clinically causes respiratory diseases in rats, particularly pneumonia and otitis media. Over the years, studies have reported that this organism is localized in the nasopharynx, oropharynx and saliva in rats.[15,21]

Currently, *S. moniliformis* is considered to be an opportunistic bacterium with low pathogenic potential for rats. Today, the incidence of *S. moniliformis* in the laboratory has been markedly reduced by higher standards of animal care and the general use of gnotobiotic-derived laboratory mice. However, it is occasionally isolated from both wild and conventional laboratory mice or its presence is detected serologically or by PCR.[28]

Laboratory mice may serve as zoonotic reservoirs for *S. moniliformis* infections.[29] It can also act as a reservoir for wild rat infections in nature.[30] Unlike rat infections, which are usually asymptomatic, *S. moniliformis* infections are not asymptomatic. Infections in mice can be characterized by generalized septicemia, lymphadenitis, osteomyelitis of the lower hind limbs and caudal vertebrae, and polyarthritis. As in humans, septicemia and polyarthritis in mice can develop following rat bites.[28] In addition to the information provided, no human-to-human transmission has been reported.[31]

Diagnosis is based on isolation and cultural characterization of *S. moniliformis*. Important confirmatory information can be obtained by PCR with swabs from multiple clinical sites such as pharynx, trachea and lymph nodes.[32] Cultural isolation of *S. moniliformis* from clinical material is difficult, especially from rats without lesions, even when known to be positive serologically or by PCR.[31] Smears from blood or other clinical material often show small (less than 1 mm wide and 1-5 mm long) Gram-negative rods and filaments.[26] PCR is seen as the main means of detecting this organism in both human and rodent clinical material.[32] PCR can be used to screen rodent colonies for the presence of *S. moniliformis* and has been used in feral rat microbiology research.[33]

Primer sets have been developed to identify *S. moniliformis* down to the genus level for screening purposes.[32]

2.4. Streptococcus pneumoniae infection

S. pneumoniae was not recognized as a pathogen of laboratory rats until the first half of the 20th century. In the second half of the 20th century, *S. pneumoniae* was recognized as the most common pathogen for acute respiratory disease in rats of all age groups. It has been reported that the nasopharyngeal microbiome of humans in contact with laboratory mice can be observed asymptomatic organisms. The incidence of *S. pneumoniae* infection has decreased considerably in recent years.[34,35]

However, the infection is not normally subtle and the clinical signs of respiratory disease with varying degrees of mortality are more pronounced. Serosanguinous to mucopurulent nasal discharges are often the first signs of clinical disease and often precede pulmonary involvement. Rhinitis, sinusitis, conjunctivitis and otitis media are common major lesions of upper respiratory tract infection. Outward protrusion of the eardrum can be recognized as a sign of pus under pressure in the middle ear. Histologically, mucopurulent exudates cover the respiratory mucosa of the turbinates, sinuses, eustachian tube, nasolacrimal duct and tympanic cavity. Acute inflammatory cells, especially neutrophils and more chronic inflammatory cells including plasma cells and lymphocytes, infiltrate the mucosa and submucosa. Numerous organisms can be seen in exudates and superficial levels of mucosa by tissue Gram stains or smears. Concomitant clinical signs may include changes in posture, abdominal breathing after pneumonia. Dyspnea, conjunctival exudation, anorexia with weight loss, depression and/or sniffling abnormal respiratory sounds. The onset of clinical signs and lesions is often acute or subchronic rather than chronic and affects rats of all ages, especially younger age groups.[17,21]

The typical progression of upper respiratory tract infections involves progression from the nasopharynx into the lung tissues. In the initial stage, fibrinous bronchopneumonias affecting specific lobes rapidly develop into fibrinous lobar pneumonia. Microscopically, the mucosa of the trachea, bronchi and bronchioles is necrotic and fluid and purulent exudates, often with blood, accumulate in the lumen. Alveolar capillaries are occluded and therefore the alveoli appear to be filled with blood, proteinaceous fluids, neutrophils or varying combinations of these. Tissue Gram stains reported abundant intracellular Gram-positive cocci in affected tissues in microcolonies, freely dispersed single cells and phagocytic cells including neutrophils. Depending on the severity of lung involvement, loss of life may occur at this stage. However, usually the organism escapes into the thoracic cavity, where lesions of fibrinous pleuritis, pleural effusion and fibrinous pericarditis are common. The organisms may progress to a septicemia originating from the thoracic organs, which is often the result. In rarer cases, the organism is embolically distributed to various organs and sites, resulting in complications such as purulent arthritis, focal necrosis or infarction of the liver, spleen and kidneys, and fibrinopurulent peritonitis, orchitis and meningitis. In serum biochemical evaluations of infected rats, changes in glutamic-pyruvic transaminase, glutamicoxaloacetictransaminase and lactate dehydrogenase enzymes can be observed.[15,36]

The diagnosis can be established by isolation of S. pneumoniae from affected tissues. It is supported by characteristic macroscopic and microscopic lesions. The diagnosis can be established by culture of nasopharyngeal swabs from rats or by washing the ear cavity and nasoturbinates. The upper respiratory tract may be more preferable as it is where the organism shows the highest growth in rats. After inoculation on blood agar, encapsulated pneumococcal colonies appear circular and 1-2 mm in diameter with steep edges. At the beginning of the incubation period the colonies are dome-shaped and shiny, but after 24-48 hours the centers collapse due to autolysis, giving the top of the colony a typical concave umbilicus. S. pneumoniae colonies are surrounded by a small (alpha) zone of hemolysis with greenish discoloration of the medium.[37]

Although *Enterococcus spp.* belonging to the *Streptococcus spp.* group have traditionally been considered a genus of *streptococci*, *fecal streptococci* are classified as a separate genus *Enterococcus. Enterococci* are catalasenegative and facultative anaerophilic, forming punctate colonies. They usually show mild a-hemolysis and occur in chains. Rodent clinical infections have been determined to occur mainly in phenotypic group III of *enterococci* species closely related to *Enterococcus faecium, including Enterococcus durans, Enterococcus dispar* and *Enterococcus hirae.* This group cannot grow on tellurite-containing media and is generally negative for mannitol, sorbitol and the acid in sorbose. Subsequent studies with new and reference *Enterococcus* isolates from rats revealed a new species, *Enterococcus ratti,* closely related to *E. durans* and *E. hirae* as a common cause of naturally occurring pathogens.[38,39]

Enterococci are ubiquitous in nature and are usually carried as bacterial commensals in the human gastrointestinal tract. They are becoming increasingly important as causes of nosocomial nosocomial infections in patients receiving antimicrobial regimens. *E. faecium* and *Enterococcus faecalis*, the most common species in humans, are likewise most commonly carried by laboratory mice. Most clinical cases have been reported to occur in accredited animal care settings in rats, where common pathogens are known to be absent. In rats, enterococci are also normally carried in the gastrointestinal tract as non-pathogenic commensals and may even be considered to have a probiotic function as their abundant presence competes with other forms to act as an inhibitor on the rise of other bacterial pathogens.[40] Probiotics are reported to lower intestinal pH through the organic acids they secrete and stimulate systems with bactericidal effect.[41]

In some cases enterococci have been identified as causative in enteropathic diarrhea syndrome in neonatal rats. Species implicated include *E. durans* and also Enterococcus-like agents that have not been further characterized.[38] However, as mentioned earlier, all of these are now recognized as possible *E. ratti* infections.[39] Clinical episodes tend to occur in the 6 to 12 day age group with high morbidity and low mortality. Clinical signs typically include abdominal distension and coarse, yellowish matted hair, perineal scalding and soiling and ulcerati-

on of the skin of the hind limbs.[39] The cages of affected fry have wet bedding and often lack formed feces. Soft feces can also be found in dams with affected fry.[42] At autopsy, the stomachs of affected puppies are consistently filled with clotted milk. Typically, the small and large intestines are distended with gas and yellowish fluid and the other internal organs are usually normal in appearance. Microscopic changes are very few. Numerous cocci can be seen in aggregates on the surface of villi and villi tips in the small intestine, often without a significant inflammatory component.[38]

All investigators emphasized that isolation of an *Enterococcus* species alone is not diagnostic of neonatal enteropathy. This is because many, perhaps all, clinically normal rats carry commensal enterococci after removal from gnotobiotic isolates, and biochemically identical isolates can differ significantly in pathogenic potential.[38] Although virulence factors such as adhesins and cytolysins are receiving increasing attention, little is known about how such factors are triggered to appear in pathogenic strains, and none have been sufficiently characterized to serve as diagnostic criteria. Considering newer information on the gut microbiome, it can be hypothesized that dysbiosis due to antibiotic treatment or microbial restriction through gnotobiotic derivation may play a role in the pathogenic potential of this commensal.[43]

2.5. Pasteurellaceae spp. infection

As a genus, Pasteurellaceae are Gram-negative, fermentative, immobile, oxidase and catalase positive coccobacilli that fail to grow on MacConkey agar. The three classical genera Pasteurella, Actinobacillus and Haemophilus can be considered as bases. In the past decade, the taxa of the rodent Pasteurellaceae have been revised very significantly through genetic and phenotypic studies. Rodent isolates classified as Pasteurellaceae have been reclassified into six new genera.[15,21] These are *Rodentibacter*[44], *Muribacter*[45], *Cricetibacter, Mesocricetibacter*[46], *Mannheimia*[47] *and Necropsobacter*[48].

The most frequently reported rodent pathogen of the *Pasteurellaceae* family is *P. pneumotropica*. It has been associated mainly with the respiratory tract of laboratory rodents. *P. pneumotropica*, It has emerged as an important and worldwide widespread infectious agent of laboratory mice since the 1950s. A report based on data from a large commercial rodent diagnostic laboratory found the prevalence of *P. pneumotropica* to be 4.81% from 8,241 cultures sampled.[18] Microbiological tests on mice from 161 universities and 101 pharmaceutical companies in Japan found the prevalence of *P. pneumotropica* to be 4.35% and 0.99%, respectively.[49]

Clinical experience reports that the immunological disorder of rats is a highly effective factor in infection 50,51. Therefore, the immune competence of the host appears to be as important as the pathogenicity of the organism in determining the outcome of infection.[52] Under conditions of natural infection, *P. pneumotropica* has been most frequently isolated from the microflora of the nasoturbinates, pharynx, conjunctiva, trachea, lungs and uterus. Less commonly, infection of deeper organs such as the liver, spleen, and kidney of laboratory mice has been observed. When the ways and methods of *P. pneumotropica's* infection spread are evaluated, it can spread to the middle ears and eyes through the Eustachian tube and nasolacrimal duct. It can also infect the preputial glands, vagina, uterus, skin (Figure 2) and breast tissues by contact with or biting the genital areas. [53]

Figure 2. Skin effects in a mouse with *P. pneumotropica.*[21]

The pathology of *P. pneumotropica* infection is not dissimilar and resembles the pathology caused by various pyogenic bacteria in similar sites. Subcutaneous abscesses of the skin, adnexal organs, or orbital structures are often encapsulated and filled with liquid exudates. Microscopically, the lesions are suppurative with fluid-active necrosis surrounded by a central zone of granulomatous inflammation. Clinically latent infections in the lungs, upper respiratory tract, uterus, and intestines often occur without histopathological evidence of epithelial inflammation. In the lungs of mice, areas of consolidation can be produced by perivascular and peribronchial infiltration of acute and chronic inflammatory cells, but this lesion is not evident.[15]

Diagnosis of *P. pneumotropica* can be made by cultural and biochemical, serological and PCR analysis/analysis. Diagnosis of *P. pneumotropica* infection is made by isolation of the organism in pure culture from infected tissues. Primary isolation of the organism can be performed on blood agar using swabs from epithelial surfaces or lesion contents or aspirates from nasoturbinates, larynx, or trachea. Isolation from feces or intestinal contents by use of a selective medium. Blood agar cultures should be incubated in a moist, microaerophilic environment. After 24 to 48 hours, colonies become smooth, convex, and greyish, 1 to 2 mm in diameter, nonhemolytic. Colonies with gram-negative, fermentative coccobacilli, nonmotile, and catalase, oxidase, and nitrate-positive organisms can be identified as *P. pneumotropica* isolates.[21]

2.6. Helicobacter spp. infection

Helicobacter spp. was detected as a result of incidental symptoms of chronic hepatitis in various rat species at the Frederick Cancer Center experimental animal production center. detected and reported.[54,55] A newly identified spiral bacterium isolated from affected livers and later named *Helicobacter hepaticus* was reported to cause the lesions. Spiral bacteria are also reported to be present in the cecum and colon mucosa of mice. The

H. hepaticus cases initiated an intensive diagnostic and research effort to characterize the nature and biology of a new genus of gastrointestinal pathogens in infectious diseases of laboratory animals.[56]

The genus Helicobacter is generally divided into two groups. These are gastric *Helicobacter* spp. and enterohepatic *Helicobacter* spp. They are separated according to their location. It is also reported that enterohepatic groups settle in the gallbladder It is stated that there are seven enteropathic Helicobacter species known to naturally inhabit mice. These are *Helicobacter bilis, Helicobacter trogontum, Helicobacter muridarum, Helicobacter rodentium, Helicobacter typhlonius, Helicobacter ganmani* and *Helicobacter pullorum.* However, only *H. bilis* has been shown to cause clinical disease in rats.[57] In their study, during the autopsy of a male rat, they detected the presence of spiral-shaped bacteria in the cecal crypts. Following this first case, autopsies were performed on 10 more males aged 5-8 months from the same colony. It was reported that 5 out of 11 rats had clinical signs such as acute perianal inflammation and mild diarrhea. Each of these 5 rats was reported to have focal or diffuse whitish thickened areas in the cecum. Among the study results, 8 of 11 rats had proliferative colitis and proctitis of varying severity. It has been reported that the most severe large intestine lesions are in the cecum. Microscopic examination revealed that the crypt epithelium was hyperplastic and there was a significant decrease in goblet cells. After staining, curved/spiral rod-like bacterial masses compatible with Helicobacter were observed. No other significant lesions were found in other major organs, including the stomach, small intestine, and liver. Identification of the isolates as *H. bilis* was achieved by PCR, amplicon sequencing and electron microscopy.[58]

H. pullorum, a known pathogen in poultry and humans, has been reported to grow naturally or experimentally in rodents.[59] In one study, mice given the organism orally were found to be *H. pullorum* positive by fecal PCR for the entire 30-week study and had an IgG antibody response to infection. It has been reported that this is the first study investigating enterohepatic *Helicobacter spp*. This organism that infects humans has also been reported to persistently infect mice. Among the results of the autopsy were that the cecum and colon were the primary sites for localization of the organism. However, the information in the study shows that no intestinal or liver pathology was observed in any of the rats infected with *H. pullorum*.[60]

Clinical findings can often be associated with inflammatory or neoplastic diseases present in the mouse. Inflammatory lesions in the cecum and colon may be encountered in rats with strong immunity. The incidence of helicobacter-induced liver neoplasia is generally higher in A/JCr mice. The most common clinical finding in Helicobacter-infected mice is prolapse of the rectum (Figure 3). *H. Hepaticus* is the most common cause of rectal prolapse in rats.[21]

Culture has been reported to be widely used as a screening method for the microbiological isolation of enterohepatic Helicobacter species. Serology is one of the methods used as a screening method.[61] However, similar to cultural isolation, it has significant limitations that prevent it from being validated as a generally useful diagnostic tool. Because antibody levels against Helicobacter are generally proportional to the intensity of the microbial challenge, positive serology is likely reliable as an indicator of exposure to the antigens used in the test. The problem is that there are several causes of negative serology. Generally speaking, this is not a reliable result for many reasons, including innate host resistance or significant antigenic challenge that prevents lesion development. However, it can be expressed as the possibility of false negatives due to initially low Helicobacter populations in the gastrointestinal reservoir, serum samples that can be taken early before detectable antibody levels develop, and perhaps most importantly, infection caused by Helicobacter species that stimulate antibodies that do not recognize the antigens used in the test.[15]

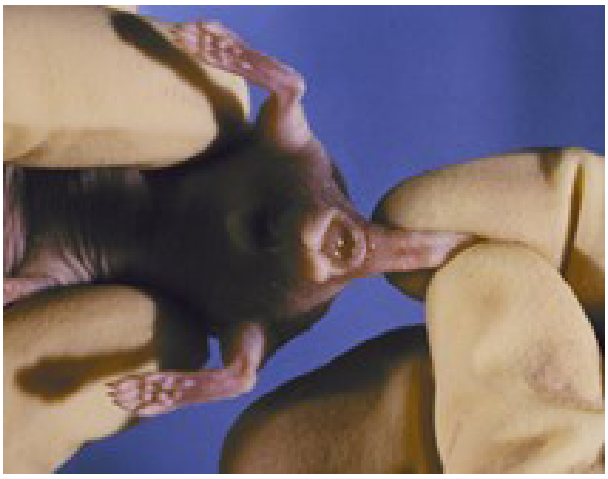

Figure 3. Rectum prolapse in a Helicobacter-infected rat.[21]

Diagnosis of Helicobacter colonization in rats is best accomplished using genus and species-specific PCR analyses. PCR is the most useful screening test for the detection of mouse helicobacteria from clinical materials, including tissue and fecal samples.[62] The method is based on the detection of unique and subsequently amplified 16S rRNA gene sequences of the agent extracted from the sample material. It can be used to identify Helicobacter DNA. The procedure is specific to one or more Helicobacter sequences determined by the primers used for amplification and is not complicated by the presence of contaminating microorganisms in the sample material. An important feature of the PCR test is the extreme sensitivity in detecting low numbers of Helicobacter in sample samples. This assay method eliminates the need for PCR post-processing, can provide greater specificity and enable quantitative determination of bacterial load. For screening programs, the combination of PCR and serology appears to offer superior detection rates.[61]

3. Conclusion

As a result, *Mycoplasma Spp., Clostridium Piliforme, Streptobacillus Moniliformis, Streptococcus Pneumoniae, Pasteurellaceae Spp*., and *Helicobacter Spp* which are frequently observed in rats. The potential effects of infections such as these on the health of mice and research should be evaluated individually. It is very impor-

tant in terms of the reliability of studies on the effects of microorganisms on rats. The effects of infections one by one or separately and their diagnostic methods should be evaluated. The selection of the diagnostic method to be used for the samples to be taken is important for the diagnostic criteria. It is very important for the reliability of the studies that the mice are germ free.

Ethical approval

This study does not require approval from the Ethics Committee for Animal Experiments.

Conflict of interest

There are no conflicts of interest associated with this re-search publication, according to the authors.

Data availability

The data that support the findings of this study are ava-ilable from the corresponding author upon reasonable request.

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This situation does not exist.

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References

1. Masoumalinejad Z, Zinatizadeh MR, Tahmasebiabdar N. A Review of Mycoplasma in Laboratory Mice. Modern Medical Laboratory Journal. 2019;2(2):127-131. doi:10.30699/mmlj17.2.1.15
2. Brenner DJ, Krieg NR, Staley JR. Bergey's Manual® of Systematic Bacteriology: Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria. Springer Science & Business Media; 2007.
3. van Kuppeveld FJ, van der Logt JT, Angulo AF, et al. Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. Appl Environ Microbiol. 1992;58(8):2606-2615. doi:10.1128/aem.58.8.2606-2615.1992
4. Sanchez S, Tyler K, Rozengurt N, Lida J. Comparison of a PCR-based diagnostic assay for Mycoplasma pulmonis with traditional detection techniques. Lab Anim. 1994;28(3):249-256. doi:10.1258/002367794780681570
5. Booth JL, Umstead TM, Hu S, et al. Housing conditions modulate the severity of Mycoplasma pulmonis infection in mice deficient in class A scavenger receptor. Comp Med. 2014;64(6):424-439.
6. Bhatt P. Viral and Mycoplasmal of Laboratory Rodents: Effects on Biomedical Research. Elsevier; 2012.
7. HILL AC. Mycoplasma collis, a New Species Isolated from Rats and Mice. International Journal of Systematic and Evolutionary Microbiology. 1983;33(4):847-851. doi:10.1099/00207713-33-4-847
8. Johansson KE, Pettersson B. Taxonomy of Mollicutes. In: Razin S, Herrmann R, eds. Molecular Biology and Pathogenicity of Mycoplasmas. Springer US; 2002:1-29. doi:10.1007/0-306-47606-1_1
9. McGARRITY GJ, ROSE DL, KWIATKOWSKI V, DION AS, PHILLIPS DM, TULLY JG. Mycoplasma muris, a New Species from Laboratory Mice. International Journal of Systematic and Evolutionary Microbiology. 1983;33(2):350-355. doi:10.1099/00207713-33-2-350
10. van Kuppeveld FJ, van der Logt JT, Angulo AF, et al. Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. Appl Environ Microbiol. 1993;59(2):655. doi:10.1128/aem.59.2.655-.1993
11. Zinatizadeh MR, Abedini F, Jafarpour M, Masoumalinejad Z. Identification of Mycoplasma Muris Isolated from Vaginal Samples of NIH Mice. Modern Medical Laboratory Journal. 2019;2(1):100-106. doi:10.30699/mmlj17.1.3.100
12. Constantopoulos G, McGarrity GJ. Activities of oxidative enzymes in mycoplasmas. J Bacteriol. 1987;169(5):2012-2016. doi:10.1128/jb.169.5.2012-2016.1987
13. Barden JA, Tully JG. Experimental arthritis in mice with Mycoplasma pulmonis. J Bacteriol. 1969;100(1):5-10. doi:10.1128/jb.100.1.5-10.1969
14. Sirois M. Laboratory Animal and Exotic Pet Medicine: Principles and Procedures. Elsevier; 2022.
15. Suckow MA, Hankenson FC, Wilson RP, Foley PL. The Laboratory Rat. Academic Press; 2019.
16. Franklin CL, Motzel SL, Besch-Williford CL, Hook RR, Riley LK. Tyzzer's infection: host specificity of Clostridium piliforme isolates. Lab Anim Sci. 1994;44(6):568-572.
17. Mansfield KG, Fox JG. Bacterial Diseases. The Common Marmoset in Captivity and Biomedical Research. Published online 2019:265-287. doi:10.1016/B978-0-12-811829-0.00016-9
18. Pritchett-Corning KR, Cosentino J, Clifford CB. Contemporary prevalence of infectious agents in laboratory mice and rats. Lab Anim. 2009;43(2):165-173. doi:10.1258/la.2008.008009
19. Livingston RS, Riley LK. Diagnostic Testing of Mouse and Rat Colonies for Infectious Agents. Lab Anim (NY). 2003;32(5):44-51. doi:10.1038/laban0503-44
20. Hansen AK, Andersen HV, Svendsen O. Studies on the diagnosis of Tyzzer's disease in laboratory rat colonies with antibodies against Bacillus piliformis (Clostridium piliforme). Lab Anim Sci. 1994;44(5):424-429.
21. Hedrich HJ, Bullock GR. The Laboratory Mouse. Elsevier Academic Press; 2004.
22. Van Andel RA, Hook RR, Franklin CL, Besch-Williford CL, Riley LK. Interleukin-12 Has a Role in Mediating Resistance of Murine Strains to Tyzzer's Disease. Infect Immun. 1998;66(10):4942-4946.
23. VAN Andel RA, Franklin CL, Besch-Williford CL, Hook RR, Riley LK. Prolonged perturbations of tumour necrosis factor-alpha and interferon-gamma in mice inoculated with Clostridium piliforme. J Med Microbiol. 2000;49(6):557-563. doi:10.1099/0022-1317-49-6-557
24. Fraise A. Currently available sporicides for use in healthcare, and their limitations. J Hosp Infect. 2011;77(3):210-212. doi:10.1016/j.jhin.2010.06.029
25. Perez J, Springthorpe VS, Sattar SA. Activity of selected oxidizing microbicides against the spores of Clostridium difficile: relevance to environmental control. Am

J Infect Control. 2005;33(6):320-325. doi:10.1016/j.ajic.2005.04.240

26. Eisenberg T, Nicklas W, Mauder N, et al. Phenotypic and Genotypic Characteristics of Members of the Genus Streptobacillus. PLoS One. 2015;10(8):e0134312. doi:10.1371/journal.pone.0134312

27. Waelsch L. Dermatologische Wochenschrift. Arch f Dermat. 1912;115(2):183-186. doi:10.1007/BF01838920

28. Easterbrook JD, Kaplan JB, Glass GE, Watson J, Klein SL. A survey of rodent-borne pathogens carried by wild-caught Norway rats: a potential threat to laboratory rodent colonies. Lab Anim. 2008;42(1):92-98. doi:10.1258/la.2007.06015e

29. Glastonbury JR, Morton JG, Matthews LM. Streptobacillus moniliformis infection in Swiss white mice. J Vet Diagn Invest. 1996;8(2):202-209. doi:10.1177/104063879600800210

30. Taylor JD, Stephens CP, Duncan RG, Singleton GR. Polyarthritis in wild mice (Mus musculus) caused by Streptobacillus moniliformis. Aust Vet J. 1994;71(5):143-145. doi:10.1111/j.1751-0813.1994.tb03368.x

31. Gaastra W, Boot R, Ho HTK, Lipman LJA. Rat bite fever. Vet Microbiol. 2009;133(3):211-228. doi:10.1016/j.vetmic.2008.09.079

32. Boot R, Oosterhuis A, Thuis HCW. PCR for the detection of Streptobacillus moniliformis. Lab Anim. 2002;36(2):200-208. doi:10.1258/0023677021912352

33. Kimura M, Tanikawa T, Suzuki M, et al. Detection of Streptobacillus spp. in feral rats by specific polymerase chain reaction. Microbiol Immunol. 2008;52(1):9-15. doi:10.1111/j.1348-0421.2008.00005.x

34. Cremers AJ, Zomer AL, Gritzfeld JF, et al. The adult nasopharyngeal microbiome as a determinant of pneumococcal acquisition. Microbiome. 2014;2(1):44. doi:10.1186/2049-2618-2-44

35. Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R. Comparative Analyses of the Bacterial Microbiota of the Human Nostril and Oropharynx. mBio. 2010;1(3):10.1128/mbio.00129-10. doi:10.1128/mbio.00129-10

36. Mitruka BM. Biochemical aspects of Diplococcus pneumoniae infections in laboratory rats. Yale J Biol Med. 1971;44(3):253-264.

37. Kontiokari T, Renko M, Kaijalainen T, Kuisma L, Leinonen M. Comparison of nasal swab culture, quantitative culture of nasal mucosal tissue and PCR in detecting Streptococcus pneumoniae carriage in rats. APMIS. 2000;108(11):734-738. doi:10.1034/j.1600-0463.2000.d01-22.x

38. Gades NM, Mandrell TD, Rogers WP. Diarrhea in Neonatal Rats. Contemp Top Lab Anim Sci. 1999;38(6):44-46.

39. Teixeira LM, Carvalho MG, Espinola MM, et al. Enterococcus porcinus sp. nov. and Enterococcus ratti sp. nov., associated with enteric disorders in animals. International Journal of Systematic and Evolutionary Microbiology. 2001;51(5):1737-1743. doi:10.1099/00207713-51-5-1737

40. Hansen AK, Nielsen DS. Handbook of Laboratory Animal Bacteriology, Second Edition. CRC Press; 2014.

41. Kucukoflaz M, Ozbek V, Sariözkan S, Kocaoğlu Güçlü B, Kara K. Growth Performance, Ruminal Volatile Fatty Acids, Health Status and Profitability in Calves Fed with Milk Supplemented with Probiotics. Kafkas Unıversıtesı Veterıner Fakultesı Dergısı. 2022;28. doi:10.9775/kvfd.2022.27203

42. Hoover D, Bendele SA, Wightman SR, Thompson CZ, Hoyt JA. Streptococcal enteropathy in infant rats. Lab Anim Sci. 1985;35(6):635-641.

43. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's Diagnostic Microbiology. Elsevier Mosby; 2007.

44. Adhikary S, Nicklas W, Bisgaard M, et al. Rodentibacter gen. nov. including Rodentibacter pneumotropicus comb. nov., Rodentibacter heylii sp. nov., Rodentibacter myodis sp. nov., Rodentibacter ratti sp. nov., Rodentibacter heidelbergensis sp. nov., Rodentibacter trehalosifermentans sp. nov., Rodentibacter rarus sp. nov., Rodentibacter mrazii and two genomospecies. Int J Syst Evol Microbiol. 2017;67(6):1793-1806. doi:10.1099/ijsem.0.001866

45. Nicklas W, Bisgaard M, Aalbæk B, Kuhnert P, Christensen H. Reclassification of Actinobacillus muris as Muribacter muris gen. nov., comb. nov. Int J Syst Evol Microbiol. 2015;65(10):3344-3351. doi:10.1099/ijsem.0.000417

46. Christensen H, Nicklas W, Bisgaard M. Investigation of taxa of the family Pasteurellaceae isolated from Syrian and European hamsters and proposal of Mesocricetibacter intestinalis gen. nov., sp. nov. and Cricetibacter osteomyelitidis gen. nov., sp. nov. Int J Syst Evol Microbiol. 2014;64(Pt 11):3636-3643. doi:10.1099/ijs.0.067470-0

47. Christensen H, Korczak BM, Bojesen AM, Kuhnert P, Frederiksen W, Bisgaard M. Classification of organisms previously reported as the SP and Stewart-Letscher groups, with descriptions of Necropsobacter gen. nov. and of Necropsobacter rosorum sp. nov. for organisms of the SP group. Int J Syst Evol Microbiol. 2011;61(Pt 8):1829-1836. doi:10.1099/ijs.0.024174-0

48. Boot R, Nicklas W, Christensen H. Revised taxonomy and nomenclature of rodent Pasteurellaceae: Implications for monitoring. Lab Anim. 2018;52(3):300-303. doi:10.1177/0023677218754597

49. Hayashimoto N, Morita H, Ishida T, et al. Current microbiological status of laboratory mice and rats in experimental facilities in Japan. Exp Anim. 2013;62(1):41-48. doi:10.1538/expanim.62.41

50. Artwohl JE, Flynn JC, Bunte RM, Angen O, Herold KC. Outbreak of Pasteurella pneumotropica in a closed colony of STOCK-Cd28(tm1Mak) mice. Contemp Top Lab Anim Sci. 2000;39(1):39-41.

51. Macy JD, Weir EC, Compton SR, Shlomchik MJ, Brownstein DG. Dual infection with Pneumocystis carinii and Pasteurella pneumotropica in B cell-deficient mice: diagnosis and therapy. Comp Med. 2000;50(1):49-55.

52. Hayashimoto N, Yasuda M, Ueno M, Goto K, Takakura A. Experimental infection studies of Pasteurella pneumotropica and V-factor dependent Pasteurellaceae for F344-rnu rats. Exp Anim. 2008;57(1):57-63. doi:10.1538/expanim.57.57

53. Nakai N, Kawaguchi C, Nawa K, Kobayashi S, Katsuta Y, Watanabe M. Detection and elimination of contaminating microorganisms in transplantable tumors and cell lines. Exp Anim. 2000;49(4):309-313. doi:10.1538/expa-

nim.49.309

54. Fox JG, Dewhirst FE, Tully JG, et al. Helicobacter hepaticus sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. J Clin Microbiol. 1994;32(5):1238-1245. doi:10.1128/jcm.32.5.1238-1245.1994

55. Ward JM, Anver MR, Haines DC, Benveniste RE. Chronic active hepatitis in mice caused by Helicobacter hepaticus. Am J Pathol. 1994;145(4):959-968.

56. Whary MT, Fox JG. Natural and experimental Helicobacter infections. Comp Med. 2004;54(2):128-158.

57. Haines DC, Gorelick PL, Battles JK, et al. Inflammatory large bowel disease in immunodeficient rats naturally and experimentally infected with Helicobacter bilis. Vet Pathol. 1998;35(3):202-208. doi:10.1177/030098589803500305

58. Barthold SW, Griffey SM, Percy DH. Pathology of Laboratory Rodents and Rabbits. John Wiley & Sons; 2016.

59. Cacioppo LD, Shen Z, Parry NM, Fox JG. Resistance of Sprague–Dawley Rats to Infection with Helicobacter pullorum. J Am Assoc Lab Anim Sci. 2012;51(6):803-807.

60. Cacioppo LD, Turk ML, Shen Z, et al. Natural and experimental Helicobacter pullorum infection in Brown Norway rats. J Med Microbiol. 2012;61(Pt 9):1319-1323. doi:10.1099/jmm.0.042374-0

61. Whary MT, Cline JH, King AE, et al. Monitoring sentinel mice for Helicobacter hepaticus, H rodentium, and H bilis infection by use of polymerase chain reaction analysis and serologic testing. Comp Med. 2000;50(4):436-443.

62. Hodzic E, McKisic M, Feng S, Barthold SW. Evaluation of diagnostic methods for Helicobacter bilis infection in laboratory mice. Comp Med. 2001;51(5):406-412.