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Laboratory animal mycoplasmosis: A mini review

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ABSTRACT

Mycoplasmas are the smallest, free-living, cell wall less prokaryotes from the class mollicutes, and these are relatively slow-growing microorganisms. Most of the laboratory animals may get the mycoplasma infection naturally. A large number of mycoplasma species (*Mycoplasma pulmonis*, *M. muris*, *M. neurolyticum, M. collis*, *M. arthritidis* and *M. caviae* etc.) have been reported from the laboratory animals. These are responsible for the development of a large numbers of clinical conditions in laboratory animals hence they may cause interference in the results of on-going research/experimental works on those laboratory animals. This review article gives insights on laboratory animal mycoplasma infections including pathogenesis, clinical signs, lesions, diagnosis, and interference in the experimental work, prevention, and control.

Keywords: CAR bacillus, Laboratory animals, Mycoplasmosis, PCR-RFLP

Laboratory animals like rats, mice, rabbits, guinea pigs, gerbil, and hamsters are most commonly used for various experimental works, however, these animals may be naturally infected by pathogenic microorganisms including mycoplasma species which may hinder the results of experimental works (Lindsey *et al.* 1971, Cassell *et al.* 1981a). The mycoplasmas are the smallest, free-living, cell wall less prokaryotes from the class mollicutes, and these are relatively slow-growing microorganisms (George 2005). Mycoplasmas may cause marked morbidity and mortality in laboratory animals intended for long-term experimental works (Simecka *et al.* 1992). Mycoplasma species are found in about 60% of barrier-maintained and nearly all rats of the conventionally housed laboratory. Number of mycoplasma species have been isolated from the laboratory rodents and most of them are regarded as commensal organisms (Davidson *et al.* 1994). Mycoplasma species like *M. pulmonis*, *M. muris*, *M. arthritidis*, *M. neurolyticum,* and *M. collis* have been reported from laboratory rodents. *Mycoplasma pulmonis* is associated with chronic respiratory disease while *M. arthritidis* is responsible for polyarthritis in rats whereas *M. neurolyticum* can cause 'rolling disease' in mice (Van Kuppeveld *et al.* 1993). The *Mycoplasma pulmonis* is a natural pathogenic agent of the respiratory as well as the reproductive system of the rodents. *Mycoplasma pulmonis* is a common causative agent of chronic persistent respiratory disease in rats which is characterized by rhinitis, pneumonia, otitis, and endometritis, while these are less frequently found in mice and causes chronic pneumonia, pulmonary abscess, suppurative rhinitis and otitis media. The susceptibility and severity of the respiratory form vary between the rat strains. The inoculation of *M. pulmonis* through the vaginal route in different strains of the rat shows differences in the susceptibility towards the *M. pulmonis* infection and in the secondary complications associated with it, which may be helpful in the determination of host-specific factors that affect the outcome of reproductive tract infection by mycoplasmas (Reyes *et al.* 2000). *Mycoplasma pulmonis* induces chronic pulmonary disease syndrome and frequently exists as a co-pathogen with CAR (Cilia-associated respiratory) bacillus. The incidence of *Mycoplasma pulmonis* is common in non-SPF laboratory animals. The transmission of *M. pulmonis* occurs directly via fomites and may also be transmitted through the placenta. Venereal transmission may also occur (Brown *et al.* 2001). Rats may be the asymptomatic carrier for *M. pulmonis* infection. Though the infection starts without any clinical signs, unfavourable environmental conditions like a rise in ammonia levels of the cage, and/or the invasion by primary bacterial or viral pathogens of the respiratory tract can cause the activation of subclinical infections. The early signs of the infection may be torticollis and occulonasal discharge. Other clinical signs are chattering, snuffling, anorexia with loss of body weight, hunched posture, rough hair coat, and reduction in fertility. *M. pulmonis* has also been isolated from the Syrian hamsters, Guinea pig, Gerbil but without any clinical manifestation. *M. pulmonis* and *M. pneumoniae* can cause acute arthritis whereas *M. arthritidis* produces chronic arthritis in rabbits (Cedillo *et al.* 1992). Some mycoplasma species (*M. caviae* and *M. pulmonis*) have been isolated from the guinea pigs. Besides *Streptococcus* spp. and *Staphylococcus*

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spp., *M. caviae* commonly cause the acute arthritis in guinea pigs. Moreover, *M. caviae* is also associated with lymphadenitis and metritis in guinea pigs. Sometimes the affected animals remain asymptomatic. Usually, guinea pigs are the carrier for the *M. pulmonis* and do not become ill (Hill 1971, 1984). *Mycoplasma caviae* has also been isolated from the nasopharynx and vagina of the guinea-pigs with unknown pathogenicity (Hill 1971). Improper living conditions, stress and contact with newly acquired animals in the shed can result in the arrival of the mycoplasma infection.

The prevalence of *Mycoplasma pulmonis* in the rats of Western Europe was 3.6% (Mahler and Kohl 2009). It has been evident from several studies that the seroprevalence of *M. pulmonis* is higher in Indian rodents. The prevalence of rodent pathogens is notably higher in the Northern region of India than in the South part (Manjunath *et al.* 2015). A study conducted in Germany found that *M. pulmonis* is normally present in the vagina of most guinea pigs (Hill 1971).

Pathogenesis

The unhygienic conditions and overcrowding inside the cage may result into an increase in the level of ammonia which irritates the respiratory tract and aggravate the risk of mycoplasma infection. After entering into the host body, mycoplasmas damages the host cells by hindering the functioning of cilia of the epithelial cells in the respiratory and genital tract. The cell surface proteins of the mycoplasma species may mimics or modulate the host's immune responses and involve in the adhesion to the host cell. Some mycoplasma species can also fuse with the cell membrane of the host cell or may invade directly into the host cell cytoplasm. Mycoplasma species show strict specificity towards the host and tissue (Razin 1992, Waites and Talkington 2005). *M. pulmonis* preferably colonizes the nasal passages and middle ears. Mycoplasma competes with host cells for nutrients and metabolites; besides this, it may also produce peroxides causing cell damage as well as nonspecific mitogens (Percy *et al.* 2001, Hodge *et al.* 2002).

Clinical signs and lesions

The clinical signs of mycoplasma infection are most prominent in older laboratory animals however younger animals mostly remain asymptomatic. Common clinical signs are ocular and nasal discharge, rales and dyspnea, snuffling and chattering, rubbing of eyes, and tilting of the head. Loss in body weight, as well as the reduction in fertility rate, may be seen in severe cases. The severity of the disease depends on the host-pathogen interaction and environmental factors like relative humidity, temperature and levels of the ammonia inside the cage (Lindsey *et al.* 1985). The immune status of the hosts, strain and age of the host, as well as the presence of concurrent infectious conditions, may aggravate the disease severity. Moreover, the disease severity may also increase due to the dietary deficiencies of some vitamins such as vitamins A and E (Percy and Barthold 2007).

The macroscopic lesions may vary with the duration of infection as well as with the type of tissue that got infected. The mycoplasma infection can lead to the suppuration in the respiratory tract, reproductive system, and sometimes in joints. In the early stage of the infection, very little exudates were seen (Suppurative rhinitis or otitis media) whereas in the case of the advanced stage of infection, accumulation of suppurative materials inside the middle ear, uterus, and dilatation of bronchi and bronchioles may be observed (Fox *et al.* 2002). Microscopic examination reveals chronic suppurative bronchopneumonia along with marked hyperplasia of lymphoid tissue of the bronchi. Suppurative inflammation and hyperplasia of lymphoid follicles of affected tissues (middle ear, uterus, and joints) may also occur (Percy and Barthold 2007).

Diagnosis

The diagnosis of laboratory animal mycoplasmosis depends on the cultural isolation of the mycoplasma organism (Cassell *et al.* 1981, Lussier 1991). The gross lesion (Suppurative rhinitis, laryngitis, otitis media, tracheitis, suppurative bronchopneumonia, bronchiectasis, atelectasis, and abscess formation in the lungs) and histopathological lesions are not very much helpful in the diagnosis of laboratory animal mycoplasmosis. Sometimes the widespread bronchiectasis and abscesses give 'cobblestone' appearance of the lung in the endstage of the disease. Microscopic examination reveals the infiltration of neutrophils, accumulation of lymphocytes and plasma cells, metaplasia, and hyperplasia of the epithelium (Percy *et al.* 2007). ELISA techniques may also be used for the detection of serum IgG and IgM levels but due to the lesser sensitivity and specificity, commercial ELISA kits are not desirable (Cassell *et al.* 1981). They may also be diagnosed by molecular methods. PCR-RFLP used for differential identification of *M. arthritidis* and *M. pulmonis*, in which amplification of mycoplasma genus-specific sequence done by using PCR and the digestion of PCR products done with the help of restriction enzyme SmaI. The PCR product of *M. pulmonis* was found to be digested into two fragments by SmaI while the PCR products of *M. arthritidis* remain undigested (Kim *et al.* 2005). DNA probes specific for different mycoplasma species are useful for the regular diagnosis of mycoplasmal infections of rodents (Ferebee *et al.* 1992).

Laboratory animal mycoplasmosis interference in research/ experimental work

The laboratory animals may naturally harbour various bacterial, viral, fungal, and parasitic agents. Usually, these agents do not develop apparent signs of disease conditions in laboratory animals; however, most of these natural pathogens may alter the physiology of the host thereby making them unsuitable for the use of any experimental/ research work (Lindsey *et al.* 1971, Nicklas *et al.* 1993). The mycoplasma infection of laboratory animals may also

interfere in the research work by making the animal clinically ill, hence the animals become unfit for any research work. The laboratory animal mycoplasmas spread widely into the body of the host and infect different organ systems of the body. The lung is the main target organ for mycoplasma, where they cause long-lasting changes (Decrease in the functions of cilia, alteration in morphology and function of endothelial cell, and disturbance in the airway innervation). The laboratory animal mycoplasmosis may also affect the immune system of the host and making them susceptible to other infections. Mycoplasma infection of laboratory animal results in the reduction of reproductive efficiency, decrease in delayed hypersensitivity responses, lymphocytosis, neutrophilia, changes in T cell subsets, and adjuvant-collagen induced arthritis (Bhatt *et al.* 1986, Ross *et al.* 1992, Baker 2003).

Treatment

Antibiotics such as Doxycycline (for targeting the *Mycoplasma* species) and Enrofloxacin (for targeting the opportunistic bacteria-causing secondary infections) shall be given for two weeks along with anti-inflammatory drugs such as meloxicam (Kohn *et al.* 2002). The treatment of infection with the drugs like Tylosin or Tetracycline may also subside the clinical signs. Antibiotics may be given into the drinking water but most of the time laboratory animals do not drink antibiotic added water either due to change in the taste of water or due to the weakness of the body, therefore oral administration (with the help of dropper or syringe) of antibiotics is advisable (Fox *et al.* 2002).

Prevention

The entry of mycoplasma infection into the laboratory animals can be prevented by focusing on the entry of animals and the biological materials inside the laboratory animal houses, therefore the laboratory animals should be procured from reputed vendors, quarantined and screened prior to introducing into the laboratory animal house (Graham *et al.* 2011). The strategies such as proper cleaning and sanitation of laboratory animal houses, cages, feeders and waterers and segregation of diseased/ill laboratory animals from a healthy animal should be followed (Fox *et al.* 2002). Decontamination should be done by using the decontaminating agent similar to use for non-spore-forming bacteria, non-essential materials should be discarded and equipment should be cleaned with appropriate disinfectant or autoclaved before the arrival of new animals (Davidson *et al.* 1994). The use of specific pathogen-free (SPF) animals has restricted the prevalence of laboratory animal mycoplasmosis (Cox *et al.* 1988). Depopulation of infected colonies may also be helpful in the prevention of laboratory animal mycoplasmosis. The control of environmental conditions, which are favourable for the development of the mycoplasma infection in laboratory animals (Like frequent sanitation of cages and reduction in the population density of laboratory animals inside the cages to reduce the ammonia level inside the cages) may also reduce the risk of laboratory animal mycoplasmosis outbreak (Graham J E *et al.* 2011).

The laboratory animal houses, additional support spaces as well as the primary enclosures should be regularly cleaned and sanitized. The solid-bottom cages for rodents should be changed 2–3 times in a week whereas for rabbits, rodents, and nonhuman primates in suspended cages over excreta pans and for mice in a ventilated cage system, cage changes at alternate week will be sufficient. In case of larger animals daily removal of excreta and soiled bedding material, and daily cleaning and sanitization of primary enclosures will be helpful in prevention of laboratory animal diseases (Graham *et al.* 2011).

The other necessary equipment of laboratory animal house like water bottles and feeders should be cleaned and sanitized at least twice in a week. Heating of cages and other equipment to 180°F or disinfection by using suitable chemical agents such as 0.1–0.2% hypochlorite solutions can kill the mycoplasma organisms as well as other non-spore forming bacteria and viruses that are pathogenic to the laboratory animals. Thorough rinsing of cages and other equipment of the laboratory animal houses should be done after treating with either detergents or with disinfectants. The effectiveness of the sanitation should be evaluated on regular basis with the help of appropriate microbiological and organic material detection systems (Davidson *et al.* 1994).

Conclusion

Since mycoplasma infection can lead to the development of an adverse effect in laboratory animals, which therefore interferes in the outcome of the on-going research works on those laboratory animals. The occurrence of mycoplasma in laboratory animals should be tested before the starting of experimental work. Mycoplasma infection of laboratory animals does not always develop prominent clinical signs; hence health monitoring programs of laboratory animals should be carried out. For the prevention of mycoplasma infection in laboratory animals, proper sanitary measures are taken into consideration. Laboratory animals are extensively used nowadays for various experimental works; hence it requires the development of cheap and easy techniques for the detection, prevention, and control of laboratory animal mycoplasmosis. By keeping in view the frequent use of laboratory animals in various research works as well as their probability of getting infection with *Mycoplasma* spp., more empahasis should be given to combat this infection in laboratory animals until some suitable immunoprophylactic is developed.

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