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EPIDEMIOLOGICAL AND DIAGNOSTIC STATUS OF *MYCOPLASMA SYNOVIAE* IN PAKISTAN AND INDIA: A REVIEW

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ABSTRACT

Poultry birds are affected with lot of infectious diseases in which *Mycoplasma synoviae* is one of vital significance. Mycoplasma infection can be diagnosed on several basis including history of disease in chickens, clinical signs of infected chickens, necropsy practices, microscopic lesions, and several diagnostic methods. Once, observing the clinical signs, necropsy practice is considered as the best method to identify the target pathogen as *Mycoplasma synoviae,* showing the specific type of postmortem lesions. Mycoplasma detection can also be done by several serological and molecular techniques. Early diagnosis of mycoplasma can be done by using serological tests. Status of Mycoplasma was observed time to time in Pakistan and India and presented in this review. Mycoplasma can be controlled by timely vaccination of layer chickens, appropriate and routine screening the breeder flock and culling of infected birds or flocks. Once the chickens are infected with mycoplasma, it remains infected for entire life, so eradicate the whole flock, to avoid the future losses to the poultry birds due to this pathogen. This review paper will explain the current status of *Mycoplasma synoviae* and diagnostic techniques in Pakistan and India.

Keywords: *Mycoplasma synoviae*, Mycoplasmosis, Chickens Diagnosis, Epidemiology, Pakistan

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1. INTRODUCTION

Poultry sector is known as one of the most energetic field of livestock sector of Pakistan. Approximately, 1.5 million people of Pakistan are getting likelihood sources by poultry sector (Hasni et al. 2020). Almost 30% of total meat production is contributed by poultry sector in Pakistan (Hussain et al. 2015; Ghonaim et al. 2020; Yasmin et al. 2020). Mycoplasmosis is considered as one of the major threats to the poultry sector among all the emerging infections. Mycoplasma infection leads to the greatest economic losses to poultry birds all over the globe. Due to severity of mycoplasma infection, the entire flock can be culled or eliminated to avoid future losses and transmission (Hennigan et al. 2012). Mycoplasma affects a wide range of species and spread worldwide in nature leading to huge financial losses to poultry sector all over the globe. Over 110 species of genus Mycoplasma have been identified and isolated in many living organisms including birds, mammals, fish and reptiles (Elgnay et al. 2013). Mycoplasmosis includes several pathogenic mycoplasma species including *Mycoplasma synoviae* (MS), *Mycoplasma gallisepticum* (MG), *Mycoplasma meleagridis* (MM) and *Mycoplasma iowae* (MI) are of utmost importance, in which MS and MG are of vital significance and known as notifiable pathogens by OIE (office of international and epizootics) (Stipkovits and Kempf 1996; Ishfaq et al. 2020; Qadir et al. 2020; Muhammad et al. 2021). MS mostly spreads disease in chickens, but it is also reported in wild and domestic birds. It leads to stunted growth, wheezing, synovitis (inflammation of synovial joints) and cough (Feberwee et al. 2009; Ghorashi et al. 2015). Mycoplasma is known as the smallest and simplest prokaryote without cell wall and included in the class Mollicutes followed by genus Mycoplasma. As well as taxonomic characterization is concerned, it can be done on several basis including phenotype, serology, and sequencing of 16sRNA (Brown et al. 2007; Baksi et al. 2016; Yi et al. 2020). Mycoplasma produces huge economic losses in the poultry industry. Out of 120 isolated species of mycoplasma, only 20 species are known as pathogenic to avian birds (Fraga et al. 2013). Mycoplasma can infect humans, animals, plants, and insects as well (Gondal et al. 2015). Mycoplasmosis is a disease of chicken, turkeys, domestic and wild birds. Adult and mature birds are less susceptible to Mycoplasmosis as compared to young birds (Ahmad et al. 2008). Fried egg appearance type typical colonies can be seen in case of mycoplasma. Eyes, mucosal membranes of respiratory tract, joints and urogenital tract are known as predilection sites of Mycoplasma (Doosti et al. 2011). Mycoplasma is characterized by sinusitis, conjunctivitis and sneezing in the turkeys. It results in low

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grade meat production and decrease in egg production in chickens. MG and MS differ in virulence and infectivity, and occasionally infection becomes unobvious (OIE 2008).

Mycoplasma is highly variable, leads subclinical infection to vibrant respiratory signs such as coryza, sneezing and coughing, rales, difficult breathing and nasal exudate oozing out through incompletely opened beak. In turkeys and game birds, unilateral or bilateral sinusitis, swelling of infraorbital sinuses and closure of eyelids can be seen. Sometimes, frothy ocular exudate with conjunctivitis can also be observed in chickens. In infected finches, swollen eyelids, ocular and nasal discharge, conjunctivitis can be seen (Bradbury, 2001; Sun et al. 2014; Mehmood et al. 2020). Mycoplasma is greatly variable on the basis of age, season, sex, flock size, production status and strain of the bacteria (Islam et al. 2015). For the first time it was reported that MG is able to invade the red blood cells. MG invasive erythrocytes are seen in both in vivo and in vitro infection (Vogl et al. 2008). This review will explain the current status of MS and diagnostic techniques used to detect MS in Pakistan and India.

2. Transmission

Transmission of *M. synoviae* commonly occurs in two ways, either vertical or horizontal. Vertical transmission occurs from parents to offspring (eggs to offspring) at hatchery level. Horizontal transmission may occur either direct or indirect contact (Feberwee et al. 2017; Mugunthan et al. 2023). Transmission through direct contact is mainly due to close contact among animals, where bacteria may penetrate via conjunctival and respiratory routes (Ter Veen et al. 2020). Mycoplasma resides in the environment for several days so indirect transmission may occur through indirect contact with wild birds, people, food, vehicles and water (Fig. 1) (Kaboudi et al. 2019).

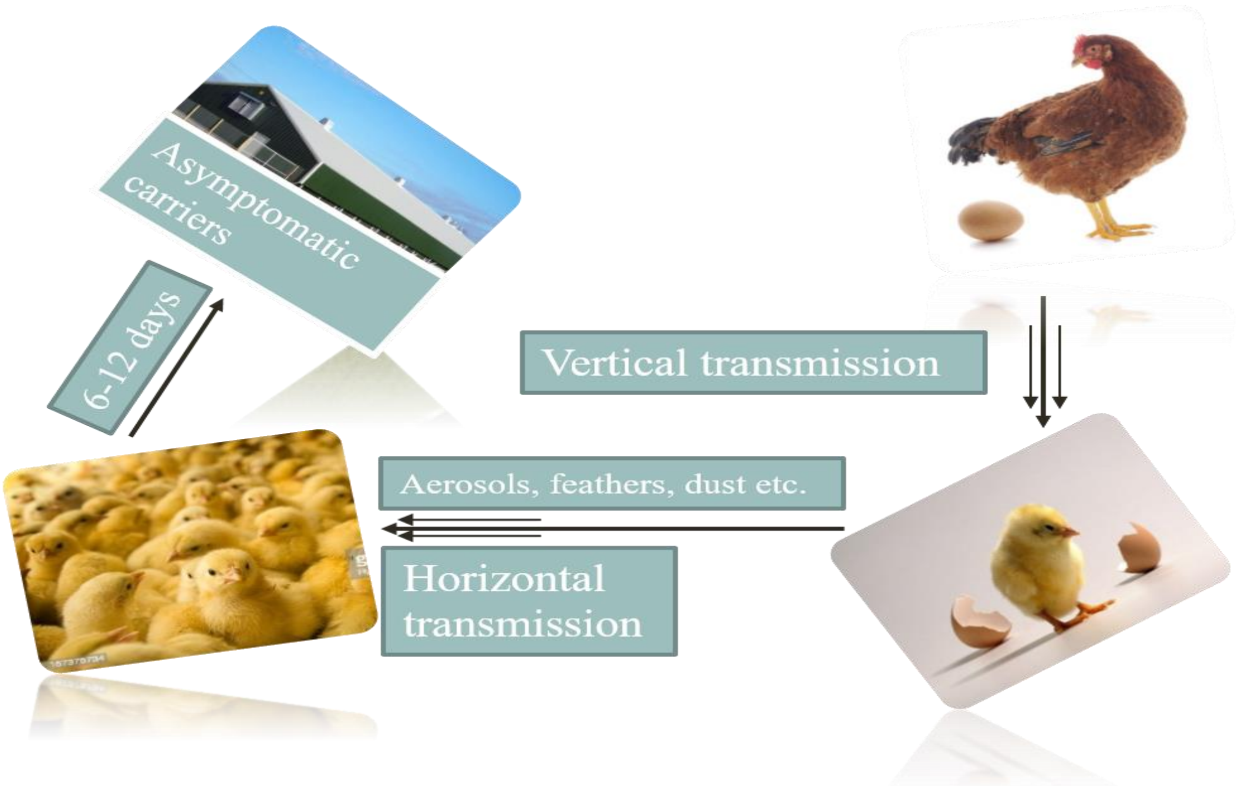

Fig. 1: Transmission routes of *Mycoplasma synoviae* in chickens.

3. MS Diagnostic Tools

MS detection is mainly based on the bird history, respiratory signs, gross and histopathological lesions, serology, organism isolation, identification, and epidemiological data of that region. Pathogen can be isolated in the infected organs of respiratory tract (lungs, air sacs, and trachea), synovial fluid, ocular and infraorbital sinus as well (Kiss et al. 1997; Hong et al. 2004). There are lot of serological techniques used to detect MS, but these methods may lead to false positive findings due to difference in specificity and sensitivity. Among these, HI test, SPAT, and ELISA techniques are included. Early detection of this disease can be done by using these methods due to its rapid and inexpensive nature (Kleven et al. 2001; Fiorentin et al. 2003; Qadir et al. 2020). Different organs or swabs

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(tracheal, cloacal swabs, lungs, spleen, and liver) are used to isolate the DNA for PCR amplification to detect the specific pathogen as MS (Hong et al. 2004). MS infection is also linked with other infections (mixed infections) so, PCR is considered as an advanced diagnostic tool to detect that specific pathogen with great accuracy. Serological tests can lead to false positive results so, for satisfactory results PCR is regarded as confirmatory technique to check the actual status of the disease in avian birds (Raviv and Kleven, 2008; Wanasawaeng et al. 2015).

4. Gross Lesions

Many chickens do not show gross changes in necropsy practices. But several chickens show many gross changes after necropsy including conjunctivitis (inflammation of conjunctiva), sinusitis (inflammation of sinuses), air-sacculitis (inflammation of air sacs and tracheitis (inflammation of trachea) with mucus, (Fig. 2A), synovitis (inflammation of synovial joint), pneumonia, osteomyelitis (inflammation of joints), and salpingitis (inflammation of fallopian tube), Pericarditis and Perihepatitis (Fig. 2B,C) swollen and congested lungs (Fig. 2D), congestion of trachea (Fig. 2E). Air-sacculitis is regarded as the typical lesion of mycoplasma. Upon necropsy practice, it can be concluded that synovial membrane, respiratory and reproductive organs are regarded as target systems in the mycoplasma affected chickens (Qadir et al. 2021).

5. Histopathology Examination

5.1. Histopathological Changes in Trachea

Trachea of healthy chicks is bounded by an epithelium known as ciliated pseudo stratified columnar epithelium followed by tracheal lumen and hyaline cartilage. Whereas complete loss of cilia and moderate epithelial degeneration can be seen in histological structure of diseased bird's trachea (Fig. 3A-A2). The epithelial region was affected by a mild level of lymphocytic infiltration followed by normal sub epithelial region. Loss of cilia with low to higher degenerative changes can be seen. Epithelium disruption with lower level of epithelial hyperplasia can be observed. Lower to higher levels of congestion in tracheal epithelial region can also be observed (Qadir et al. 2021).

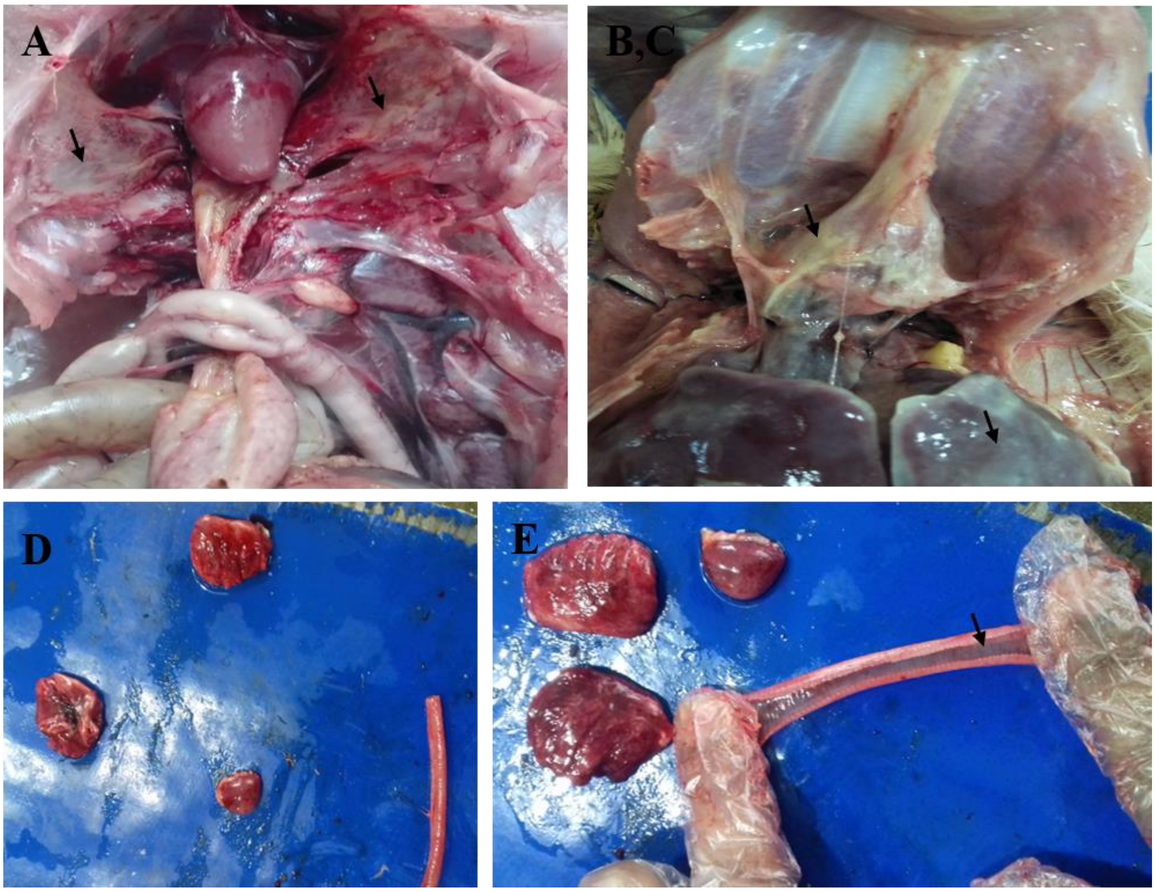

Fig. 2: A: Photograph of airsacculitis (arrow), **B, C:** Pericarditis, Perihepatitis (arrows), **D:** Congestion of spleen and lungs, and **E:** Congestion of trachea from Mycoplasma morbid birds.

5.2. Histopathological Changes in Lungs

Lower to moderate levels of congestion can be observed in the lungs, but the severity of congestion increases at the level of bronchiole and alveoli. Mild and thick degree (level) of fibrosis can be seen in alveolar septa. However, alveolar parenchyma was thoroughly congested. Lower to moderate levels of necrotic changes with inflammatory cells and congestion can be seen. Lung's appearance looks like liver, which is known as Red hepatization (Fig. 3; B-B2). Emphysematous zones (areas) are present at some places and interalveolar septa become thickened (Qadir et al. 2021).

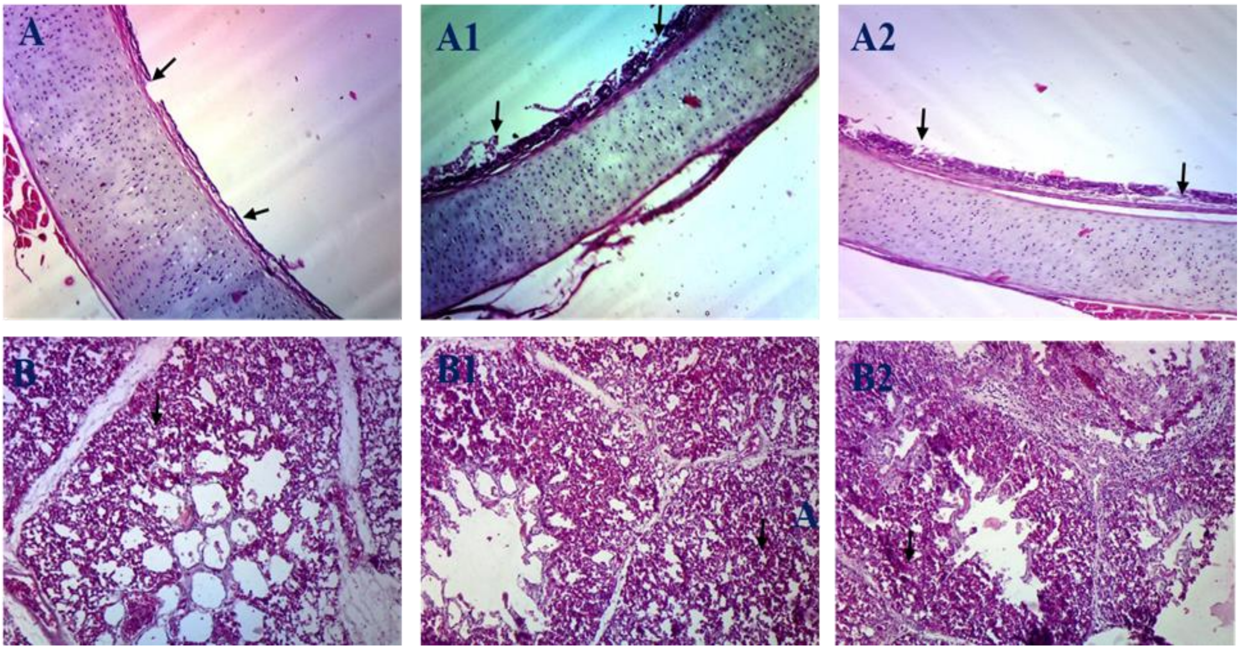

Fig. 3: A-A2: Photomicrograph of trachea from Mycoplasma (morbid birds) showing congestion of epithelial region with loss of cilia (arrows). **B-B2:** Photomicrograph of lungs (morbid birds) where lungs appearance looks like liver (red-hepatization) and some necrotic changes with severe congestion at bronchiole and alveoli level (arrow). H & E Staining; 200x.

6. Control Measures

Regular screening of entire flocks is mandatory to detect the disease at early stages, to minimize the disease and economic loss to poultry sector worldwide. If some positive flocks are detected, culling of entire positive flock is mandatory to eliminate the disease in next flock because mycoplasma infection transmitted by two routes either vertical or horizontal. Horizontal spread can be controlled by culling of diseased birds and good management practices or biosecurity measures, but vertical spread can be minimized by screening of breeder flocks which is mandatory to eliminate the disease in next generation of broiler and layer chickens (Kleven 2008).

Three basic control measures including serological monitoring, security measures and immediate culling of infected chickens were planned by the National Poultry Improvement Plan (NPIP) to prevent *Mycoplasma* infection (Levisohn and Kleven 2012). Mycoplasma is resistant to penicillin but susceptible to several antibiotics such as quinolones, macrolides, and tetracycline. Trans- ovarian transmission and clinical signs are reduced by usage of antimicrobials. Currently, prevention of disease in layer chickens can be done by inactivated and live attenuated vaccines. Moreover, these types of vaccines are not recommended for breeder flocks because, these disrupt the diagnosis and monitoring of Mycoplasma in parental flock (Nascimento et al. 2005).

7. MS Prevalence in Pakistan

Different epidemiological status was reported at different places in Pakistan (Table 1). In Faisalabad district of Pakistan, samples were collected from 142 commercial broiler flocks to investigate the MS prevalence by using SPAT and PCR techniques. Out of total samples collected from 142 flocks, 76.57 and 98.82% samples were tested positive by SPAT and PCR respectively (Ehtisham et al. 2011). Atique et al. (2012) collected 600 samples from broiler and layer flocks from Quetta, Pishin and Kuchlak districts of Balochistan, Pakistan. They reported 7.86 and 11.19% in broilers while 8.16 and 15.33% MS prevalence in layers by SPAT and ELISA techniques respectively. In

Table 1: *Mycoplasma synoviae* prevalence in Pakistan

City/State/Region	Bird's type	Samples collected	Diagnostic tests	MS prevalence or incidence (%)	References
Faisalabad district	Commercial broiler flocks	142 broiler flocks	SPAT and PCR	76.57% and 98.82% positive samples by SPAT and PCR respectively.	Ehtisham et al. (2011)
Quetta, Pishin and Kuchlak districts of Balochistan, Pakistan	Broiler and layer flocks	600	SPAT, ELISA	7.86 and 11.19%, in broilers while 8.16 and 15.33% in layers by SPAT and ELISA respectively.	Atique et al. (2012)
Pakistan	Poultry farms	100 field and 250 experimental samples (n=350)	Duplex PCR	92% MS in field and 100% in experimental samples	Arshad et al. (2013)
Khushab District, Pakistan	Broilers and commercial layers 360 poultry farms	Data were collected from 360 poultry farms during four quarters of the year	Growth on McConkey agar, biochemical and sugar fermentation tests	Seasonal incidence (%) of MS in broilers on quarterly basis was 5.68%. Seasonal incidence (%) of MS in commercial layer on quarterly basis was 5.52%.	Abbas et al. (2015)
Pakistan	Poultry flocks	200 samples for RSA and 92 samples for ELISA	RSA, ELISA by local and imported antigens	69.5 and 70% by local and imported antigens by RSA respectively. 89.13 and 80.43% by local and imported antigens by ELISA respectively.	Rasool et al. (2017)
Five Districts of Khyber Pakhtunkhwa-Pakistan	Broilers and backyard poultry	648 serum samples	SPAT	23.33, 20, 18.26, 12.67 and 11.88% in Peshawar, Dera Ismail Khan, Mansehra, Tank and Abbottabad 11.88%.	Rehman et al. (2018)
Faisalabad district	commercial chicken	124 suspected Cases of MS	PCR	13.70%	Khatoon et al. (2018)
Rawalpindi, Pakistan	Breeder broiler and layer birds	1667 sera samples	SPAT	10, 42.6 and 50.14% MS in broiler, layer and broiler breeder flocks respectively. While, 23.53, 52.09% MS was seen in 0-20 and above 21 week old layers birds, and 42.10 and 48.04% MS was seen in 0-20 and above 21 week old broiler breeder flocks respectively.	Shoaib et al. (2019)
Pakistan	Chicken infected farm	25 samples (10, 6, 4, 5) of trachea, air sac, oral swab and lung tissues)	Culture, DNA-based PCR kits	100% mycoplasma isolation findings. Highest number of isolations yielded by biochemical test with Mycoplasma was10 out of 25. DNA based commercial PCR kit was established as diagnostic technique for Mycoplasma.	Raza et al. (2022)

another report, 100 field and 250 experimental samples (n=350) were collected from different poultry farms in Pakistan and 92% MS in field and 100% in experimental samples were tested positive by duplex PCR (Arshad et al. 2013). Abbas et al. (2015) collected samples from 360 poultry farms during four quarters of the year from Khushab district, Pakistan. 5.68% and 5.52% seasonal incidence of MS was reported in broilers and commercial layer on quarterly basis by using culture and biochemical techniques. Another study was reported by Rasool et al. (2017), they collected 200 samples for SPAT and 92 samples for ELISA from different poultry flocks. Flocks were tested by local and imported antigens. 69.5 and 70% samples were tested MS positive by local and imported antigens by SPAT respectively, and 89.13 and 80.43% samples were tested positive by local and imported antigens by ELISA, respectively. 648 serum samples were collected from broilers and backyard poultry from five districts of Khyber Pakhtunkhwa-Pakistan. 23.33, 20, 18.26, 12.67 and 11.88% samples were tested positive in Peshawar, Dera Ismail

Khan, Mansehra, Tank and Abbottabad 11.88% by SPAT (Rehman et al. 2018). Khatoon et al. (2018) collected 124 suspected cases from commercial chickens from Faisalabad district of Pakistanand13.7% of samples were reported positive by using PCR. Shoaib et al. (2019) collected 1667 sera samples of breeder, broiler, and layer birds from Rawalpindi, Pakistan.in which10, 42.6 and 50.14% MS prevalence was reported in broiler, layer and broiler breeder flocks respectively. While 23.53 and 52.09% MS prevalence was seen in 0-20 and above 21-week-old layers birds, and 42.10 and 48.04% MS was seen in 0-20 and above 21 week old broiler breeder flocks by SPAT, respectively. In another study, 25 samples (10, 6, 4, 5 of trachea, air-sac, oral swab, and lungs tissue) were collected from chicken infected farms in Pakistan in which100% positive mycoplasma was reported by culture technique. The highest number of isolations yielded by biochemical test, with Mycoplasmosis account was 10 out of 25 (Raza et al. 2022).

8. MS Prevalence in India

MS was prevalent in different regions in India and reported time to time (Table 2). Senthilnathan et al. (2013) collected 144 samples from different broiler breeder farms in Tamil Nadu. Culture and PCR techniques were used to investigate the MS prevalence in chickens. Some samples were destroyed during handling and processing and 166 samples were left for PCR. 15.5% showed fried egg appearance and 49.1% (57 out of 166) were tested positive by PCR. In another report, 1354 samples were collected from seven different states of India. ELISA was done to detect the MS prevalence in these states and overall, 41.1% MS prevalence was reported by Baksi et al. (2016).

Table 2: Mycoplasma synoviae prevalence in India

City/district/Region	Samples collection	Diagnostic techniques	MS prevalence/incidence (%)	References
Tamil Nadu	144 samples (broiler breeder farms), 166 samples were left for PCR	Culture, PCR	15.5% showed fried egg appearance. 49.1% (57 out of 166) were positive by PCR.	Senthilnathan et al. (2013)
Different states of India	1354 samples	ELISA	41.1%	Baksi et al. (2016)
Haryana	Total 382 serum samples, 284 (day old) and 98 6-8 weeks old broiler chickens	SPAT	10.56% MS in day old broiler chickens and 18.36% in 6-8 weeks old broiler chickens	Tomar et al. (2017)
Haryana	92 (tissue) Samples	PCR	2.1%	Tomar et al. (2017)
5-States (Karnataka, Telangana, Himachal Pradesh, West Bengal and Gujrat)	635 (serum) Samples	ELISA	52.1 %	Rajkumar et al. (2018)
7-States (Karnataka, Telangana, Tamil Nadu, Himachal Pradesh, Gujarat, Odisha and West Bengal)	309 Choanal Swabs	PCR	33.0%	Rajkumar et al. (2018)
Western Maharashtra, India	60 tissue samples from 60 flocks	PCR	20% MS, 3.03% MG+MS, 3.03% MG+MS+E. coli.	Bagal et al. (2019)
Various districts of Haryana	100 tissue samples (trachea, lungs and air sacs)	PCR	19%	Vaishali et al. (2020)
Haryana	100	PCR	19%	Vaishali et al. (2020)
Namakkal Region of Tamil Nadu	Samples from 24 flocks of 14 different farms	Culture and PCR	16.6% and 12.5% MS and MS+MG respectively.	Srinivasan et al. (2020)
Haryana	92 tissue samples	PCR, Growth inhibition test	3.24%	Tomar et al. (2020)
Poultry farms of Bihar, Andhra Pradesh, Gujrat, Goa, Kerala, Haryana, Jharkhand, Odisha, Karnataka, Maharashtra, Rajasthan, Punjab, Tripura, Tamil Nadu, Telangana, and West Bengal	3620 tracheal or choacal swabs	PCR	23.61 and 15.49% were MS positive and MS+MG positive respectively.	Giram et al. (2022)

Tomar et al. (2017) collected 382 serum samples in which 284 serum samples were from day old chicks from 18 hatcheries and 98 samples were collected from 6-8 weeks old broiler chickens to investigate the MS prevalence in Haryana region. Out of 98 samples, 18 samples 18.36% were reported positive for MS in 6-8 weeks old broiler chickens by SPAT. While 30 samples (10.56%) were tested positive for MS out of 284 total samples in day old broiler chickens by SPAT. In another report, 92 tissue samples were collected, and 2.1% samples were detected positive for MS by PCR (Tomar et al. 2017). Rajkumar et al. (2018) collected 635 serum samples from 5-States (Karnataka, Telangana, Himachal Pradesh, West Bengal and Gujrat) India and 52.1% MS Prevalence was recorded by ELISA method. In the second report, they collected 309 choanal swab samples from 7-States (Karnataka, Telangana, Tamil Nadu, Himachal Pradesh, Gujarat, Odisha and West Bengal) and 33% samples were reported positive for MS by PCR (Rajkumar et al. 2018). Another study by Bagal et al. (2019) was done to investigate the MS prevalence in Western Maharashtra, India. During this study, 60 tissue samples from 60 flocks were collected and PCR was conducted to check the MS prevalence. 20% MS, 3.03% MG+MS, 3.03% MG+MS+*E. coli* individual and mixed infection was observed. Vaishali et al. (2020) investigated MS prevalence in two different reports separately in the year 2020. In the first report, they collected 100 tissue samples (trachea, lungs and air sacs) and 19% samples were observed positive for MS by PCR. In the second report, again 100 samples were collected, and 19% MS prevalence was recorded by PCR. Another study was done at Namakkal Region of Tamil Nadu region, during which samples from 24 flocks of 14 different farms were collected to investigate the MS status. Culture and PCR techniques were done to record the MS prevalence. 16.6 and 12.5% samples were tested MS positive and MS+MG mixed infection respectively (Srinivasan et al. 2020). Tomar et al. (2020) collected 93 tissue samples and 3.24% MS prevalence was investigated in Haryana region in the year 2020. In another recent study, 3620 tracheal or cloacal swabs were sampled from several poultry sheds of Bihar, Andhra Pradesh, Gujrat, Goa, Kerala, Haryana, Jharkhand, Odisha, Karnataka, Maharashtra, Rajasthan, Punjab, Tripura, Tamil Nadu, Telangana, and West Bengal to check MS status in these regions. PCR test was conducted to investigate the MS prevalence and 23.61 and 15.49% samples were MS positive and MS+MG positive, respectively.

9. Conclusion

MS is prevalent in many countries leading to huge financial losses to the poultry sector and poultry farmers. Firstly, infection should be controlled at hatchery level and secondly, by proper biosecurity measures horizontal transmission can be controlled. Proper screening and monitoring of entire bird's flock should be done to investigate the disease status time to time and to eliminate the morbid chickens. In other case, entire flock should be eliminated to control the future loss and transmission of infection. Serological techniques are regarded as very fast to investigate MS status and screening of flocks. Early detection by screening and vaccination is very helpful to control and prevent the infection at initial stages. It is urgent need of time to educate the farmers related to biosecurity measures and vaccination protocols to decrease the financial losses happening because of this disease. This review explained the epidemiological status of MS in different provinces, districts and regions of Pakistan and India.

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