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Occurrence and molecular characterisation of respiratory bacterial infections in pet birds: A gender and age-specific analysis#

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Abstract

The study examined 76 pet bird carcasses at the Department of Veterinary Pathology, CVAS, Mannuthy. The birds were categorised into four major groups: Psittaciformes, Passeriformes, Columbiformes and Galliformes. It was identified Escherichia coli infections in 16 out of 42 psittacine birds (38.1%), 2 out of 7 passerines (28.6%), 6 out of 18 columbid birds (33.33%), and 4 out of 9 ornamental birds (11.84%). Salmonella Typhimurium was detected in 28.6% of both psittacine and passerine birds, while 22.2% in columbid and ornamental birds tested positive. Pasteurella spp. was detected in 28.6% of both psittacine and passerine birds, and 16.7% in columbid birds. Mycoplasma spp. infections were detected in 11.84% of 76 birds, with psittacine and passerine birds showing positivity rates of 7.1% and 42.1%, respectively. Chlamydia spp. infections were detected in 23.68% of psittacine birds, 28.6% in columbid birds, and 11.1% in ornamental chickens. The occurrence of E. coli infection was the highest in adult passerine birds (50%), and lowest in adult psittacine birds (18.18%). Infection with S. typhimurium was diagnosed in nestling psittacine birds, with no infections in nestling ornamental birds (35.48%). Pasteurella spp. was detected in 100% of adult passerine birds, while no infections were found in ornamental chickens. Mycoplasma spp. was observed in nestling passerine birds, with no infections detected in adult psittacine, passerine, columbid or ornamental birds.

Keywords: *Pet birds, Escherichia coli, Salmonella Typhimurium, Pasteurella spp., Mycoplasma spp.*

Pet bird farming is globally significant because it serves as a reservoir for many multidrug-resistant zoonotic bacteria and highly pathogenic viruses, which pose serious risks to both birds and humans. Respiratory infections in pet birds are significant and cause severe respiratory distress and mortality among affected birds (Modi and Bhanderi, 2024). The pathogens, including various viruses and bacteria, can quickly spread among bird populations, especially in environments with poor ventilation or high bird density (Furian *et al*., 2018). This study aimed to detect bacteria in respiratory infection in per birds by molecular methods.

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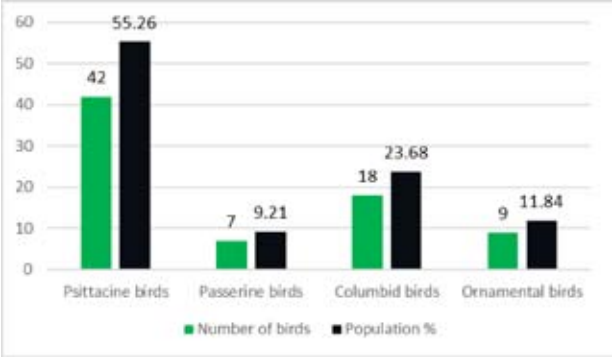
Materials and methods

This study focused on 76 pet birds from various orders, including Psittaciformes, Columbiformes, Passeriformes, and Galliformes (Ornamental chickens), which were presented for necropsy at the Department of Veterinary Pathology in Thrissur district, Kerala. Respiratory organs showing lesions *viz*., larynx, trachea, syrinx, lung and air sacs were collected for PCR studies. The tissue samples were collected in cryovials and stored at -20ºC till further use. DNA isolation was done using the commercially available kit (Qiagen). The PCR detection using primers specific for various bacteria in this study was standardised by different concentration of reaction mix and cycling conditions. Positive DNA from the repository in the laboratory were used for standardisation.

Results and discussion

In this study, samples were collected from 76 pet bird carcasses presented for necropsy which included 42 psittacines (55.27%), 7 passerines (9.21%), 18 columbid birds (23.68%), and 9 ornamental chickens (11.84%), as illustrated in Fig. 1.



Polymerase chain reaction revealed 477 bp sized amplicons of *E.coli*, 660bp sized amplicons of *Salmonella* Typhimurium, 560bp sized amplicons of *Pasteurella spp.,* 715 bp sized amplicons of *Mycoplasma spp.* and 230 bp sized amplicons of *Chlamydia* spp. (Fig 2-6). The study identified *Escherichia coli* infections in 16 out of 42 psittacine birds (38.1%), 28.6% of passerines, 33.33% of columbid birds and 11.84% of ornamental birds. Thomas (2019) has reported that incidence of *Chlamydia* spp. and *Mycoplasma gallisepticum* infection was the most prevalent among the pet bird population of Kerala. The present study identified that *E. coli* infection had a highest occurrence in psittacine birds which was higher than the previous studies conducted by Graham and Graham (1978) and Ahmed *et al*. (2021) who reported 13.6% and 17.6% as the prevalence rate in captive psittacine birds. Identification of *Salmonella* typhimurium revealed a 28.6% positivity rate in both psittacine and passerine birds, with a total of 12 out of 42 psittacine and 2 out of 7 passerine birds. In columbid and ornamental birds, 22.2% tested positive for *S.* typhimurium. Ibrahim *et al*. (2019) reported the prevalence rate of *E. coli* and *Salmonella* spp. in psittacine birds as 34.11% and 12.9% and the current study identified almost similar prevalence rate of *E. coli* and a higher rate for *Salmonella* spp.

PCR analysis for *Pasteurella* spp. indicated a 28.6% positivity rate in both psittacine and passerine birds and 16.7% in columbid birds. The report of Raji *et al*. (2010) in Nigeria, stated that the prevalence rate of *Pasteurella* spp. in pet birds increased from 2.3% to 7.4% during 2001 to 2005, which was found similar to the current study which documents the occurrence rate of 7.1% of *Pasteurella* in psittacine birds. Overall, 9 out of 76 birds (11.84%) tested positive for *Mycoplasma* infection, with psittacine and passerine birds showing positivity rates of 7.1% and 42.1%, respectively. Columbid and ornamental birds both recorded an 11.1% positivity rate for *Mycoplasma*. The highest occurrence of *Mycoplasma* infection was found in passerine birds. The current study was almost similar to the identification of Khafagy *et al*. (2017) who reported that the prevalence rate of *Mycoplasma* species in chickens was 59%, with 15.3 % of *M. gallinarium*.

PCR analysis for *Chlamydia* spp. detected a total positivity rate of 23.68% in psittacines (35.7%), passerines (28.6%), and ornamental chickens (11.1%). The highest prevalence of *Chlamydia* was observed in psittacine birds. The occurrence rate from current study was found to be lesser than the report of Meyst *et al*. (2022) who identified

Table 1. Details of the specific primers used

Organism	Primer sequence (5'- 3')	Target gene	Size	Reference
Escherichia coli	**F:** GGGTAGAAAATGCCGATGGTG	*fimC*	477 bp	Janben *et al*., (2001).
	R: GTCATTTTGGGGGTAAGTGC			
***Salmonella* Typhimurium**	**F:** GGAACTGAGACACGGTCCAG	*16S rRNA*	660 bp	Kaabi *et al*., (2019)
	R: CCAGGTAAGGTTCTTCGCGT			
Pasteurella spp.	**F:** GCAGTGAAAGARTTCTTTGGTTC	*rpoB*	560 bp	Korczak *et al., (*2004)
	R: GTTGCATGTTNGNACCCAT			
Mycoplasma spp.	**F:** ACTCCTACGGGAGG CAGCAGTA	*16S rRNA*	715 bp	Kuppeveld *et al*., (1992)
	R: TGCACCATCTGTCACTCTGTTAACCTC			
***Chlamydia* spp.**	**F:** GCCTACCGGCTTACCAAC	*16S rRNA*	230 bp	Parut *et al*., (2019)
	R: GGCGCAATGATTCTCGAT			

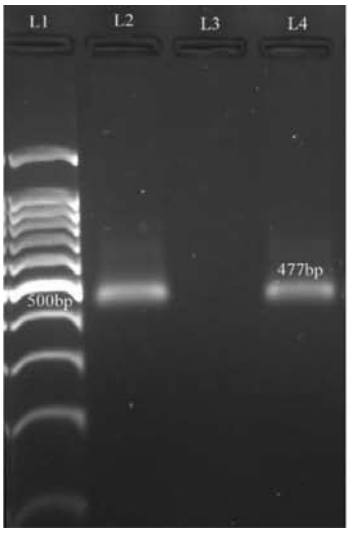


Fig. 2. Agarose gel electrophoresis, Identification of *Escherichia coli at* 477bp. Lane 1. 100 bp DNA molecular weight markers; Lane 2. Positive control; Lane 3. Negative control; Lane 4. Positive sample.

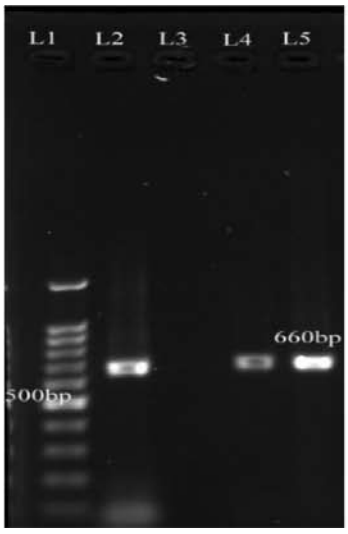


Fig. 3. Agarose gel electrophoresis, Identification of *Salmonella* Typhimurium at 660bp. Lane 1. 100 bp DNA molecular weight markers; Lane 2. Positive control; Lane 3. Negative control; Lane 4 & 5. Positive samples

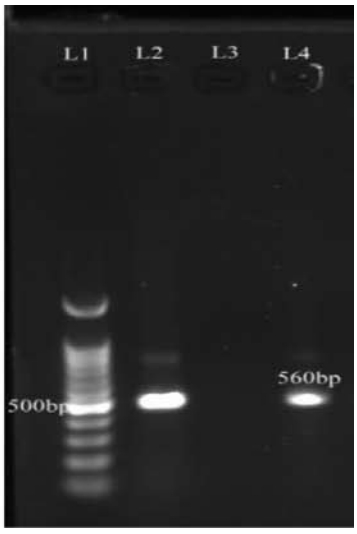


Fig. 4. Agarose gel electrophoresis, Identification of *Pasteurella* spp. at 560bp. Lane 1. 100 bp DNA molecular weight markers; Lane 2. Positive control; Lane 3. Negative control; Lane 4. Positive sample

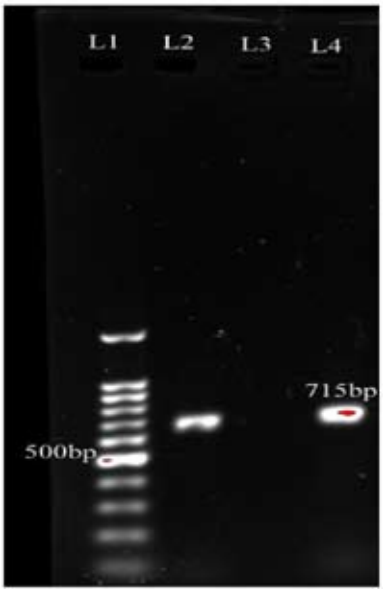


Fig. 5. Agarose gel electrophoresis, Identification of *Mycoplasma* spp. *at* 715bp. Lane 1. 100 bp DNA molecular weight markers; Lane 2. Positive control; Lane 3. Negative control; Lane 4. Positive sample

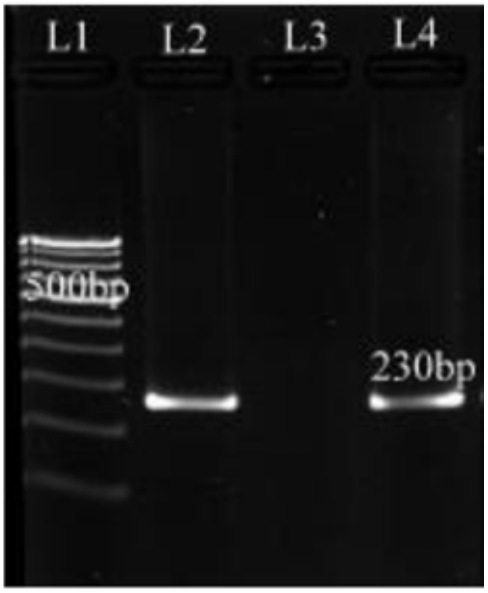
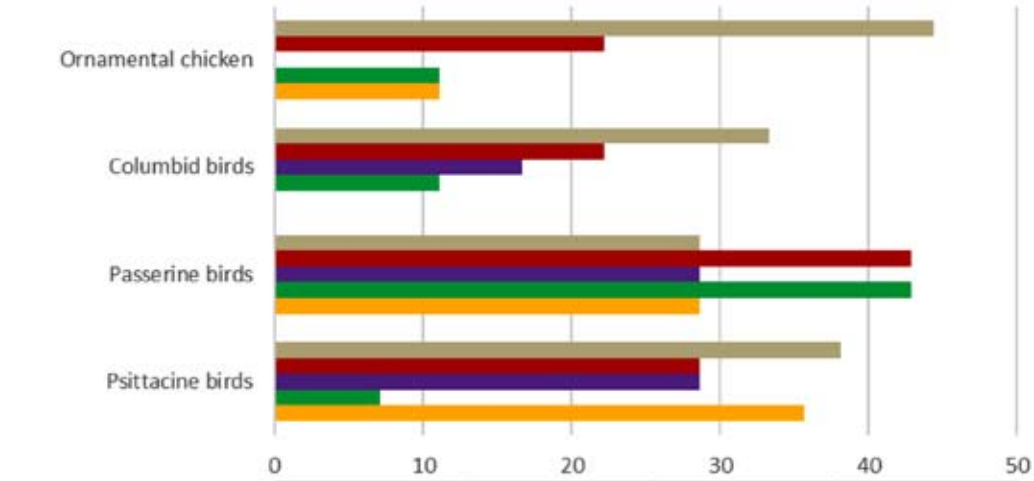


Fig. 6. Agarose gel electrophoresis, Identification of *Chlamydia* spp. *at* 230bp. Lane 1. 100 bp DNA molecular weight markers; Lane 2. Positive control; Lane 3. Negative control; Lane 4. Positive sample

an overall positivity of 39.3% with 26.2% for *Chlamydia psittaci* and 13.1% for *Chlamydia avium*. The sample details and positivity percentages are provided in Fig.7.

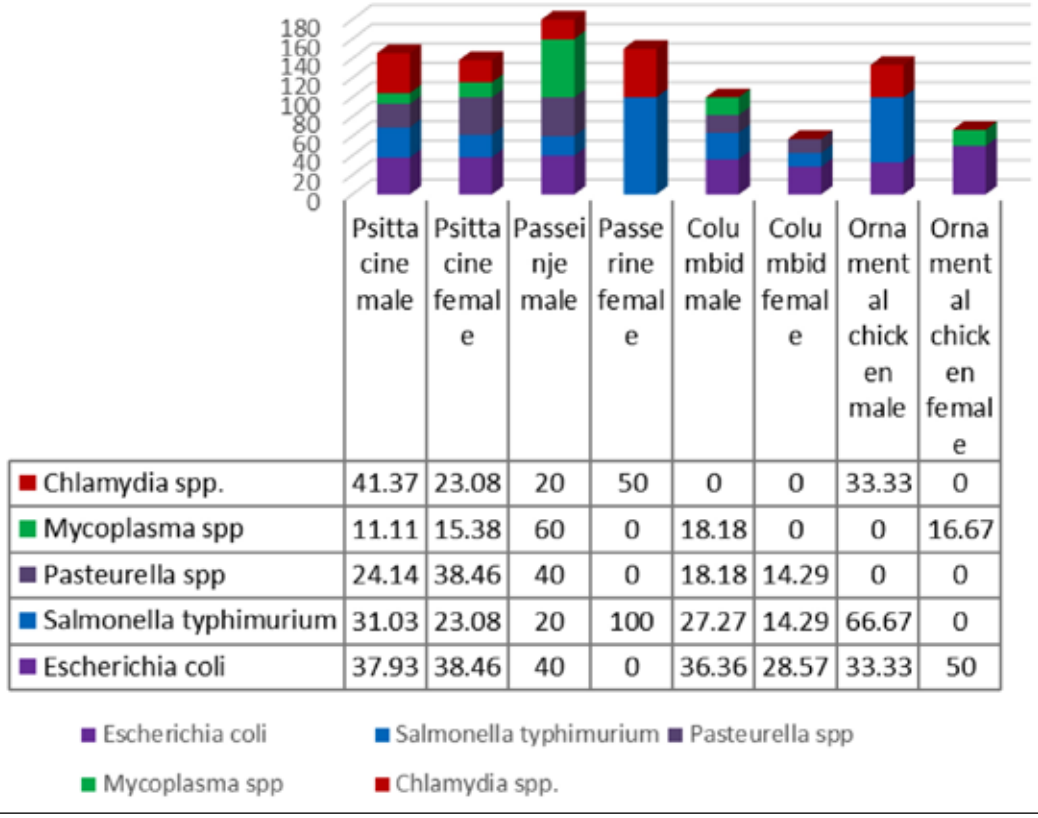
In this study, 11 out of 29 male (37.93%) and 5 out of 13 female (38.46%) psittacine birds were found positive for respiratory *E. coli* infection. The highest prevalence for *E. coli* infection was found in male psittacine. In passerine birds 40% positivity in male and no positivity in female birds for *E. coli* infection was found. In columbid birds, 36.66% and 28,57% positivity for *E. coli* was recorded in male and female birds, respectively. Among the ornamental birds with highest occurrence of *E. coli* infection was noted in female birds (66.67%). Nupur *et al*. (2023) suggested that the gender does not play a crucial role in infection rates of *E. coli* in birds which was found in connection with the current study that no drastic differences in infection rate among different sexes of birds. The highest prevalence for *S.* typhimurium infection was recorded in female passerine birds with 100% positivity. Salmonellosis in psittacine birds were identified with 31.03% and 23.08% in male and female birds, respectively. Chen *et al*. (2016) suggest that male and female birds exhibit different immune responses to Salmonella infections, with males showing a higher



	Psittacine birds	Passerine birds	Columbid birds	Ornamental chicken
Escherichia coli	38.1	28.6	33.3	44.4
Salmonella typhimurium	28.6	42.9	22.2	22.2
Pasteurella spp.	28.6	28.6	16.7	0
Mycoplasma spp.	7.1	42.9	11.1	11.1
Chlamydia spp.	35.7	28.6	0	11.1

Escherichia coli Salmonella typhimurium Pasteurella spp.
Mycoplasma spp. Chlamydia spp.

Fig. 7. Sample details and positivity percentages



	Psittacine male	Psittacine female	Passeinje male	Passerine female	Columbid male	Columbid female	Ornamental chicken male	Ornamental chicken female
Chlamydia spp.	41.37	23.08	20	50	0	0	33.33	0
Mycoplasma spp	11.11	15.38	60	0	18.18	0	0	16.67
Pasteurella spp	24.14	38.46	40	0	18.18	14.29	0	0
Salmonella typhimurium	31.03	23.08	20	100	27.27	14.29	66.67	0
Escherichia coli	37.93	38.46	40	0	36.36	28.57	33.33	50

Escherichia coli Salmonella typhimurium Pasteurella spp
Mycoplasma spp Chlamydia spp.

Fig 8. Gender-wise prevalence of respiratory bacterial infections

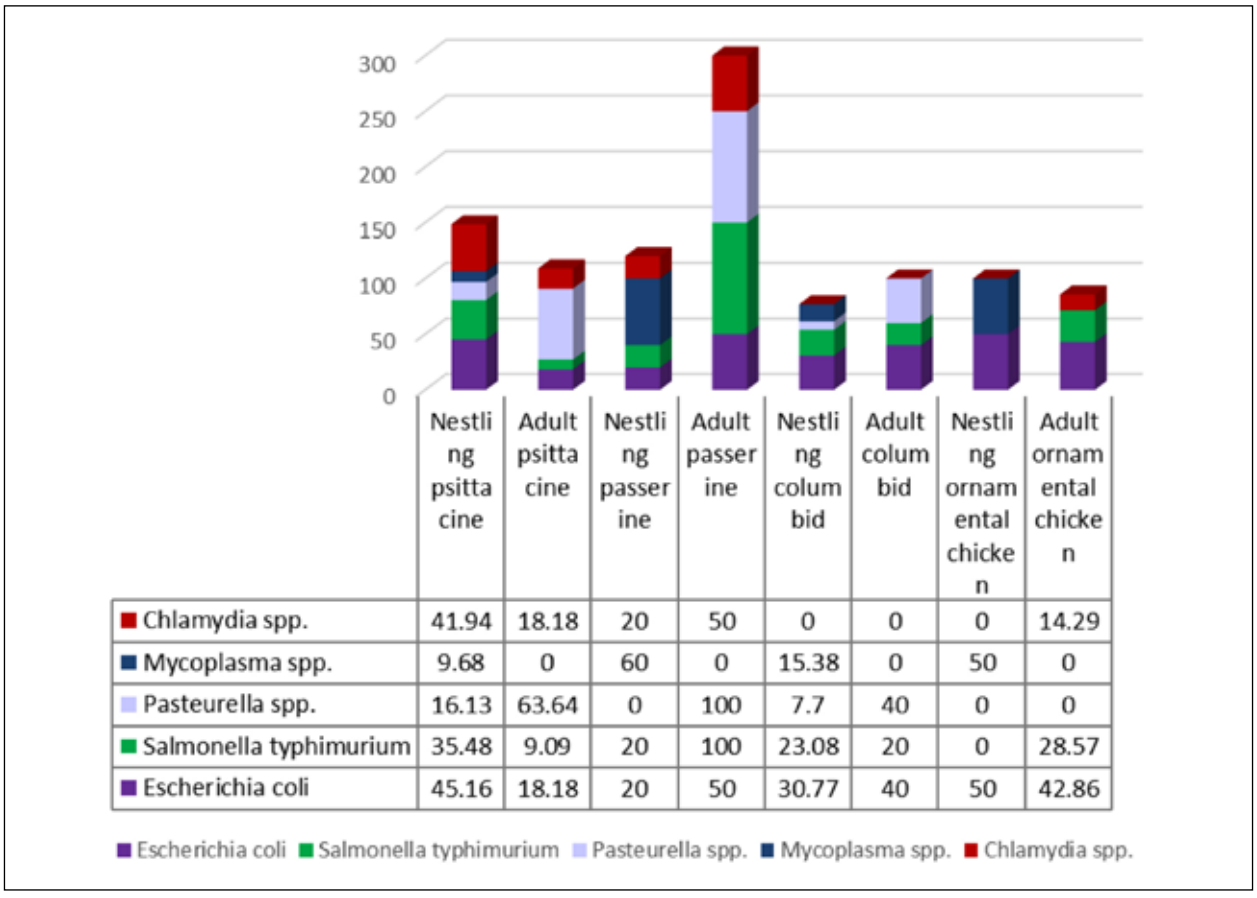


Fig. 9. Age-wise prevalence of respiratory bacterial infections

production of certain antibodies and reactive oxygen species which was resemblance to the infection rate of salmonellosis in passerine birds of current work

PCR identification of *Pasteurella* spp. revealed the highest occurrence (40%) in male passerine birds, while no positivity was observed in ornamental chickens. Among psittacine birds, 38.46% of females and 24.14% of males tested positive for *Pasteurella* spp. The identification of *Mycoplasma* spp. showed the highest occurrence in male passerine birds (60%), followed by 18.18% in male columbid birds and 16.67% in female ornamental chickens. The higher mycoplasma incidence was found in male passerine birds. Nolan *et al*. (1998) and Yancey *et al*. (2001) stated that immune response to respiratory mycoplasma infections varies by gender, with males often exhibiting more severe pathology due to factors like testosterone levels, which may compromise immune function. PCR identification of *Chlamydia* spp. revealed the highest prevalence in female passerine birds (50%), followed by male psittacine birds (41.37%) (Fig. 8).

The birds were categorised into two age groups: nestlings and adults. In this study, *E. coli* infection was found to be more among adult passerine birds (50%) and nestling ornamental birds (50%), with the lowest occurrence observed in adult psittacine birds (18.18%). The highest occurrence of *Salmonella* typhimurium was detected in nestling psittacine birds (35.48%). For *Pasteurella* spp., 100% positivity was recorded in adult passerine birds. Nestling columbid birds showed a 15.38% positivity rate for *Mycoplasma* spp. Among the 31 nestling psittacine birds examined, 13 were found positive for *Chlamydia* spp., representing the highest prevalence, followed by adult psittacine birds at 18.18%. In columbid birds, both nestling and adult birds, as well as nestling ornamental chickens, tested negative for chlamydial infections (Fig. 9). The age of pet birds plays a significant role in their susceptibility to bacterial infections, influencing both the colonisation patterns and immune responses. Younger birds often exhibit higher colonisation rates and more severe infections compared to older birds, which tend to have more developed immune systems capable of mounting stronger responses. This age-related variation in infection dynamics is crucial for understanding and managing bacterial infections in avian species.

Conclusion

This study examined 76 pet bird carcasses categorised into four major groups: Psittaciformes, Passeriformes, Columbiformes, and Galliformes. The study identified *E. coli and Chlamydia* spp. infections found mostly in psittacine birds, *Salmonella* typhimurium was detected in psittacine and passerine birds. *Salmonella* typhimurium, *Pasteurella* spp., *Mycoplasma* spp.

were detected in both psittacine and passerine birds. *Escherichai coli* infection were most common in adult passerine birds (50%), with the lowest occurrence observed in adult psittacine birds (18.18%). *Salmonella* typhimurium was detected in nestling psittacine birds, with no infections found in nestling ornamental birds (35.48%). *Pasteurella* spp. was 100% positive in adult passerine birds, while no infections were found in ornamental chickens. *Mycoplasma* spp. was observed in nestling passerine birds, with no infections detected in adult psittacine, passerine, columbid or ornamental birds.

Conflict of interest

The authors declare that they have no conflict of interest.

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