

Reproductive disorders in domestic canaries (*Serinus canarius domesticus*): a retrospective study on bacterial isolates and their antimicrobial resistance in Italy from 2009 to 2012

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Keywords

Antimicrobial resistance, Aviculture, Embryo mortality, Gram staining, Italy, Microbiology, *Serinus canarius domesticus*.

Summary

Reproductive disorders are responsible for significant economic losses in canary aviculture due to embryo and newborn chick mortality. Most of the time, deaths are caused by bacterial pathogens, however little published data exist about the prevalence of bacterial isolates that are identified during diagnostic protocols. This study reports on data collected from previous investigations carried out on cloacal swabs (n 456), unhatched eggs (n 52), and dead newborn chicks (n 68) collected from canary aviaries with a history of reproductive disorders. Of the examined samples, 41% were positive for the presence of pathogenic or potentially pathogenic bacteria, with particular regard to Gram negative species during bacteriological investigations. The most prevalent microorganisms were Gram-negative (55%). A predominance of *Klebsiella*, *Escherichia*, and *Pantoea* genera was observed. These are usually associated to pathological conditions in pet birds. Among Gram-positive bacteria, *Bacillus* spp. and *Staphylococcus* spp. were most prevalent. The antimicrobial susceptibility testing carried out on bacterial isolates showed a multiple resistance, especially against amoxycillin, erythromycin, spiramycin, tiamulin, and tylosin. This study represents a first attempt to provide an update on microbial causes of embryonic and neonatal mortality in canary aviaries in Italy; in addition, it provides further understandings about the efficacy of antimicrobial therapy.

Disordini riproduttivi nei canarini domestici (*Serinus canarius domesticus*). Uno studio retrospettivo sugli isolati batterici e la resistenza antimicrobica in Italia dal 2009 al 2012

Parole chiave

Resistenza antimicrobica, Avicoltura, Mortalità degli embrioni, Colorazione di Gram, Italia, Microbiologia, *Serinus canarius domesticus*.

Riassunto

I disordini riproduttivi sono responsabili di significative perdite economiche nell'avicoltura dei canarini per la mortalità dell'embrione e dei pulcini. I decessi sono causati principalmente da patogeni batterici ma sulla prevalenza degli isolati batterici identificati durante i protocolli diagnostici esistono pochi dati pubblicati. Questo studio riporta i dati raccolti da indagini precedenti condotte su tamponi cloacali (n. 456), uova non schiuse (n. 52) e pulcini nati morti (n. 68) provenienti da voliere di canarini con una storia di disturbi riproduttivi. Alle indagini batteriologiche è risultato positivo il 41% dei campioni esaminati: il 55% dei microrganismi apparteneva ai Gram-negativi con una predominanza dei generi *Klebsiella*, *Escherichia* e *Pantoea*, di solito associati a condizioni patologiche negli uccelli da compagnia. Tra i batteri Gram-positivi, sono risultati prevalenti *Bacillus* spp. e *Staphylococcus* spp. I test di sensibilità antimicrobica condotti su isolati batterici hanno mostrato una resistenza multipla, specialmente contro amoxicillina, eritromicina, spiramicina, tiamulina e tilosina. Questo studio è un primo passo verso l'aggiornamento sulle cause microbiche della mortalità embrionale e neonatale nelle voliere di canarini in Italia; fornisce, inoltre, informazioni sull'efficacia della terapia antimicrobica.

Reproductive disorders in domestic canaries (*Serinus canarius domesticus*) can cause economic losses in commercial flocks (Schmidt *et al.* 2003). Predisposing factors such as nutrition, housing, and overcrowding with different avian species can influence the onset of diseases associated with these disorders (Dorrestein 2003, Joseph 2003).

The examination of carcasses and unhatched eggs usually reveals macroscopic findings consistent with infection in tissues and organs (Dorrestein 2003). Bacterial aetiology is often involved in the outcome of diseases, with a predominance of Gram-negative species. There is currently no data about the incidence of disease caused by specific pathogenic microorganism in Italian aviaries (Glünder 1981, Gerlach 1994). Anatomopathological examination and microbial analysis carried out on specific specimens are essential in order to assess effective therapeutic protocols and prophylaxis measures.

This study provides an update on the most frequent microbial agents detected in the reproductive disorders of domestic canaries in Italy during 2009–2012. In addition, bacterial isolates were tested for antimicrobial susceptibility to the antimicrobial drugs that are routinely employed.

A retrospective analysis was performed on canary birds (*Serinus canarius domesticus*) that were submitted for microbiological investigations in the laboratories of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), Italy from 2009 to 2012. The samples were collected from canary colonies, in which reproductive disorders, including those causing high mortality in embryos and young birds, were observed. Data (aviary environment, light source, diet, pharmacological treatments) were collected for each colony. Samples were represented by cloacal swabs collected from adult female canaries with reproductive disorders as well as by unhatched eggs after the 13 day of

incubation or newborn chicks died within the first ten days of life.

Cloacal swabs were suspended in peptone water and stored at +4 °C along with the unhatched eggs and carcasses. The carcasses were submitted for necropsy and anatomo-pathological examination in order to collect tissue samples suitable for microbiological analysis. Cultures were taken under aerobic, microaerophilic, and anaerobic conditions. Gram staining bacterial colonies and the identification of bacterial species were performed through biochemical testing using Vitek system (BioMérieux, Bruz, France). Finally, the Antimicrobial Susceptibility Testing (AST), based on the disc diffusion method, was applied to assess bacterial isolates susceptibility to amoxycillin, colistin, erythromycin, neomycin, trimethoprim/sulfamethazole, tiamulin, aminosidin, ceftiofur, enrofloxacin, lincomycin-spectinomycin, spiramycin, tetracycline, and tylosin antibiotics (Bauer *et al.* 1966). The results of AST were expressed as a percentage of Resistant, Susceptible, and Intermediate strains tested for each bacterial species.

A Bayesian approach based on beta distribution (95% confidence interval) was applied to estimate the prevalence of bacterial genera.

In total, 162 aviaries were evaluated. The aviaries size ranged from a minimum of 15 up to 400 reproducing canary pairs. The breeding facilities were characterised by indoor cages, with different canary breeds (Fife, Border, Gloster, Gibber, Lizard, etc.) kept in pairs, often located within a garage, sometimes inside the house of the breeder, with natural or artificial lighting. Exotic or captive finches were also present. A mixture of seed (canary, niger, hemp, and linseed seed) and occasionally extruded foods were used for feeding the animals. Commercially available dry or wet mash, vegetables, and hard-boiled chicken eggs were also used, especially before the breeding season until the end of the molt period. At clinical examination, adult birds exhibited sporadic episodes of diarrhoea, abdominal swelling, weight loss, and dirty feathers around the vent. Wet and dirty cage bottoms – which suggest abnormal droppings in newborn canaries – were uncommon.

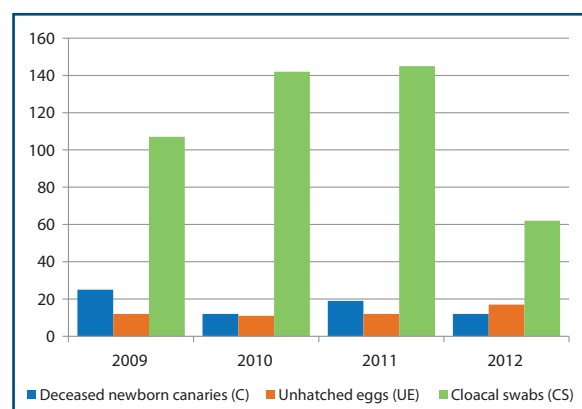


Figure 1. Cloacal swabs (CS), unhatched eggs (UE) and dead newborn chicks (C) collected from aviaries with reproductive disorders during 2009–2012 in Italy.

Table I. Results of microbiological investigations carried out on the cloacal swabs (CS), unhatched eggs (UE) and dead newborn chicks (C) collected from the aviaries under study (2009–2012, Italy).

Samples	No growth	No-specific bacterial flora	Bacterial isolates	Total (%)
CS	98	157	201	456 (79.17)
UE	29	6	17	52 (9.03)
C	38	7	23	68 (11.81)
Total (%)	165 (28.65)	170 (29.5)	241 (41.84)	576

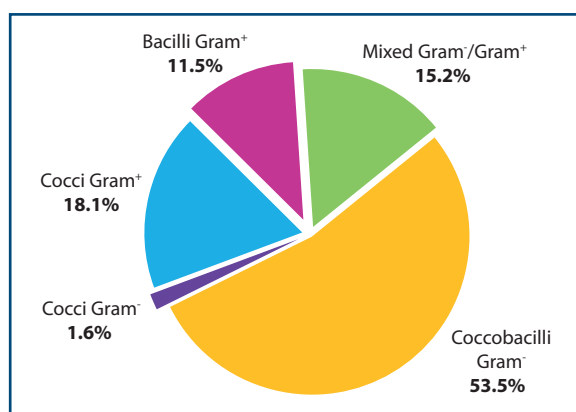


Figure 2. Gram staining of microbial isolates obtained from investigated specimens (2009–2012, Italy).

The mortality rate in embryos and young birds ranged from 10% to 30%.

A total of 576 samples, including 456 cloacal swabs, 52 unhatched eggs, and 68 carcasses were collected (Figure 1). At necropsy of the carcasses, abdominal swelling associated with cutaneous hyperemia, liver and spleen congestion, siero-haemorrhagic or colliquative enteritis were found. Microbial results are summarised in Table I. No microbial growth was observed in 28.65% of samples, while mixed bacterial flora has been identified in 29.5% of the specimens.

The remaining cultures were submitted to the Gram staining and biochemical test for bacterial identification. The most prevalent microorganisms were Gram-negative (55%) with predominance of coccobacilli, while the most common genera identified by biochemical analysis were *Bacillus*, *Staphylococcus*, *Klebsiella*, *Escherichia* and *Pantoea* (Figures 2 and 3). Vitek identification system showed predominance of *E. coli*, *S. sciuri* and *B. cereus* (Table II). Antimicrobial resistance was observed especially against amoxycillin, erythromycin, spiramycin, tiamulin, and tylosin in *B. cereus*, *Klebsiella* spp., *E. coli*, *Pseudomonas* spp. and *E. amnigenus*. Some macrolides (tylosin and erythromycin) and tiamulin are ineffective against many bacterial strains, especially those that are Gram-negative. Among Gram-positive bacteria (*Bacillus* and *Staphylococcus*) the most ineffective molecules are represented by amoxicillin, colistin, and tiamulin (Table III).

This study represents a first attempt to evaluate the role of microorganisms on embryonic and neonatal mortality in canary in Italy. The predominance of Gram-negative bacteria obtained from cloacal swabs could suggest their involvement in the mortality episodes reported in the aviaries under study. Historically, the normal intestinal flora of passerine birds should be predominantly composed of Gram-positive bacteria, while the Gram-negative

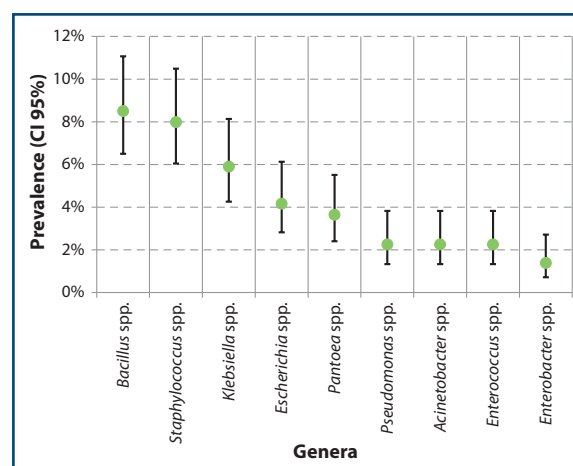


Figure 3. Prevalence of bacterial genera identified from the investigated specimens (2009–2012, Italy).

occurrence is frequently associated with disease conditions in these animals (Glünder 1981, Gerlach 1994, Reavill 1996, Dorrestein 2009). The diet can also influence the distribution of enteric bacteria, since a diet based on seeds and grains seems to be not suitable for intestinal colonisation by *E. coli* or *Klebsiella* spp. (Glünder 2002, Styles 2005). Therefore, the detection of enterobacteria from cloacal samples of granivore birds should be considered more carefully, as this suggests favorable conditions for the development of potential pathogens.

Table II. Bacterial species identified by biochemical test and Vitek system (BioMérieux, France), from cloacal swabs (CS), unhatched eggs (UE) and dead newborn chicks (C) collected from the aviaries under study (2009–2012, Italy).

Genus	Species	Positive samples	CS	UE	C
<i>Bacillus</i> spp. (n. 49)	<i>B. cereus</i>	13	9	2	2
	<i>S. sciuri</i>	17	17		
<i>Staphylococcus</i> spp. (n. 46)	<i>S. gallinarum</i>	7	3	3	1
	<i>S. aureus</i>	4	3		1
	<i>S. xylosus</i>	4	3	1	
<i>Klebsiella</i> spp. (n. 34)	<i>K. oxitoca</i>	4	3		1
	<i>K. pneumoniae</i>	3	3		
<i>Escherichia</i> spp. (n. 24)	<i>E. coli</i>	22	13		9
	<i>P. agglomerans</i>	8	5	2	1
<i>Pantoea</i> spp. (n. 21)	<i>Pseudomonas</i> spp. (n. 13)	5	5		
	<i>P. putida</i>	5	5		
<i>Acinetobacter</i> spp. (n. 13)	<i>A. iwoffii</i>	7	5		2
<i>Enterobacter</i> spp. (n. 8)	<i>E. amnigenus</i>	5	3	1	1

Table III. Number of antimicrobial Resistant (R), Susceptible (S) and Intermediate (I) strains for each genus and species identified. The values were expressed as percentage of tested strains. Values bigger than 50% of resistant and susceptible strains are in bold.

	Staphylococcus spp. (n. 23)			E. coli (n. 17)			B. cereus (n. 11)			Pseudomonas spp. (n. 10)			P. agglomerans (n. 8)			Klebsiella spp. (n. 7)			A. lwoffii (n. 4)		E. amnigenus (n. 4)	
Antibiotic	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	R	S
Aminosidin	4.3	56.5	0	0	29.4	5.9	0	54.5	0	30	30	0	0	25	0	0	14.3	14.3	0	0	40	20
Amoxycillin	60.9	34.8	4.3	76.5	17.6	5.9	81.8	18.2	0	90	10	0	37.5	50	12.5	100	0	0	50	50	20	80
Ceftiofur	0	47.8	52.2	0	100	0	72.7	27.3	0	80	0	20	0	100	0	0	100	0	0	100	0	100
Colistin	65.2	21.7	13	47.1	52.9	0	81.8	18.2	0	50	40	10	12.5	87.5	0	28.6	57.1	14.3	0	100	40	60
Enrofloxacin	8.7	39.1	13	11.8	82.4	5.9	0	90.9	9.1	50	40	10	0	87.5	0	0	100	0	25	75	20	80
Erythromycin	21.7	60.9	13	94.1	0	5.9	18.2	72.7	9.1	80	20	0	87.5	12.5	0	100	0	0	50	50	80	0
Lincomycin/ Spectinomycin	21.7	69.6	8.7	0	100	0	18.2	72.7	9.1	40	40	20	0	100	0	14.3	85.7	0	0	100	0	100
Neomycin	4.3	95.6	0	35.5	52.9	11.8	0	90.9	9.1	30	60	10	0	100	0	0	85.7	14.3	25	75	40	60
Spiramycin	26.1	30.4	43.5	76.5	17.6	5.9	36.4	36.4	27.3	70	0	0	62.5	12.5	25	100	0	0	50	50	100	0
Tetracycline	39.1	60.9	0	41.2	47.1	11.8	18.2	63.6	18.2	40	60	0	0	100	0	14.3	85.7	0	50	50	40	60
Tiamulin	65.2	34.8	0	88.2	11.8	0	72.7	27.3	0	90	10	0	12.5	87.5	0	100	0	0	25	75	80	20
Trimethoprim/ Sulfamethazole	8.7	91.3	0	11.8	88.2	0	63.6	36.4	0	60	40	0	0	87.5	0	0	100	0	25	75	0	100
Tylosin	26.1	69.6	0	82.4	11.8	0	18.2	81.8	0	90	10	0	75	12.5	0	100	0	0	100	0	100	0

A similar distribution of bacterial isolates, with a predominance of Gram-negative bacteria was also observed in the carcasses under study, reinforcing the hypothesis of a causative association between these microorganisms and neonatal mortality. These results are consistent with a previous survey conducted on 22 canary farms with a history of nestlings and embryo mortality. Bacterial tests performed on feed components employed in the 22 aviaries showed that the source of contamination was through the enterobacteria of the seeds, suggesting food as potential source of infection for canaries (Conzo *et al.* 1998). Similarly, the role of food contamination in the occurrence of Gram-negative infections was highlighted in passerine birds living in English gardens. A longitudinal study of four years demonstrated that *Salmonella typhimurium* and *E. coli* were the most common bacterial species isolated from the carcasses of wild birds during the winter season, when supplementing food supplies for these animals is predominant (Pennycot *et al.* 1998). Unfortunately in our study, no microbial investigations were performed on the feed used by farmers, and thus we cannot confirm this hypothesis.

Among the Gram-negative bacteria, *Klebsiella*, *Escherichia* and *Pantoea* spp. were more frequently detected by means of biochemical and Vitek system identification. *Klebsiella* genus includes potentially pathogenic bacteria for small passerines. This is responsible for septicemia, which is characterised by scattered petechial hemorrhage in various organs

at necropsy. The final stage of *Klebsiella* infection is characterized by encephalomyelitis associated to neurological symptoms (Gerlach 1994). *Escherichia coli* is one of the most pathogenic bacterial species in canaries. Liquid stools in nestlings (the nests are wet and pullus are called 'hard-earned'), often accompanied by mucus, lethargy, and death within 10 days of birth are the main symptoms observed in infected birds (Sandmeier 2006). *E. coli* can also invade the egg through the reproductive system of the infected female, or through the shell contaminated by fecal material. In these cases, the yolk sac appears watery, yellow-green, or yellow-brown. *Pantoea agglomerans*, previously classified as *Enterobacter agglomerans*, along with *Enterobacter amnigenus*, is not significant in adult birds but should be considered carefully in those cases involving very young subjects (Gerlach 1994, Dorrestein 2009, Conzo *et al.* 1998).

Pseudomonas fluorescens and *P. putida* are ubiquitous in aviaries, and are usually detected in drinking water or feed. Nevertheless, under favorable conditions, they can play a significant role as opportunistic pathogens. In particular, *Pseudomonas fluorescens* has been recognised as being responsible for hepatic necrosis in birds (Fudge *et al.* 1992, Jackson and Phillips 1995). Similarly, *Acinetobacter* spp. are considered opportunistic agents, able to determine cutaneous infection in humans; but septicemia and meningitis can also occur (Jiménez-Mejías *et al.* 2002, Mathews *et al.* 2005, Guerrero *et al.* 2010). Many avian species

can harbour these types of microorganisms in their respiratory or intestinal tracts, where transmission to the egg is possible. Therefore, it is assumed that *Acinetobacter* spp. has low pathogenicity in birds, and, therefore, the infection usually occurs in immunocompromised subjects (Gerlach 1994).

The pathologic role of Gram-positive bacteria in birds is considered controversial. In this study, most Gram-positive species belong to *Bacillus* and *Staphylococcus* genera. A recent study conducted on 12 psittacine carcasses kept in a Brazilian zoo, reports the presence of *Bacillus cereus* in various organs associated with lung hemorrhage, hepatic congestion, hemorrhagic enteritis, and congestive heart failure (Godoy *et al.* 2012). A study conducted by Hermansand and colleagues suggests that *Staphylococcus aureus* may represent an occasional pathogen for psittacine birds but it is not clear whether it is a primary pathogen or the infection is concomitant with other microorganisms (Hermansand *et al.* 2000). In canaries, *S. aureus*, *S. intermedius*, and *S. hyicus* are associated with *Megabacterium ornithogaster*, *Atoxoplasmaserini*, *Chlamydiophila psittaci*, or Canary poxvirus (Devriese *et al.* 1994).

Of special concern here are the results obtained from the analysis of antimicrobial resistance. Multiple antimicrobial resistance was observed for many isolates belonging to *E. coli*, *B. cereus*, *Pseudomonas* spp., and *Klebsiella* spp. In avian medicine, data regarding drug resistance are available for many species, especially in the poultry industry (Nemeghaire *et al.* 2014, Usui *et al.* 2014, Van Hoorebeke *et al.* 2011). This is not the case with canary or cage bird flocks. Our results suggest that antimicrobial resistance in canaries is relevant, and thus should be taken into account in order to establish the most appropriate treatment for bacterial diseases.

In conclusion, this study represents a first attempt to determine the impact of bacterial infections on the reproductive performance of canaries. Further investigations are needed to define more in depth the pathological role of each microorganism in more detail, and identify other potential causative agents such as viruses, parasites, or fungi that could play a synergistic role with bacterial microorganisms in diseases occurrence.

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