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Case Report

Treatment of Infectious Coryza in Canaries: Antibiotic Sensitivity and Clinical Outcomes

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ABSTRACT

Introduction: Infectious coryza is a respiratory disease that affects poultry and other avian species. It is caused by *Avibacterium paragallinarum*. Common clinical signs of infectious coryza include rhinitis, facial swelling, anorexia, and retarded growth in young poultry.
Case report: This report delves into a specific infectious coryza outbreak among a herd of 120 ornamental canaries in Iran in February 2021, where 15 canaries succumbed to the disease. The canaries indicated symptoms, such as swollen heads, closed eyes, severe sinusitis, weight loss, loss of appetite, and reduced ovulation. After the necropsy, a clumpy discharge in the eyes and sinuses and minor bleeding were observed in the trachea. Samples from the sinuses of dead canaries were taken to the laboratory (Mashhad, Iran), and it was determined that the bacteria responsible for the deaths belonged to the genus *Avibacterium*. *Avibacterium* spp. are slow-growing and require a specific factor, nicotinamide adenine dinucleotide, for growth. The antibacterial susceptibility of the bacteria was tested using 18 different antibiotics. Based on the results, fosfomycin and amikacin were selected for treatment. The birds were administered oral fosfomycin (160 milligrams per kilogram) and injection amikacin (10 milligrams per kilogram) for 7 consecutive days. However, a recurrence of symptoms was observed a week after the initial treatment (second outbreak), prompting a further 5 days of treatment. The isolate was completely sensitive to fosfomycin, trimethoprim-sulfamethoxazole, vancomycin, penicillin, amikacin, and furazolidone. The bacterium showed intermediate susceptibility to other antibiotics which tested. After 72 hours of treatment, casualties ceased, and clinical symptoms were reduced. Complete resolution of symptoms was observed within a week. In the second outbreak of the disease, no casualties occurred, and the symptoms vanished within 48 hours of initiating treatment.
Conclusion: This case report underscored the infection of canaries flock by *Avibacterium* spp., which was sensitive to fosfomycin and amikacin in laboratory conditions, and and effectively facilitated the recovery of the infected birds *in vivo*. The antibiotic sensitivity test provided useful information for finding an effective treatment against bacterial infection, emphasizing the significance of collaborating with laboratories for optimal results. Furthermore, continuous monitoring of this isolate is imperative, as it may potentially play a role in upper respiratory disease outbreaks across diverse avian species.

1. Introduction

Infectious coryza (IC) or snot is an infectious upper respiratory disease affecting poultry and other avian species[1-3]. This disease is caused by *Avibacterium paragallinarum* (*A. paragallinarum*), previously known as *Haemophilus paragallinarum*[4]. Some clinical signs that are commonly seen in IC cases are rhinitis, facial swelling or edema, anorexia, and retarded growth in young poultry[5-7]. *Avibacterium* spp. cause a sudden inflammation of the nasal

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passages and sinuses, accompanied by an inflammation of the conjunctiva and swelling of the face and wattles. Pneumonia and airsacculitis are rarely observed in these cases[8]. The large economic losses due to IC include increased culling, decreased egg production (10-80%), decreased body weight, stunting growth, and some mortality (2-10%)[4].

Factors, such as the simultaneous occurrence of respiratory infections, the presence of nicotinamide adenine dinucleotide (NAD)-independent strains, the overgrowth of fast-growing bacteria that can mask the growth of *A. paragallinarum*, the need for specialized media for culturing, and the existence of various biovars all contribute to the challenge of confirming the diagnosis of the disease[9].

Natural reserves, such as golden pheasant, have established a significant role in spreading the infection, as they provide habitat for many endemic threatened species, and the outbreak of *A. paragallinarum* infection in some birds could have implications for other species[10].

Avibacterium paragallinarum generally requires reduced nicotinamide adenine dinucleotide (NADH) as a growth factor[11]. For this reason, isolation requires co-cultivation with *Staphylococcus epidermidis*[11] or *Staphylococcus hyicus* as a feeder[12] to supply this requirement. The *A. paragallinarum* is a relatively slow-growing organism that produces tiny dewdrop colonies and can be overgrown by other bacteria in diagnostic samples[13].

Antimicrobial agents may be used to treat the infected birds to reduce the severity and the spread of the disease. However, IC prevention mainly relies on good biosecurity practices and vaccination in poultry flocks. Despite these measures, sporadic IC outbreaks continue to occur and pose significant economic losses to the poultry industry, especially in developing countries. Otherwise, vaccination against IC in other avian species is not widely recommended.

The aim of the current study was to report the outbreak of *Avibacterium* spp. infection in ornamental canaries (*Serinus canaria*) in Iran, along with a description of the clinical, pathologic, and antibiotic susceptibility of the isolated strain. Additionally, the treatment program implemented for the infected flock was outlined.

2. Case report

During winter 2021, 15 male and female fatalities from a herd of 120 ornamental common canaries of different ages were referred to the Aban Veterinary Clinic, Mashhad, Iran. The symptoms before dying were head swelling, closing of the eyes, severe sinusitis, weight loss, loss of appetite, and reduced ovulation. During post-mortem examination, distinct changes were noticed in the affected birds, including the presence of a noticeable quantity of lumpy discharge in the sinus areas around the eyes. Additionally, minor bleeding was observed in the airway, known as the trachea. However, no significant abnormalities were found in the lungs.

Fresh casualties were immediately sent to the laboratory for further analysis. In the laboratory, the contents of the nose and sinuses around the eyes were cultured on McConkey, chocolate agar, and blood agar along with *Staphyococcus spp*. (*Staphyococcus spp*. on top streaked to provide nutrients [NAD])[11].

After incubating the samples at 37°C for 72 hours, small and clear colonies grew around *Staphyococcus spp*. bacteria. These colonies were subsequently purified and subjected to gram staining, morphological examination, oxidase activity assessment, and catalase reaction testing. Based on these analyses, it was determined that the bacteria belong to the genus *Avibacterium*. The sample exhibited tiny droplet-shaped formations and non-hemolytic colonies during the growth process, even during the initial cultivation period. Additionally, these isolates did not show growth when placed in MacConkey agar culture media under normal air incubation conditions.

Avibacterium species are slow-growing and fastidious bacterium that requires NAD for growth. There is no standardized medium for susceptibility testing; therefore, enriched chocolate agar was used for the antibacterial susceptibility tests by Kirby-Bauer disk diffusion method following the guidelines of Clinical and Laboratory Standard Institute[14]. A suspension of the isolates was prepared (adjusted to 0/5 McFarland turbidity standard) and used to perform the antibiogram[14].

Eighteen different antibiotic discs (Padtan Teb, Iran) were selected for testing. The size of the growth inhibition zone around each antibiotic disc was measured after incubation, and the results were recorded as susceptible, intermediately susceptible, or resistant according to the manufacturer's guide table.

The antibiotics tested included amikacin (30µg), cephalexin (30µg), ceftriaxone (30 µg), cefixime (5µg), cefazolin (30µg), doxycycline (30µg), florfenicol (30 µg), fosfomycin (200 µg), gentamycin (10 µg), enrofloxacin (5 µg), penicillin (10 µg), trimethoprim-sulfamethoxazol (1.25 + 23.15 µg), oxytetracycline (30 µg), tetracycline (30µg), vancomycin (30µg), chloramphenicol (30µg), danofloxacin (5 µg), and furazolidone (50µg).

Based on the results of the antibiogram and considering the available drugs, as well as the constraints associated with medication use in ornamental bird medicine, the treatment involved daily oral administration of fosfomycin at a dose of 160 mg/kg (according to the manufacturer's recommendation; FOSBAC®, BEDSON S.A., Argentina) and amikacin at a dose of 10 mg/kg twice a day for injection[15].

For drug therapy, the weight of the canaries in each cage was calculated, and the drug was prepared according to the instructions. The birds were kept thirsty for 3 hours once a day for one week, and water containing medicine was immediately provided to them. The medicated water was administered daily at an 8-hour interval, and the water in the drinking container was changed. During the remaining hours of the day, supportive treatment, including AD_3E and B-Complex vitamins was used at a dose of 1 milliliter per liter[15] of drinking water on a rotating basis.

Birds that experienced a loss of appetite and displayed lethargy were treated by administering the fosfomycin drug orally based on their body weight. Additionally, the Amikacin drug was also administered intramuscularly to

Table 1. Antibiogram results against *Avibacterium* infection in canary

Drug Name	Abbreviations	Sensitivity*	Drug Name	Abbreviations	sensitivity
Amikacin	AN	S	Gentamycin	GN	I
Cephalexin	CN	I	Enrofloxacin	NFX	I
Ceftriaxone	CRO	I	Penicillin	P	S
Cefixime	CFM	I	Trimethoprim-sulfamethoxazol	STX	S
Cefazolin	CZ	I	Oxytetracycline	OTC	I
Doxycycline	D	I	Tetracycline	TE	I
Florfenicol	FF	I	Vancomycin	V	S
Fosfomycin	FOS	S	Furazolidone	FZ	S
Chloramphenicol	C	I	Danofloxacin	DFX	I

* S: Susceptible, I: Intermediately susceptible

the birds.

One week after the initial treatment ended, the symptoms of the disease reoccurred . Immediately upon the onset of clinical signs, drug therapy was reinstated following the previous regimen and continued for a consecutive 5-day period.

The antibiotic sensitivity of the isolated bacteria is described in Table 1. After 72 hours of initiating treatment, casualties stopped clinical symptoms decreased. Within a week, all symptoms were resolved. In the second period of the disease, no casualties were observed, and the symptoms disappeared after 48 hours from the start of the treatment.

3. Discussion

Avibacterium paragallinarum is a bacterium of the Pasteurellaceae family that is a Gram-negative facultative anaerobe. It is known to cause acute infectious respiratory disease in chickens, with clinical signs resembling swollen-head syndrome[4]. One important feature of *A. paragallinarum* pathogenicity is its ability to adhere to and colonize the nasal mucosa during the early stages of infection. The disease caused by *Avibacterium*, IC, typically exhibits high morbidity and low mortality, but its severity can be worsened when complicated with other diseases, such as fowlpox, infectious bronchitis, laryngotracheitis, *Mycoplasma gallisepticum* infection, and pasteurellosis[8,16-18]. Most *A. paragallinarum* isolates require NAD as a growth factor[19].

The clinical signs of the chickens suspected of IC are facial swelling or facial edema, discharge from nasal sinuses, conjunctivitis, and decreased egg production[20,21]. In the current study, infected canaries that show typical signs produced a foul smell in chronic infection. The researchers' findings indicated that canaries are vulnerable to this disease at all stages of their lives.

In this study, the *Avibacterium spp*. colony on blood agar or chocolate agar was circular, transparent, and smooth dewdrops. This bacterium had relatively slow growth and could be detected after being incubated for 36-48 hours[22]. Besides *Avibacterium spp*., *Staphylococcus sp*. can also grow on this medium and be well observed with white circular morphology. Gram stain of *Avibacterium spp.* indicated that bacteria were coccobacilli morphology and red color (Gram-negative). The obtained result is in line with the study reported by Akhter et al.[23] and Priya et al.[24]. Colonies with morphological characteristics leading to *A. paragallinarum* colonies were recultured until pure colony. The suspected colony was isolated to blood agar and chocolate agar medium and added with *Staphylococcus spp*. as bacterial feeder[12,25]. The *A. paragallinarum* that needs V factor would grow alongside the bacteria feeder and form satellite colony[3,4,26], while some *A. paragallinarum* species could grow even without V factor. The addition of *Staphylococcus spp*. onto blood agar medium in this study showed that *Avibacterium spp.* isolates from canary were NAD-dependent and showed satellite colonies[27].

The isolate showed biochemical characteristics of *A. paragallinarum*, negative catalase, negative oxidase, which was also reported by Blackall and Soriano[28].

The isolate was completely sensitive to fosfomycin, trimethoprim-sulfamethoxazol, vancomycin, penicillin, amikacin, and Furazolidone. The bacterium showed intermediate susceptibility to oxytetracycline, doxycycline, tetracycline, ceftriaxone, cefazolin, cefixime, cephalexin, enrofloxacin, florfenicol, chloramphenicol, gentamycin and danofloxacin. While the isolated *Avibacterium spp.* was not resistant to any antibiotic, the susceptibility pattern was almost similar to other studies[27,29]. In a study conducted by Rajurkar et al.[30] in India, six *A. paragallinarum* were all resistant to tetracycline.

4. Conclusion

In the current study, *Avibacterium spp*. strain was isolated from canary using culture method. Antimicrobial susceptibility pattern of the isolate was determined. Antibiotic sensitivity test provides useful suggestion for appropriate treatment that is effective and efficient against the bacterial infection It is crucial to seek assistance from laboratory analysis for improved results. In the present study, according to the results of antibiogram, fosfomycin and amikacin antibiotics were selected for treatment and used for one week in the infected herd. Continued monitoring is necessary due to the potential involvement of this *A. paragallinarum* isolate in upper respiratory disease lesions across various bird species.

Declarations

Competing interest

There is no conflict of interest.

Authors' contribution

The initial examination and sampling for the project were carried out by Omid BehrouziNasab. Laboratory tests were conducted by a team consisting of Alireza Koochakzadeh, Majid Kazemnezhad, and Mahyar Yarahmadi Khorasani. The conceptualization of the project was a collaborative effort between Alireza Koochakzadeh and Omid BehrouziNasab, while the investigation phase was led by Alireza Koochakzadeh and supported by Omid BehrouziNasab. Project administration responsibilities were shouldered by Alireza Koochakzadeh, and both Alireza Koochakzadeh and Omid BehrouziNasab were responsible for the original draft of the project. The final stage of writing, including review and editing, was also jointly undertaken by Alireza Koochakzadeh and Omid BehrouziNasab. All authors read and approved the final draft of the manuscript.

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Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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