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THE INCIDENCE OF *SALMONELLA ARIZONAE* IN DIFFERENT POULTRY SPECIES IN NEW VALLEY GOVERNORATE, ITS SENSITIVITY AND ITS PATHOGENICITY IN TURKEY POULTS

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ABSTRACT

Arizonosis is a septicemic disease of turkey poults; one of the most frequently identified salmonella serotypes with significant mortality and morbidity causing serious outbreaks. The present work aimed to detect the incidence of *Salmonella arizonae* in different bird species in New Valley Governorate, its sensitivity and/or resistance to antibacterial drugs, and its pathogenicity for turkey poults. A total of 250 suspected samples from dead and sick birds of different species in the New Valley Governorate were collected. Isolation was carried out following standard methods, primarily through standard laboratory culture media, followed by biochemical identification. All obtained isolates were subjected to a set of 6 antibacterial agents to study their antibiogram sensitivity using the disk diffusion method. Experimental infection of seven-day-old turkey poults with the isolated *Salmonella arizonae* was carried out by oral and intraocular routes. Results revealed that 23 isolates were identified as *Salmonella arizonae* (9.2%) from turkey poults, pigeons, and quail of different ages. High sensitivity of *Salmonella arizonae* isolates was recorded for gentamicin, followed by colistin sulphate, doxycycline, and Trimethoprim/Sulfamethoxazole. High resistance of *Salmonella arizonae* isolates was recorded for florfenicol and amoxicillin. Clinical signs of experimentally infected turkey poults include depression, off food, whitish and greenish diarrhea, emaciation, ruffled feathers, and reluctance to move. Post-mortem examination showed that mild arthritis, bronzy coloration of the liver, typhlitis, pneumonia, hemorrhage in the brain, severe enteritis, and enlargement of spleen and gallbladder. Mortality rates in experimentally infected turkey poults reached 80% and 50% with both ocular and oral routes, respectively. We concluded that *Salmonella arizonae* could be detected with an incidence rate 9.2% from turkey, pigeons, and quail in New valley Governorate. *Salmonella arizonae* is sensitive for gentamicin, colistin sulphate, doxycycline, and Trimethoprim/ Sulfamethoxazole, while it is resistant to florfenicol and amoxicillin. Experimental infection of *Salmonella arizonae* in turkey poults results in high mortality and sever clinical signs and postmortem lesions.

Keywords: *Salmonella arizonae*, incidence, pathogenicity, sensitivity, resistance.

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INTRODUCTION

A diverse number of serovars of *Salmonella*, including *Salmonella arizonae* (*S. arizonae), have been* registered in all countries of the world. Despite the rapidly growing poultry industry, the periodic outbreaks due to *S.arizonae* infections led to huge problems in production and egg hatchability (Jones *et al.*, 2002).

Poultry species were important reservoirs of many zoonotic pathogens, of which *S.arizonae* is of prime importance to human by consumption of contaminated poultry meat and eggs. It causes gastroenteritis in young infants and immune-compromised individuals. Furthermore, the invasive form leads to complications such as meningitis, septicemia, and osteomyelitis (Cristina, 2017).

The genus *Salmonella* consists of only two species, *Salmonella bongori* and *Salmonella enterica*, with the latter divided into six subspecies; *S. enteric* subsp. *enterica*, *S. enteric* subsp. *salamae*, *S. enteric* subsp. *arizonae*, *S. enteric* subsp. *diarizonae*, *S. enteric* subsp. *houtenae*, and S. *enteric* subsp. *Indica* (More *et al.*, 2017). *S. arizonae* is a Gram-negative, non-spore-forming, motile, rod-shaped, facultative anaerobic bacterium (Wang *et al.*, 2015). *S. arizonae* infection is quite common among different poultry species including turkeys, chickens, ducks, quail, and pigeons (Özkalp, 2012). Turkeys were the principal species, especially turkey poults for infection with *S. arizonae* (Hafez, 2013; Tracy *et al.*, 2020). *S. arizona is* transmitted through vertical and horizontal transmission (More *et al.*, 2017).

In acute *S. arizonae* infection, young birds showed septicemia, neurologic signs, and mortality with a duration typically of 3-5 weeks, while adult turkeys exhibit asymptomatic chronically intestinal carriage and fecal shedding for extended periods (Shivaprasad, 2013). Infection of turkeys with *S. arizonae* seriously impairs fertility, hatchability, and egg production (Ibrahim *et al.*, 2013).

In the past few decades, the emergency use of antibiotics for the prevention and control of different species of bacteria in poultry industry has been on the rise (Davies, 2010). Many antibiotics currently have a broad spectrum of activity and high efficacy in a wide range of Gram-negative bacteria infections, such as aminoglycosides, tetracycline, and chloramphenicol (Shivaprasad and Barrow, 2008). Antibacterial susceptibilities of *S. arizonae* are not constant, but vary from time to time, with different environments, antimicrobial susceptibility testing has been conducted worldwide (Mir *et al*.,2015).

There was little information on the incidence and antibiotic susceptibilities of *S. arizonae* in Egypt. Thus, our study aimed to isolate and identify *S. arizonae* from different poultry species, including turkeys, chickens, ducks, quail, and pigeons in New Valley Governorate, to detect its sensitivity and/or resistance pattern against antibacterial agents, and to study its pathogenesis in turkey poults.

MATERIALS AND METHODS

1. Specimens collection:

A total of 250 suspected samples were collected from poultry farms and back yard birds in New Valley Governorate, including 84 intestines, 83 livers, 24 yolk sacs, 9 ovaries and 50 cloacal swabs; they collected from 91 turkeys aged from one day up to 7 months old, 79 chickens aged from one day up to 6 months, 30 ducks aged from one day to 15 days old, 25 pigeons older 6 months age, and 25 quail older 6 months age; as shown in **Tab(1).**

2. Isolation and identification of *Salmonella arizonae*:

Samples were cultured in brain heart infusion (BHI) broth (Oxoid, UK) to encourage the growth of *S .arizonae* and incubated at 37°C for 24 hours, then sub-cultured on bismuth

sulphite agar (Oxoid, UK) and incubated at 37°C for 48 hours (Shivaprasad *et al.*, 2006). Individual colonies were obtained and stained with Gram stain. Suspected colonies were subjected to different biochemical tests, including lactose and dulcitol fermentation, hydrogen sulfide production, indole, catalase, malonate, and gelatin hydrolysis (Mahajan *et al.*, 2003).

Table 1: Types and number of samples collected from different poultry species:

species	Age of birds	Tissue samples					Total
		Ovary	Liver	Yolk sac	intestine	cloacal swabs	
turkeys	From 1 to 7 days		20	10	20		91
	Over 7 months	7	5		4	25	
chickens	From 1 to 7 days		20	14	20		79
	One month		3		5	15	
	Over 6 months	2					
ducks	From 1 to 15 days		15		15		30
pigeons	Over 6 months		10		10	5	25
quails	Over 6 months		10		10	5	25
Total		9	83	24	84	50	250

3. Detection of antibacterial sensitivity and/or resistance patterns of *S.arizonae* isolates :

All *S. arizonae* isolates were subjected to *in vitro* antibiotic susceptibility testing against 6 antibacterial agents of different classes, including doxycycline, gentamicin, florfenicol, colistin sulphate, amoxicillin, and trimethoprim/sulfamethoxazole (Oxoid, UK) using disk diffusion test. The diameters of inhibition zones were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS sub-Committee's recommendation, 2002).

4. Experimental Infection:

Sixty-one-day old turkey poults were maintained in separate units at the Poultry Diseases Department, Animal Health Research Institute, New Valley Governorate, Egypt, with approval from the National Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt according to the OIE Standards for use of animals in research, following ARRIVE guidelines (Under the No.06/2023/0105). They randomly divided into three equal groups; 20 poults for each group and provided with feed and water ad libitum. Turkey poults were challenged with 0.5 ml of *S. arizonae* containing 2 $x10^8$ colony-forming units/mL (CFU/mL), in group (1) by ocular route and in group (2) by oral route, at 7^{th} days old of age. Turkey poults of group (3) were kept as non- infected control group. Turkey poults in all groups were observed daily for signs, mortalities, and postmortem lesions for 14 days post-infection.

RESULTS

1. Incidence of *S. arizonae*:

Twenty-three suspected *S. arizonae* isolates were obtained from different poultry species in the New Valley Governorate, with a

incidence rate 9.2%. A high rate of *S. arizonae* incidence was reported in turkey with a percentage of 20.8% (19 out of 91 examined turkeys), especially in turkey poults aged from 1 to 15 days. Lower incidence rates of *S. arizonae* in pigeons and quails were recorded with a percentage of 8% for both, especially in adult birds older than 7 months. Negative trials were done to detect *S. arizonae* in chickens and ducks.

In the present study, the results revealed that the highest rate of *S. arizonae* isolation was from the intestines (14.3%), followed by the liver (9.6%), and yolk sac (8.3%), respectively. *S. arizonae* was isolated from cloacal swabs with incidence rate 2%. No isolation of *S. arizonae* could be obtained from ovaries, as shown in **Tab(2).**

Table 2: Incidence of *S. arizonae* in different poultry species.

Species	No. of examined samples	Suspected *S. arizonae* isolates					Total No. of isolates	Incidence
		Liver	Yolk sac	Intestine	Ovary	cloacal swabs		
Turkey	91	8	2	8	0	1	19	20.8%
Pigeon	25			2			2	8%
Quail	25			2			2	8%
Chicken	79			0			0	0%
Duck	30			0			0	0%
Total	250	8	2	12	0	1	23	9.2%

2. Colonial, cellular, and biochemical characteristics of *S. arizonae*:

The obtained colonies of suspected *S. arizonae* appeared on bismuth sulphite agar as minute circular brown-black colonies with a metallic sheen that turned into black when time of incubation increased as shown in **Figure (1)**. Gram-stained films which made from suspected *S. arizonae* colonies revealed gram-negative bacilli.

Figure (1): *Salmonella arizonae* colonies on Bismuth sulphite agar

The results of biochemical characterization revealed that suspected *S. arizonae* isolates were positive for lactose fermentation, hydrogen sulfide production, catalase, malonate, gelatin liquefaction and negative for indole and dulcitol fermentation, as shown in **Table (3) and Figure (2).**

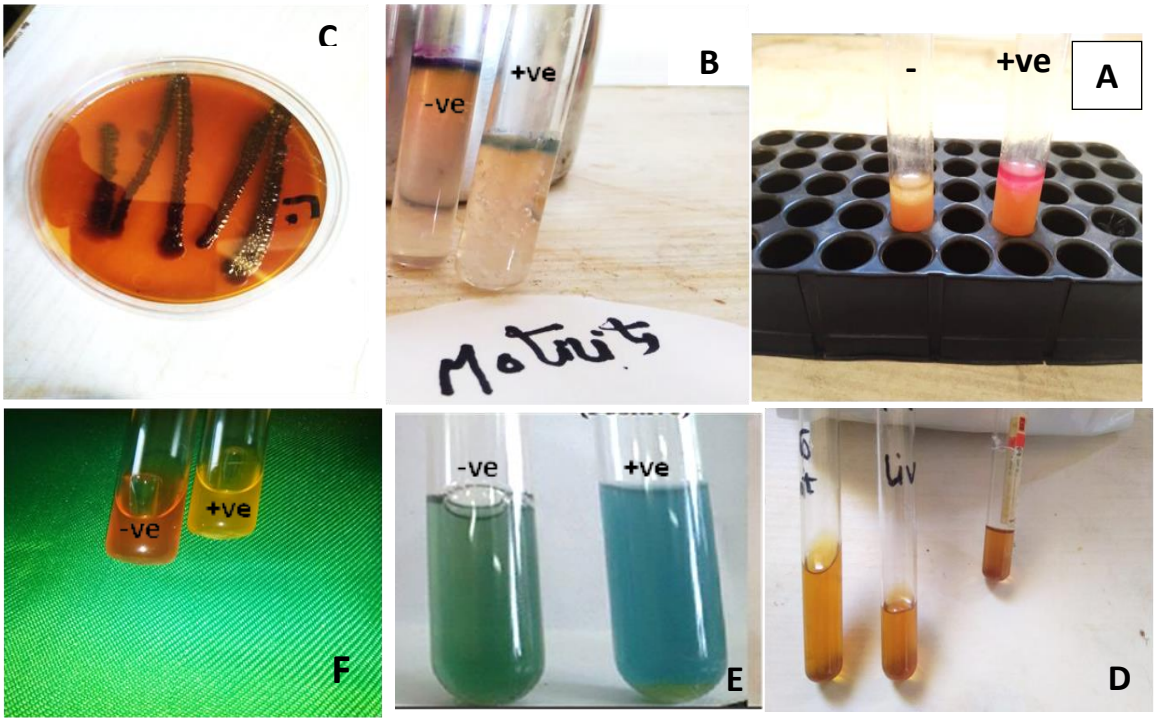

Figure (2): A: Hydrogen sulfide production, B: Motility test, C: Indole test, D: Gelatin liquefaction test, E: Sodium malonate test, F: Dulcitol hydrolysis

Table 3: Biochemical characters of *S. arizonae*.

Tests	*S. arizonae* isolates
Hydrogen sulfide production	+
Motility test	+
Lactose fermentation test	+
Indole production test	-
Dulcitol hydrolysis test	-
Gelatin hydrolysis test	+

3. Antimicrobial sensitivity:

In vitro antibiotic susceptibility assay showed that all suspected *S. arizonae* isolates were sensitive to Gentamicin (10%), Colistin (10%), Doxycycline (30%), and Trimethoprim/Sulfamethoxazole. While they were resistant to Florfenicol (10%) and Amoxicillin (25%), as shown in **Figure (3).**

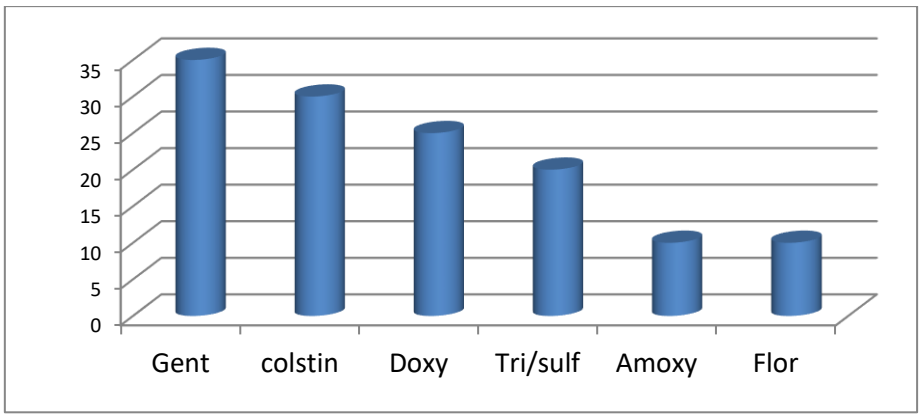

Figure (3): Antibacterial sensitivity testing of suspected *S. arizonae* isolates. Gent: gentamycin, Doxy: doxycycline, Tri/sul: trimethoprim/sulfamethoxazole, Amox: amoxicillin, and Flor: florfenicol.

Experimental infection:

In group (1), after 48 hours of ocular challenge with *S. arizonae, a* high mortality rate was detected (80%), and the living infected poults showed depression, off-food, whitish diarrhea, emaciation, ruffled feather, and mild arthritis. In group (2), the mortality rate reached 50% after 72 hours of oral infection and infected poults showed depression, off food, and greenish diarrhea. Bronzy discoloration of the liver, typhlitis, and pneumonia were detected in post-mortem examination in both groups, but in group (1), there was hemorrhage in the brain, severe enteritis, and mild arthritis. Enlargement of the spleen and gallbladder was observed in PM inspection in group (2). All turkey poults in the control negative group (3), showed no clinical signs and no post-mortem lesions until the end of the experiment, results of experimental infection are shown in **Table(4) and Figure (4,5).4.**

Table 4: Clinical signs and PM lesions of *S. arizonae* isolates experimental infection in turkey poults.

Group	Inoculation rout	Clinical signs	PM lesions
1	intraocular	A high rate of mortality was detected (80%) after 48 hours of infection with signs of ruffled feathers, whitish diarrhea, and emaciation.	Bronzy coloration of the liver, typhlitis, pneumonia, hemorrhages in the brain and severe enteritis, mild arthritis.
2	orally	Low rate of mortality, mortality reached 50% after 72 hrs of infection, off food, greenish diarrhea, Emaciation.	Emaciation, the bronzy coloration of the liver, typhlitis, severe enteritis, enlarged spleen and gallbladder.
3	Control	No mortalities and no clinical signs.	No PM lesions

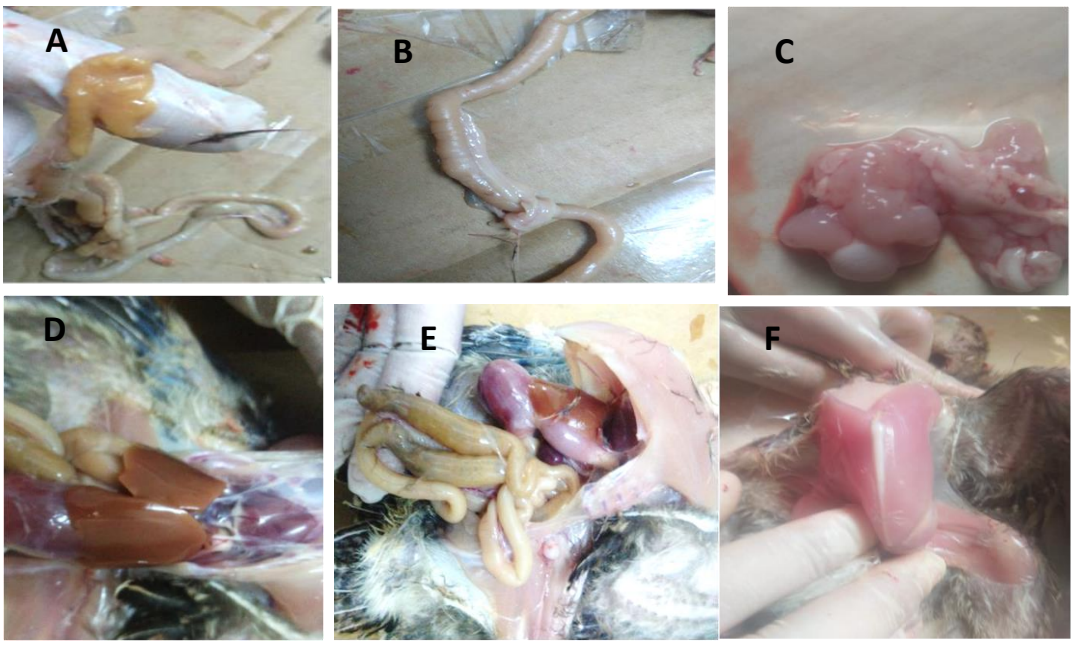

Figure 4: A,B: Severe enteritis, C: Hemorrhages in brain (ocular infection), D: Bronzy coloration of liver, E: Typhilitis (cecal core), F:Emaciation.

Figure (5): A: Sever emaciation, B: Mild arthritis (ocular infection), C: greenish diarrhea (oral infection).

DISCUSSION

Arizonosis, a disease that causes high mortality and morbidity rates in young turkey poults, resulting in severe economic losses through continuous outbreaks. *Salmonella arizonae* was isolated from turkey poults, pigeons, and quail in New Valley Governorate with an incidence rate of 9.2%. High rate of isolation was obtained from turkey poults (19 out of 91 samples). These results matched with a previous study, in which turkeys were the principal species for infection with *S. arizonae* (Park *et al.*, 2008). More *et al., (*2017) also reported that *S. arizonae* infection was confined to the first few weeks of life in different poultry species, especially young turkeys. Carrique-Mas and Davies (2008) reported that *S. arizonae* infection in turkeys was around one month of age. However, they also noticed infection in laying hens with an incidence rate (6%). Our results revealed that the rate of *S. arizonae* isolation in pigeons and quail was (8%), this low rate of isolation may be due to the low number of quail and pigeon farms in New Valley Governorate, strict isolation and biosecurity around farms, and intermittent shedding of organisms through fecal samples, these findings agree with Shivaprasad *et al. (*2006) and Ahmad *et al., (*2019).

In the present work, negative trials were done to isolate *S. arizonae* from suspected cases of chicken and duck, and this was in line with Cardinale *et al.* (2005) and El-Tawab *et al.* (2015), who attributed these results to the period of sample collection, different examined organs, the hazards of antibiotics for chickens, the variable fecal excretion of the organisms, and/or the limited reliability of cloacal swab cultures, although there was another report about *S. arizonae* infection in chicken and duck in which the pathogen was completely identified and the lesions studied in some details (Izat *et al.*, 2014).

Among the examined internal organs, the highest rate of isolation was from the intestine (14.3%), followed by the liver (9.6%). These results were agreed with Shivaprasad *et al.* (2006), who indicated that *S. arizonae* could be isolated from the liver of infected poults, and with Bhunia (2007), who stated that *S. arizonae* was isolated mostly from the intestinal tract, indicating that the main reservoir of *S. arizonae* was the intestinal tract of animals and birds. Acute infections

cause septicemia, and the liver was the first affected organ.

Ibrahim *et al*. (2013) identified one isolate of *S. arizonae* from the liver of broiler chickens during their investigation of 102 samples from Omdurman farms. In another study, Lenev *et al*. (2016) examined 371 samples from different broiler houses in Russia, and the isolation rate of *S. arizonae* was 6.5% of the total isolates. The percent of *S. arizonae* isolation by Klishchova and Nazarenko (2021), was 6%. In our study, the lowest rate of *S. arizonae* isolation from tissues was the yolk sac (8.3%); this matched with Lamas *et al.,* (2016) and Klishchova and Nazarenko (2021), who isolated *S. arizonae* from the yolk sac (6%); this may be explained by the severity of infection and/or antibiotics used at the point of lay, which transferred from adults to poults.

In the present study, collected samples were cultured in brain heart infusion broth to enhance the growth of *S. arizonae*, then sub-cultured on bismuth sulphite agar for differentiation of *S. arizonae* from other *Salmonella spp.* The bacterial colonies appeared minute and circular, brown black with metallic sheen because of hydrogen sulfide production by *S. arizonae*. The colonial and cellular morphologies and biochemical properties were similar to those reported by Murray *et al.* (1999). The total *S. arizonae* incidence rate was 9.2%, and this recent increase in its incidence among turkey poults could cause serious impediments to the poultry industry, especially in developing countries in Asia and Africa.

Our results of sensitivity and/or resistance pattern of all *S. arizonae* isolates against gentamicin and colistin sulphate were similar to those observed by Evangelopoulou *et al.* (2014), while they disagree with Irfan *et al*. (2015), who found that most of *S. arizonae* isolates were resistant to colistin sulphate; this may be due to the presence of resistance genes to colistin sulphate in some *S. arizonae* isolates.

Our results of *S. arizonae* sensitivity to doxycycline was comparable to Al-Salauddin *et al.,* (2015), who reported 100% resistance to tetracycline in their study. Resistance to tetracycline has been attributed to its irrational use of it as a growth promoter in poultry feed. In recent years, the use of tetracycline has been limited in food animals, which explains the change in the pattern of resistance.

Another finding of interest is the resistance of *S. arizonae* isolates to florfenicol and amoxicillin. This agreed with the study by Diarraa and Malouin (2014), who reported in their study a similar pattern of resistance against amoxicillin. These findings may be due to the growing resistance towards beta-lactam antibiotics, that has been prevalent worldwide among members of the Enterobacteriaceae of animal origin, especially in *Salmonella spp*., associated with various antibiotic-resistance gene determinants. This observation draws serious attention, as poultry serves as an important source of transmission of these multidrug-resistant *S. arizonae* genes to human.

Results of our study about sensitivity of *S. arizonae* to florfenicol did not match with a study by Mei *et al.,* (2021), who used florfenicol in poultry production to prevent and treat *Salmonella* infection, reducing intestinal colonization and decreasing susceptibility to Salmonella infection. Routine use of florfenicol for prophylactic and therapeutic purposes in the poultry industry leads to bacterial resistance to this antibacterial agent and selective pressure on various bacteria, such as *S. arizonae*.
Turkey poults, the most susbtable species for arizonosis, were experimentally infected with *S. arizonae* isolates, leading to developing of clinical signs and PM lesions similar to those reported by Tracy *et al*. (2020).

Hemorrhages in the brain were occurred in turkey poults infected by the ocular route, which may be attributed to the vascular

spread of the infection to the brain, undoubtedly following primary replication of *S. arizonae* outside the nervous system, which comes close to results obtained by Jortner and Larsen (1984). Also, the inflammation of the hock joints which was observed in birds that probably suffered from bacteremia and localization of the microorganisms in certain parts of the skeletal system as joints, that agrees with finding reported by Oh *et al.* (2010).

CONCLUSION

The present study demonstrates that *S. arizonae* caused serious mortality in turkey poults, as a first report in New Valley Governorate farms (turkey poults, pigeons, and quail). *S. arizonae* was detected in turkey poults, pigeons, and quail in the New Valley Governorate, with an incidence rate of 9.2%. The sensitivity and resistance to antibacterial agents in this study draw serious attention to the poultry sector in Egypt and New Valley Governorate, especially for the real need to prevent and control *S. arizonae* in poultry farms. Such data is essential for developing appropriate treatment of *S. arizonae* using high-efficacy antibacterial agents after conducting a sensitivity test. Proper scientific and public health regulations are needed to control the non-judicial use of antibacterial agents, that may reduce the emergence of microbial bugs, which are spreading worldwide and responsible for fatal disease outcomes in different parts of the world.

Ethical approval

The Animal Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt according to the OIE Standards for the use of animals in research following ARRIVE guidelines has approved the present study under permission No: 06/2023/0105.

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الكشف عن نسبة الإصابة ببكتيريا السالمونيلا أريزونا في أنواع الدواجن المختلفة بمحافظة الوادي الجديد وحساسيتها وقدرتها المرضية في الديك الرومي

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تعتبر عدوى الأريزنوزز في طيور الرومي الصغيرة واحدة من الأنماط المصلية للسالمونيلا التي تتميز بالإمراضية والنفوق العالي خلال تفشي المرض على نطاق واسع. تم جمع 250 عينة مشتبه بها من الطيور النافقة والمريضة من مختلف الأنواع في محافظة الوادي الجديد. تم إجراء العزل بالطرق القياسية من خلال وسائط الاستزراع المختبرية القياسية متبوعة باختبارات الكيمياء الحيوية. تم عمل اختبار حساسية لجميع المعزولات التي تم الحصول عليها باستخدام 6 مضادات بكتيرية لدراسة حساسية المعزولات للمضادات البكتيرية باستخدام طريقة أقراص المضادات الحيوية. تم عمل تجربه للكشف عن امراضية المعزولات من السالمونيلا أريزونا لصغار الديك الرومي البالغة من العمر 7 أيام تمت العدوى عن طريق الفم وداخل العين. ثلاثة وعشرون عزلة تم تحديدها على أنها سالمونيلا أريزونا بنسبة (9.2٪) من طيور الديك الرومي والحمام والسمان من مختلف الأعمار. تم تسجيل حساسية عالية المعزولات للجنتاميسين يليه كوليستين سلفات ودوكسيسيكلين وتريميثوبريم / سلفاميثوكسازول. تم تسجيل مقاومة عالية للعزلات للفلورفينيكول والأموكسيسيلين. لوحظت اعراض وآفات ما بعد الوفاة في كل من مسارات العين والفم بعد الإصابة التجريبية بالسالمونيلا أريزونا مع معدلات وفيات وصلت إلى 80 ٪ و 50 ٪ على التوالي.