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RESEARCH ARTICLE

Prevalence and Molecular Characterization of *Salmonella* Serovars Isolated from Diarrheic Cattle and Buffalo-Calves

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Abstract

In the first 10 weeks of life, bovine salmonellosis is the most serious infection typically affects calves. The aim of this work was to study the prevalence, antimicrobial susceptibility profile, attributes of some virulence and resistance genes of Salmonella isolated from diarrheic cow and buffalo-calves. A total of 200 fecal samples from cow and buffalo-calves were bacteriologically examined for isolation of Salmonella species. The percent of positive cases (n= 65 /200) was 32.5%. Serological typing of the recovered Salmonella isolates produced eight serotypes, Salmonella Typhmurium (13.8%), S. Anatum (7.6%), S. Sanktjohann (1.5%), S. Salami (20%), S. Mississippi (24.6%), S. Stratford (13.8%), S. Enteritidis (7.6%) and S. Saintpaul (10.7%). Upon ower knowledge, this is the first record of isolation of S. Sanktjohann from diarrheic calves in Egypt. The results revealed a higher incidence of salmonellosis in Spring (57.6%) followed by Winter (27.9%). Also, the incidence of salmonellosis was more recorded in cow calves (43.58%) than buffalo calves (16.86%). Antimicrobial susceptibility testing showed that the highest sensitivity levels were found for nalidixic acid (75%), enrofloxacin (62.5%), and chloramphenicol (50%) whereas, all isolates (100%) were resistant to ampicillin, gentamicin, streptomycin, and doxycycline. The 4 virulence genes (invA, avrA, stn, spvC) were found in the 8 examined Salmonella isolates. The blaTEM and tetA(A) resistance gene were detected in all isolates that were resistant to ampicillin and doxycycline. Tetracycline resistance gene (floR) was identified in 5 isolates; the sul1 gene was present in Sulphamethoxazole resistant isolates and the dfrA gene was present only in 2 isolates (S. Sankjohan and S. Mississippi) which existed resistance to trimethoprim. By comparing the stn gene sequence data of both S. Sanktjohann and S. Stratford with other Salmonella strains from the GeneBank the point mutation (Threonine 371 to Serine) was identified. In conclusion, this study proved the presence of different virulent and MDR salmonella isolates in diarrheic calves that make persistence shedding of microorganism into the environment. Moreover, antimicrobial sensitivity testing should be performed prior to treatment of Salmonella infection.

Keywords: Bovine salmonellosis, Calves, *Salmonella* serotypes, Antimicrobial resistance, Virulence genes.

Introduction

Salmonellosis is a major endemic disease of calves, which has been reported by an increase in incidence, in particular that caused by Salmonella Typhimurium in calves of intensive rearing systems [1]. Salmonellosis is a zoonotic disease that can cause serious infection in both calves and adult cattle. Clinical symptoms of bovine salmonellosis may include diarrhea fever, anorexia, dehydration, abortion, and endotoxemia evidence though many infections remain subclinical [2]. The monitoring of drug resistance patterns among Salmonella isolates not only gives vital clues to the clinician on the best therapeutic regime in each individual case, but is also an important tool in devising a comprehensive chemoprophylactic and chemotherapeutic drug schedule within a geographical area [3]. PCR is important tool to

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develop a highly sensitive and precise diagnostic method for rapid detection of Salmonella species in calves [4]. The invasion A (invA) is one of the most studied virulence factors that is also used as a biomarker for Salmonella spp. detection as it contains sequences that are unique to the genus Salmonella [5]. The spvC is virulence-related gene on the plasmid required for survival within host cell [6]. AvrA gene is an effector protein of the type III secretion system (TTSS) complex that contributes to the virulence of Salmonella spp. by limiting the host's inflammatory responses through the inducement of cell apoptosis, especially of macrophages, and by the innibition of IL-8 and TNF-α [7]. Salmonella enterotoxin gene that encoded stn induces more loss of intestinal fluids causing diarrhea [8]. Resistance to β-lactam antimicrobial agents in E. coli is primarily mediated by β-lactamases, which hydrolyze the β- lactam ring and thus inactivate the antibiotics [9]. At least nine different florfenicol resistance genes have been identified including floR [10]. Resistance to tetracycline is governed by tet genes, which are involved in either active efflux of the drug, ribosomal protection or enzymatic drug modification [11]. Thus, in this study we investigated the prevalence rate of salmonellosis among newly born cow and buffalo calves. In addition, the phenotypic resistance pattern of the recovered isolates, some antimicrobial resistance genes and virulence genes were determined.

Materials and Methods

Animals and clinical samples

Over the period from December 2018 to October 2019, a total of 200 diarrheal calves (117 cows and 83 buffalo) from large farm animals and sporadic cases from three governorates (Gharbia, Menoufia, Qaliubiya) attended the veterinary clinic. Rectal swabs were collected from diarrheal calves for bacteriological examination. All samples have been sent to the laboratory in an ice box, with minimal delay for bacteriological testing.

Bacteriological isolation

The rectal swabs were inoculated into tubes contain buffered peptone water for pre enrichment then each culture of pre-enrichment has been inoculated into Selenite F broth (Oxoid, UK) then each enrichment culture was streaked on a selective agar as into the xylose lysine deoxycholate (XLD, Oxoid, UK) agar for the isolation of Salmonella [12]. Suspected colonies were tested biochemically (urease production, methyl red (MR) and voges-proskaure (VP) tests, lysine decarboxylase production, citrate utilization, H_2S production and indole production) as documented previously [13].

Serological identification

Serological typing of Salmonella isolates was done using the modified Kauffman-White scheme as documented previously [14].

Antibiotic susceptibility testing

All the recovered Salmonella isolates (n=8) from diarrheal calves have been tested against 10 antimicrobial disks (Oxoid, UK) for their antimicrobial sensitivity using the standard disc diffusion methods [15]. The tested antimicrobials included Gentamicin (10 µg), Streptomycin (10 µg), Doxycycline (30 µg), Norfloxacin (10 µg), Enrofloxacin (5 µg), Nalidixic acid (30 µg), Ampicillin (10 µg), Ampicillin (10 µg), Levofloxacin (5 µg), Chloramphenicol (30 µg), and Trimethoprime-sulphamethoxazole (1.25 + 23.75 µg). The results were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines [16].

Molecular characterization of some virulence and resistance genes

The recovered salmonella isolates were tested for 4 virulence (invA, Stn, avrA and spvC) and 5 antibiotic resistance (blaTEM, floR, Sul1, tetA(A), and dfrA) genes (Table 1). The bacterial DNA was extracted using QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. The PCR reaction mixture consisted of 12.5 µL of Emerald Amp GT PCR master mix (Takara),1 µL of each set of forward and reverse primers (20 pmol), (Eurofins Pvt. Ltd., Bangaluru), 5 µL of DNA as a template and nuclease free water to make 25 µL of reaction volume. The PCR cycling conditions were programmed according to the reference of the primer (Table 1). The amplified PCR products were resolved by agarose gel electrophoresis, using 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL) and visualized and documented using UV gel documentation system (Alpha Innotech, Biometra).

Table 1: Oligonucleotide primer sequences used in investigation of *Salmonella* species isolated from diarrheic calves

Primer use and target gene	Sequence (5'→3')	Amplified product (bp)	References
Resistance genes	ATCAGCAATAAACCAGC	516	[17]
bla$_{TEM}$	CCCCGAAGAACGTTTTC		
floR	TTTGGWCCGCTMTCRGAC	494	[18]
	SGAGAARAAGACGAAGAAG		
Sul1	CGGCGTGGGCTACCTGAACG	433	[19]
	GCCGATCGCGTGAAGTTCCG		
tetA(A)	GGTTCACTCGAACGACGTCA	576	
	CTGTCCGACAAGTTGCATGA		
dfrA	TGGTAGCTATATCGAAGAATGGAGT	425	[20]
	TATGTTAGAGGCGAAGTCTTGGGTA		
Virulence genes	TTG TGT CGC TAT CAC TGG CAA CC	617	[21]
Stn	ATT CGT AAC CCG CTC TCG TCC		
invA	GTGAAATTATCGCCACGTTCGGGCAA	284	[22]
	TCATCGCACCGTCAAAGGAACC		
avrA	CCT GTA TTG TTG AGC GTC TGG	422	[23]
	AGA AGA GCT TCG TTG AAT GTC C		
spvC	ACC AGA GAC ATT GCC TTC C	467	
	TTC TGA TCG CCG CTA TTC G		

blaTEM: β-lactamases resistance gene, *floR* :chloramphenicol resistance gene, *Sul1*: Sulphamethoxaxole resistance gene, *tetA(A)*: Tetracycline resistance genes, *dfrA*: trimethoprim resistance gene. Stn: enterotoxin gene, *invA*: invasion gene, *avrA*: virulence associated effector protein gene, and *spvC*: *Salmonella* virulence plasmid gene.

DNA sequencing and phylogenetic analysis

The obtained PCR products of two isolates were purified by Qiaquick PCR purification kit (Qiagen Inc. Valencia CA) according to the manufacturer's Guidelines. Sequence analysis was performed to determine nucleotide composition of the strain detected for genotypic analysis and this was applied in both directions using the previously mentioned forward primer and reverse primers of *stn* gene by 3730 DNA Analyzer, Applied Biosystems, USA. Big Dye Terminator v3. 1 cycle sequencing kit (Applied Biosystem, UK). The manufacturer's protocol had been used as recommended. Sequences alignment (224 bp fragments of VP1) and Creating phylogenetic tree (Neighbor-joining) to detect genetic similarity of the strain tested of the current study compared to other strains worldwide registered in GeneBank were carried out using BioEdite software program V.5.0.9 [24] and MEGA-7 software program [25].

The identified strains were *S.* Stratford_*SH_QS1* with accession NO. MT019960 and *S. enterica*_SH_AS2 (*S.* Sanktjohann) with accession NO. MT019961.

Results

Prevalence of Salmonella serotypes among diarrheic calves

The clinical examination of 200 diarrheic calves revealed variable consistency of diarrhea (watery, pasty, mucoid and bloody), fever, with different grades of dehydration, and paleness in mucous membrane. Some animals suffered from respiratory manifestation.

Out of 200 bacteriologically examined rectal swabs, 65 samples were positive. *Salmonella* isolates on XLD media were pink with black center and categorized to 8 serogroups. All isolates were positive for catalase, methyl red, and lysine decarboxylase tests and negative for indole, VP, oxidase test and urea hydrolysis. On TSI agar *Salmonella* not ferment lactose and produced red slant and yellow butt with H_2S production. All *Salmonella* isolates were motile on semisolid agar media. The rate of *Salmonella* infection was 16.86% (14/83) and 43.58% (51/117) among the examined diarrheic buffalo and cow calves, respectively. The prevalence rates in Spring were 72.4% and 39.13% and in Winter were 40.42% and1 2.8%, while in Summer

were 32.35% and 0% in cow and buffalo calves, respectively. The identified serotypes were *S.* Typhmurium and *S.* Anatum from the examined diarrheic buffalo calves, *S.* Salami, *S.* Mississippi, *S.* Sanktjohann, *S.* Stratford, *S.* Saintpaul, and *S.* Enteritidis from cow calves (Table 2).

Table 2: Serotypes, resistance phenotype, virulence and resistance genes of the isolated *Salmonella* from the examined calves

Specie	Age (days)	Sex	Locality	Clinical signs	Serovar	Resistance profile	Virulence genes	Antimicrobial resistant genes
Buffalo calf	120	Male	Gharbia	Watery diarrhea, weakness, anorexia, fever (41,5 °C)	Typhmurium	Am, CN, S, DO, ENR, SXT and C	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*, *TetA(A)* and *Sul1*
	60	Female	Gharbia	mucoid diarrhea with fetid odor, increase temp (39,9 °C)	Anatum	Am, CN, S, DO and SXT	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*,*TetA(A)* and *Sul1*
Cow calf	15	Female	Monofia	Profuse watery diarrhea, subnormal temperature (36 °C) dehydration	Salami	Am, CN, S, DO, SXT and C	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*, *TetA(A)* ,*Sul1* and floR
	25	Female	Mmonofia	mucoid diarrhea with soiled tail, offensive odor, normal temp (39,2 °C)	Mississippi	Am, CN, S, DO, LEV, NOR, ENR, SXT, C and NA	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*, *TetA(A)* ,*Sul1* and dfrA
	21	Female	Monofia	mucoid diarrhea, fever (41 °C), red m.m	Sanktjohann	Am, CN, S, DO, LEV, NOR, ENR, SXT, C and NA	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*, *TetA(A)* ,*Sul1*, floR and dfrA
	45	Female	Monofia	Pasty diarrhea, normal temperature (39,2 °C)	Stratford	Am, CN, S, DO, NOR and SXT	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*, *TetA(A)* ,*Su1* and *floR*
	60	Female	Qulubia	mucoid diarrhea, fever (41,7 °C) high respiratory rate, nasal discharge	Saintpaul	Am, CN, S, DO, LEV, NOR and SXT	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*,*TetA(A)* ,*Sul1* and *floR*
	30	Male	Qulubia	watery diarrhea, normal body temp (38,9 °C)	Enteritidis	Am, CN, S, DO, NOR and SXT	*Stn*, *invA*, *spvc* and *avrA*	*blaTEM*,*TetA(A)* ,*Sul1* and *floR*

Am: ampicillin, C: chloramphenicol. CN: gentamycin, DO: doxycycline, ENR :enrofloxacin, LEV: levofloxacin, NA: nalidixic acid, NOR: norfloxacin, and SXT :Sulphametaxozle+ trimethoprim.

Resistance phenotypes of the recovered Salmonella isolates

All tested *Salmonella* isolates (100%) were resistant to ampicillin, gentamicin, streptomycin, doxycycline and sulphamethaxozle + trimethoprim, Meanwhile, 62.5% of the isolates were resistant to norfloxacin, and 50% to chloramphenicol. The lowest resistance rate was observed against levofloxacin and enrofloxacin (37.5%) and nalidixic acid (25%).

Virulence characteristics and determinants of antibiotic resistance

PCR technique was used as a molecular tool in this study to detect 4 virulence genes (*invA, avrA, stn, spvC).* It was found that, all 4 virulences genes were detected in all the 8 salmonella isolates with percentage of 100% **(**Figure 1**).** All isolates which were resistant to doxycycline, ampicillin, and sulphamethaxozle were positive for *tetA(A)*, *blaTEM*, and *sul1* genes **(**Figure 2). Two isolates which existed resistance to trimethoprim yielded 425bp amplicons for *dfrA* gene and 5 tetracycline resistant isolates produced 494 bp amplicons for *floR* gene (Table 2).

PCR successfully amplified the *stn* gene with band of amplification size at 617 bp from the isolates. After sequencing and analysis of the 617 bp PCR products of *S.* Sanktjohann and *S.* Santipaul with the other *Salmonella* strains on the GeneBank database, the point mutation (Threonine 371 to Serine) was identified. The phylogenetic analysis indicated that *S.* Sanktjohann that belongs to *S. Enterica* (GeneBank accession NO. MT019961) has identity percent of 99.7% with *S.* Sloterdijk ATCC15791 (CP012349), *S. Paratyphi* A ATCC9150 (CP000026), and *S. Paratyphi* A ATCC11511 (CP019185). The identified *S.*Stratford (MT019960) has 100% identity with *S.* Typhmurimum ATCC 13311 (CP009102), *S.* Typhmurimum PIR00538 (CP025555), and *S.* Typhmurimum 01ST04081 (CP029840) (Figure 3).

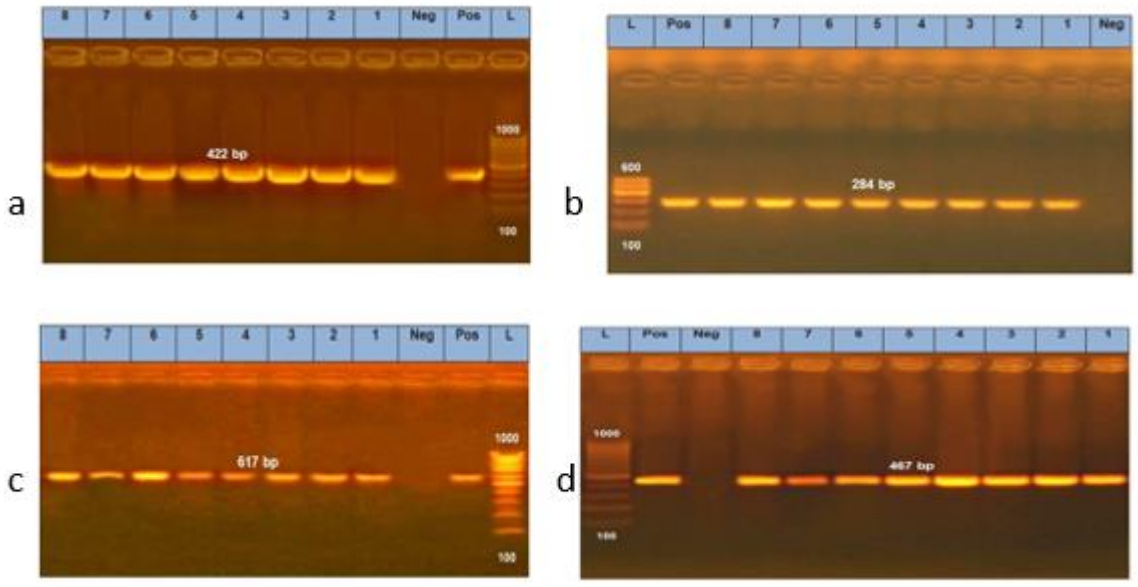

Figure 1: Agarose gel electrophoresis reveled amplification product for the *avrA* gene at 422bp (a), *invA* at 284bp (b), *stn* at 617 bp (c), and s*pvC* gene at 467 bp (d). Lane L 100bp DNA molecular size marker, lanes Pos. and Neg. positive and negative controls, respectively. Lanes (1-8) referred to the examined *Salmonella* isolates from diarrheic calves.

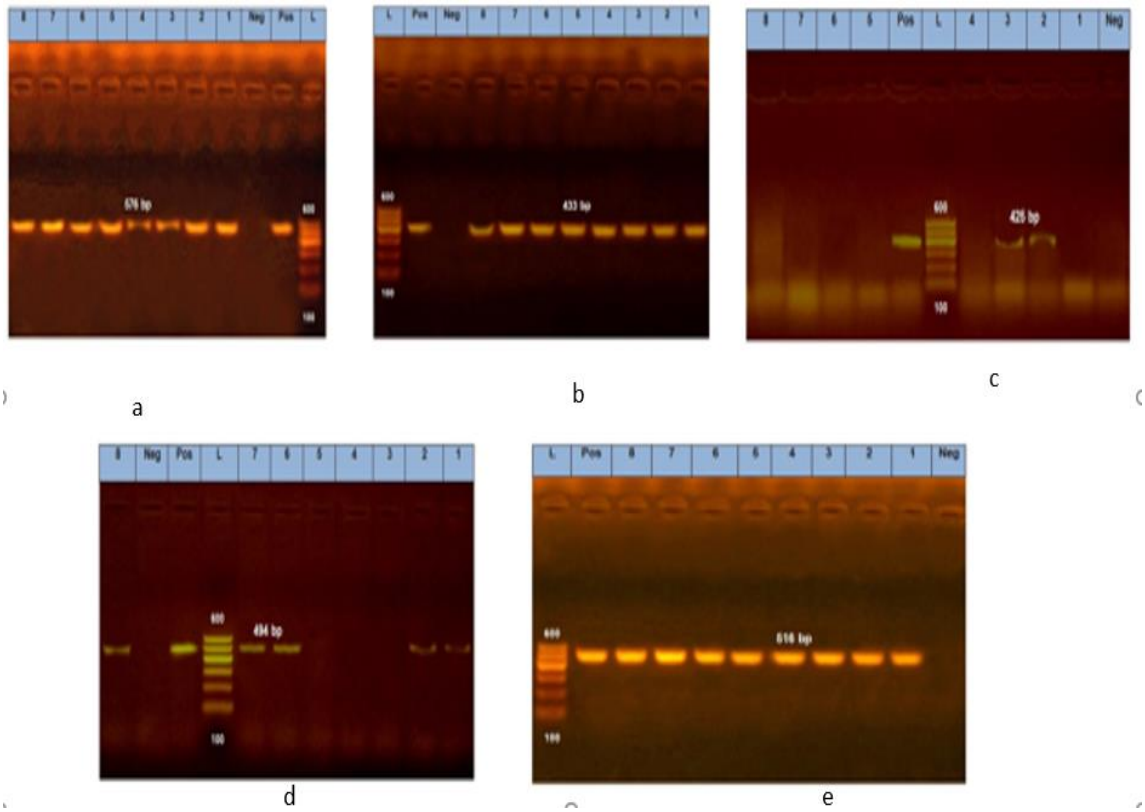

Figure 2: Agarose gel electrophoresis reveled amplification product for the *tetA(A)* gene at 576 bp (a), *sul1* at 433 bp (b), *dfrA* at 425 bp (c), *floR* at 494 bp (d) and *blaTEM* antibiotic resistant gene at 516 bp (e). Lane L 100bp DNA molecular size marker, lanes Pos. and Neg. positive and negative controls, respectively. Lanes (1-8) referred to the examined *Salmonella* isolates from diarrheic calves.

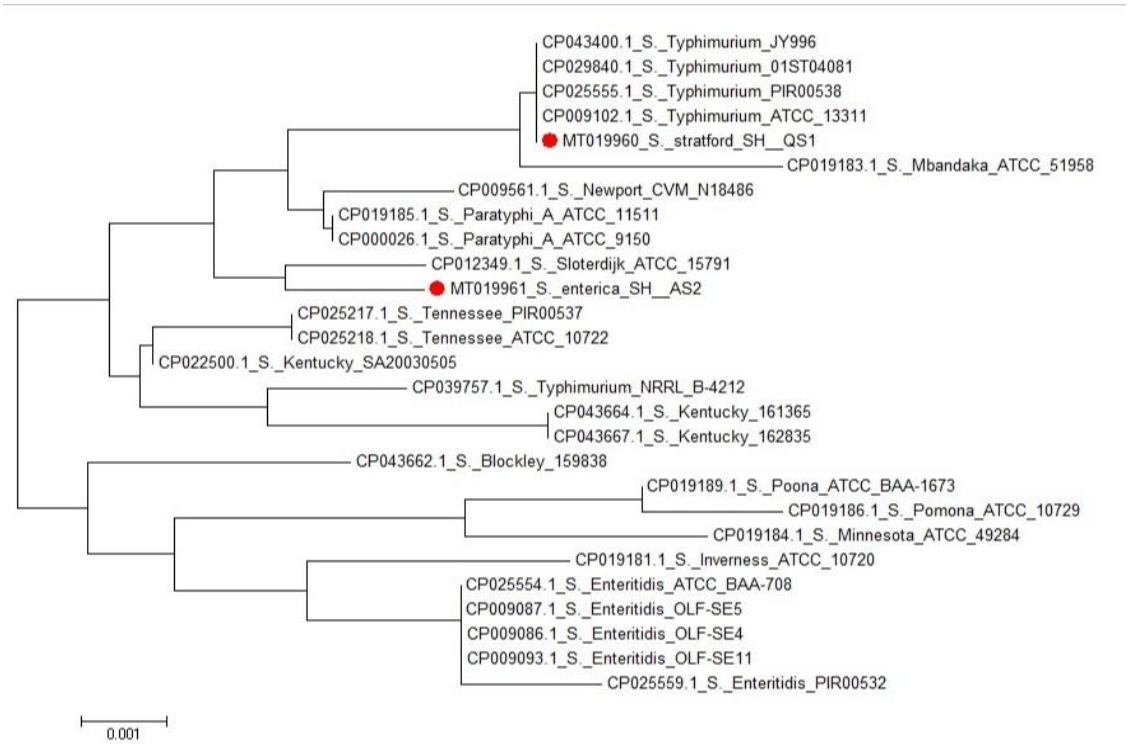

Figure 3: Phylogenetic tree of *stn* virulence gene showing the genetic relationship of *Salmonella S.* Sanktjohann (accession NO. MT019961) and *S.* Stratford (MT019960) isolated from diarrheic calves and the other *Salmonella* spp. available from the GeneBank.

Discussion

Salmonellosis is a common disease of bovine and calves [26]. This study was planned to identify the prevalence of *Salmonella* among cases of cattle diarrhea with special reference to some of their virulence genes and antimicrobial sensitivity profile. Upon clinical examination of diarrheic calves, the diarrhea was graded in 4 classes according to the consistency of the fecal matter and it was found that, the majority of cases suffered from mucoid diarrhea and increase in body temperature and this findings was as before mentioned [27] that calves infected with salmonellosis showing clinical symptoms include fever, sluggish mentation, loss of appetite and scores that often include increased mucus and blood. In the present study, *Salmonella* Enteritidis was isolated from a male cattle calf at one month of age suffering from watery diarrhea, normal body temperature (38.9C) with dehydration. It was also isolated from diarrheic calves [28, 29]. As previous report [30], *S.* Saintpaul was recovered from a male at one-month of age exhibiting mucoid diarrhea, fever (41.7C), rapid respiratory rate, nasal discharge and congested mucous membrane.

Unlike some other studies [31] the highest seasonal rate of salmonellosis among the examined diarrheic cow and buffalo calves were in spring season followed by winter and summer. These variations may be due to the exposure to stressors in winter and spring seasons such as transport, starvation, overcrowding and change in temperature. Out of 200 samples, 65 (32.5%) was positive for salmonellosis, 14 swabs from buffalo calves and 51 swabs from cow calves. This percent is higher than previous study (10.7%, 21/195) [32].

The antimicrobial resistance of *Salmonella* species associated with horizontal transmission of antibiotic-resistant genes among *Salmonella* strains and other *Enterobacteriaceae* or clonal spread of antimicrobial drug-resistant serovars that are successful in worldwide dissemination [33]. The *invA* gene was present in all isolates as detected 284 bp PCR amplicon **[**34**]**. The *avrA* is an SPI-1 effector protein involved in the enteritis pathway, with critical roles in inhibiting inflammation and apoptosis and *AvrA* is secreted by both type three secretion system (T3SS)-1 and T3SS-2 **[**35**].** In our study, *AvrA* gene was present in all recovered 8 *Salmonella* isolates. our findings are in accordance with previous results **[**35**]** that *avrA* gene was present in 100% of the isolates. The *stn* gen was detected in all tested isolates (617 bp). This result differs from previously mentioned that *stn* gene was detected only in 20% of isolates **[**36**]**. Also, the *spvC* gene was detected in all tested *Salmonella* strains (100%) at a 467 bp which is in agreement with Giacomodonato *et al***.** [35], who found that *spvC* gene was present in 92% of tested isolates. Moreover, the *tetA(A)* gene was detected in all 8 recovered *Salmonella* strains at (576 bp) which showing resistance to doxycycline that result was not as mentioned previously [4].

There are more records of antibiotic resistance and multiple drug-resistant salmonellosis in developing countries as 31.8% of *Salmonella* isolates in sheep and goats, 44.4 %t in camel isolates, and 52 % in bovine isolates were resistant to the widely used antimicrobials [37]. In this study all *Salmonella* isolates were resistance to ampicillin as mentioned before [4]. However, another study [38] found the resistance to ampicillin was 58%. The result of antimicrobial sensitivity test of *Salmonella* isolates against aminoglycosides group showed that gentamycin and streptomycin not had any sensitivity against all *Salmonella* isolates (resistant rate 100%). Nevertheless, previous study [39] declared that 53.2% of *Salmonella* isolates were multidrug resistant (MDR) and 76.9% were resistant to streptomycin while the majority of the isolates were susceptible to gentamycin. In addition, Abd El-Rahman *et al*. [38] detected that the highest sensitivity was observed for streptomycin (80%) and gentamycin (75%).

All *Salmonella* isolates were resistant to doxycycline. This result was in contrast to that reported by Abd El-Rahman *et al*. [38`] who found that the resistance rate against tetracycline was 67%. However, Atyabi [40]

revealed that all *salmonella* isolates were resistant to doxycycline and erythromycin. In contrast to previous findings [4], 75% of the isolates were susceptible to nalidixic acid.

Sulfonamides make their action through interfering with the synthesis of folic acid of microorganisms by competing with P-aminobenzoic acid (PAPA) in the biosynthesis of Dihydrofolate [41]. Our results showed that, the sensitivity of the isolates to sulphamethoxazole/ trimethoprim was 0% and these results was completely agreed with Shekhar and Singh [42] who found that the maximum resistance was observed against sulphamethoxazole was 100%. Although 50% of the tested isolates were sensitive to chloramphenicol, Shekhar and Singh [42] reported that the highest level of sensitivity among salmonella isolates was to chloramphenicol (100%).

The percent of *tetA (A)* gene was 83.7% in all tested *Salmonella* isolates. However, Adesiji *et al*. [43] detected *tetA (A)* gene at a percent of 100%. *Sul* genes are those genes responsible for conferring resistance to sulfonamide drugs. Similar to previous findings [4], the *sul1* gene was detected at a 433 bp in all tested *Salmonella* strains.

Conclusion

The obtained results proved the detection of virulent and multidrug resistant *Salmonell*a serotypes from diarrheic calves. Therefore, the use of specific antimicrobial drug for treating *Salmonella* infection after application of sensitivity test is still a must.

Conflict of Interest

The authors have no conflict of interest to declare.

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الملخص العربي

معدل الاصابه والتوصيف الجزيئي للسالمونيلا المعزولة من عجول الابقار والجاموس المصابة بالاسهال

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في الأسابيع العشرة الأولى من الحياة ، يعد داء السالمونيلا من أخطر الامراض التي تصيب العجول. كان الهدف من هذا العمل هو دراسة معدل انتشار المرض قابلية الإصابة بمضادات الميكروبات وسمات بعض جينات الضراوة والمقاومة للسالمونيلا المعزولة من المصابة بالاسهال. تم فحص ما مجموعه 200 عينة برازية من عجول الابقار والجاموس وذلك لعزل أنواع السالمونيلا المختلفة. أوضحت النتائج ارتفاع نسبة الإصابة بمرض السالمونيلا في الربيع (57.6٪) يليها فصل الشتاء (27.9٪). كما تم تسجيل حالات الإصابة بالسالمونيلا بشكل كبير في عجول الابقار (43.58٪) عن عجول الجاموس (16.86٪). النسبة المئوية للحالات الإيجابية من العدد الإجمالي هي 32.5٪. اوضحت الدراسة وجود ثمانية انماط مصلية لعزلات السالمونيلا ، (13.8٪) *S.* Typhmurium ، (7.6٪) *S.* Anatum ، (1.5٪) *S.* Sanktjohann ، *S.* Salami (20٪) *S.* stratford ، (24.6٪) *S.* Mississippi (13.8٪) و *S.* Enteritidis (7.6) و (10.7٪) *S.* Saintpaul ، وعلى حد علمنا فانه لاول مرة يتم عزل السالمونيلا سانكتجوهان من عجول الإسهال في مصر. أظهر اختبار الحساسية لمضادات الميكروبات أنه تم العثور على أعلى مستويات الحساسية لحمض النالیديكسيك (75٪) ، وإنروفلوكساسين (62.5٪) ، والكلورامفينيكول . ووجدت أعلى مقاومة للأمبيسلين والجنتاميسين والستربتوميسين والدوكسيسيكلين (100٪). تم العثور على جينات الضراوه الاربعه *invA* ، *avrA*، *stn*، *spvC* في 8 معزولات السالمونيلا التي تم فحصها

وتم اثبات وجود جين مقاومة *blaTEM* و *tetA (A)* في جميع العزلات التي كانت مقاومة للأمبيسيلين والدوكسيسيكلين. وتم تحديد جين مقاومة التتراسيكلين في 5 عزلات. كان الجين *sul1* موجودًا في عزلات مقاومة لسلفاميثوكسازول وكان جين *dfrA* موجودًا في عزلتين فقط (S.Sankjohan و S.Mississippi) التي كانت موجودة مقاومة لمضادات ترامیسوبريم. بمقارنة بيانات تسلسل الجين *stn* لكل من *S.* Sanktjohann و *S.* Stratford مع سلالات السالمونيلا الأخرى في بنك الجينات وجدت طفرة (Threonine 371→Serine) .أثبتت هذه الدراسة وجود العديد من عزلات السالمونيلا المقاومه للعديد من المضادات الحيوية في العجول المصابة بالإسهال التي تجعل استمرار التخلص من الكائنات الحية الدقيقة في البيئة صعب. علاوة على ذلك ، يجب إجراء اختبار الحساسية لمضادات الميكروبات قبل علاج عدوى السالمونيلا.