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Microbiological and molecular characterization of *E. coli* and Salmonella isolated from diarrheic calves

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Abstract

Diarrhea is one of the most common fatal disease of neonatal calves regardless of farm welfare. What makes treatment of these cases challenging is the increase in the incidence of multi-drug resistant bacteria, including *E. coli* and Salmonella. To isolate, identify, molecular characterize of *E. coli* and salmonella in diarrheic calves, 50 fecal samples were collected and analyzed aseptically. The preliminary result showed that 62% and 14% samples were positive for *E. coli* and salmonella respectively. Among the *E.coli* isolates O86:K59, O128:K-, O55:K59 (2), O86:K61, O119:K58, O08:K61, O126:K71 serotypes was detected in eight isolates while one isolate was untypeable. Three salmonella serotypes, Salmonella Typhimurium, Salmonella Anatum and Salmonella Florida was detected in the isolated samples . All of the *E. coli* strains had *eaeA* genes while 16.7 and 11.1% of them harbored *stx1* and *stx2* genes respectively. About 71.4% of salmonella isolates were positive for all five pathogenicity island genes from SPI-1 to SPI-5, while SPI-1, SPI-2 and SPI-5 were detected in 14.3% and SPI-2, SPI-3, SPI-4 and SPI-5 were positive in 14.3%. All *E. coli* strains were resistant to Ampicillin, Amoxicillin/Clavulanic, Cefazolin and Aztreonam., and all Salmonella serovars were resistant to Cefazolin, Chloramphenicol, Gentamycin, Kanamycin and Aztreonam. The present study identified multidrug resistant *E. coli* and salmonella as the common pathogen of calf diarrhea in the study area and the pathogens harbored common virulence markers of human diarrheic strains which might cause a food borne outbreak in the future.

Keywords:; calves, diarrhea, *E. coli*, Salmonella, multi-drug resistant, PCR.

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Introduction

Calves diarrhea is a global dilemma and it is considered one of the most threatening diseases of a newly born calf below one month in the whole world (Wei et al. 2021), as well as being one of the most common diseases of calves under the age of three months (Svensson et al., 2003).

Its threat is not only because of its drastic economic impact on farmers, as it causes enormous economic and productivity losses (Cho and Yoon, 2014), but also because of their public health and zoonotic importance as food born source of infection. Calf diarrhea is a multifactorial disease characterized by being a seasonal disease. In Egypt, according to Ezzat et al, 2023 most cases take place in the winter.

Several infectious agents are associated with calf diarrhea, such as viruses, bacteria and protozoa (Smith, 2009). Among bacterial pathogens, *E. coli* and Salmonella are the most important causative agents (Achá et al., 2004). However, *Clostridium perfringens*, Pasteurella spp., Klebsiella spp., Proteus spp. and *Pseudomonas aeruginosa* could also cause diarrhea in calves (Diwakar et al. 2014).

The importance of *E. coli* is attributed to causing severe watery diarrhea in the first four days of a newly born calf resulting in death within 24 hours (Cho et al, 2010). According to the virulence properties, *E. coli* is divided into 6 pathotypes; Enterohaemorrhagic (EHEC) or Shiga toxin-producing (STEC), Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC) and diffusely adherent *E. coli* (DAEC). Many of these pathotypes represent a risk to public health and are responsible for several deadly outbreaks (Pakbin et al. 2020). Salmonella enterica causes severe symptoms in calves below 3 months because it is characterized by watery and mucoid diarrhea with the presence of blood and fibrin. The severity of the illness determines how long and how frequently calves shed salmonella (Molossi et al. 2021). Moreover, recovered cases become carriers and shed the bacteria to the environment for their lifetime (Radostits et al., 2007). There are about 2500 salmonella serovars (Davies, 2008), and many of them are implicated in causing calf diarrhea. Salmonella Typhimurium and Salmonella Dublin are incriminated of causing enteritis, diarrhea and septicemia (Costa, et al, 2012). Salmonella enterica and *E. coli* are two major zoonotic foodborne pathogens in the entire world (WHO, 2015). Salmonella was the most prevalent foodborne pathogen identified in the European Union in 2019 and caused 926 outbreaks (European Food Safety Authority, 2021). Furthermore, Salmonella Typhimurium and Salmonella Anatum are among the most prevalent serovars found in animal-based food (Ferrari et al, 2019).

This study aimed for detecting *E. coli* and salmonella as causative agents for calf diarrhea, exploring their antimicrobial sensitivity and the occurrence of the main virulence genes, from fecal samples, which were taken from calves suffering from diarrhea in farms located in Qena, Egypt.

Materials and methods

Sample preparation

A total of 50 fecal samples were aseptically collected in sterile plastic tubes from diarrheic calves of less than 3 months old age, during the winter and spring seasons of 2020-2022, and subjected directly for laboratory of Microbiology, faculty of Veterinary Medicine ,South Valley University for bacteriological identification .

Isolation and biochemical identification

The fecal samples were inoculated into 10 ml Buffered Peptone Water and incubated aerobically at 37°C for 24 hrs., a loopful from bacterial suspension were subcultured on onto MacConkey agar (Oxoid, England) and incubated at 37°C for 24 hrs. The pink colonies were subcultured on Eosin methylene blue (EMB) agar (Oxoid, England). In the same time a loopful from bacterial suspension were inoculated on Tetra Thionate broth (TTB) (Oxoid,England) at 37°C for 24 hrs, then a loopful from tetrathionate suspension were streaked onto Xylose Lysine Deoxycholate (XLD) media (Oxoid, England). . Suspected isolates were morphologically identified by Gram's stain and biochemical tests; IMViC, TSI and Urease test (Varnam and Evans, 1991) and (ISO 6579, 2002).

Serological identification

Serological testing was performed on 10 *E. coli* isolates out of 31 *E. coli* isolates (10\31), which were randomly selected according to farm location and clinical signs, and were carried on all Salmonella isolates (7). They underwent serotyping using standard polyvalent and monovalent *E. coli* antisera on basis of somatic (O) and capsular (K) for *E. coli* isolates and somatic (O) and flagellar (H) salmonella antisera for salmonellae (Sifin diagnostics GmbH, Germany).

Antibiotic susceptibility testing

The antimicrobial susceptibility test were carried by disc diffusion method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018), using the following selected discs; ampicillin (AMP, 10 µg), amoxicillin/clavulanic (AMC, 30 µg), cefazolin (CZ, 30 µg), chloramphenicol (C, 30 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), tetracycline (TE, 30 µg), gentamicin (GEN, 10 µg), kanamycin (K, 30 µg), azithromycin (AT, 15µg), aztreonam (AZT, 30 µg), nitrofurantoin (F, 30 µg), ciprofloxacin (CIP, 5 µg). Results of zone of inhibition were interpreted according to (CLSI, 2018).

Polymerase chain reaction (PCR)

DNA extraction

Bacterial DNA were extracted using WizPrep^{TM} gDNA Mini Kit (Wizbiosolutions, Republic of Korea), following the manufacturer's instructions.

Amplification of *E. coli* virulence genes

PCR was carried out to detect Shiga toxin genes (*stx1, stx2*) and intimin gene (*eaeA*) in (18\31) randomly selected *E. coli* isolates, based on farm location. Both *stx1* and *stx2* genes were prepared to be done in duplex PCR, which was performed in volume 50 µL containing: 25 µL Emerald Amp GT PCR master mix (Takara, France) Code No.RR310A, 1 µL of each forward and reverse primer (Metabion Germany) as shown in table (1), 6 µL DNA template and 15 µL PCR grad water. The thermal profile of PCR were performed as following: Initial denaturation at 94 ºC /5 min followed by 35 cycles of denaturation at 94ºC/30 seconds, annealing at 58 ºC /40 seconds and extension at 72 ºC /45 seconds followed by final extension at 72 ºC /10 min. Regarding *eaeA* gene, it was prepared in uniplex PCR in volume of 25 µL, which contains 12.5 µL Emerald Amp GT PCR master mix (Takara, France) Code No.RR310A, , 1 µL of forward primer and 1 µL of reverse primer (Metabion Germany) as shown in table (1), 5 µL DNA template and 5.5 µL PCR grad water. The temperature and time conditions of PCR were done as following: Primary denaturation at 94 ºC /5 min followed by 35 cycles of denaturation at 94 ºC /30 seconds, annealing at 51 ºC /30 seconds and extension at 72 ºC /30 seconds followed by

final extension at 72 ºC /7 min. PCR for *E. coli* genes was performed using Thermal cycler (Biometra, Germany). Documentation was done by Gel documentation system (Alpha Innotech, USA).

Table (1): Oligonucleotide primers sequences of *E. coli* genes.

Gene	Sequence	Amplified product	Reference
E. coli Stx1	ACACTGGATGATCTCAGTGG	614 bp	Dipineto *et al.*, 2006
	CTGAATCCCCCTCCATTATG		
E. coli Stx2	CCATGACAACGGACAGCAGTT	779 bp	
	CCTGTCAACTGAGCAGCACTTTG		
E. coli eaeA	ATG CTT AGT GCT GGT TTA GG	248 bp	Bisi-Johnson *et al.*, 2011
	GCC TTC ATC ATT TCG CTT TC		

Amplification of Salmonella virulence genes

PCR assay was performed to detect 5 salmonella pathogenicity island genes; *InvE/A* for SPI-1, *ssaQ* for SPI-2, *mgtC* for SPI-3, *spi4R* for SPI- 4 and *sopB* for SPI-5 in all Salmonella isolates (7). PCR primers that used for the amplification of virulence genes are listed in table (3). PCR tests were carried out in volume of 20 µL containing: 10 µL WizPure^{TM} PCR 2X Master (Wizbiosolutions, Republic of Korea). 1 µL of each forward and reverse primer (Metabion Germany) as shown in table (2), 5 µL DNA template and 3 µL nuclease-free H2O. The thermal conditions of PCR were performed as following: Initial denaturation at 95 ºC /5 min followed by 30 cycles of denaturation at 95 ºC /1 min, annealing at 51 ºC /1 min (*invaE/A* and *spidR*), 53 ºC /1 min (*sopB*), 54 ºC /1 min (*mgtC*) or 58 ºC /1 min (*ssaQ*) and extension at 72 ºC /1 min before a final extension at 72 ºC /5 min. A 200 Gradient Thermal cycler (Japan) was used to perform PCR tests. PCR products were separated by gel electrophoresis in 1.5% agarose in Tris–acetate–EDTA (TAE) buffer at 100 V. And Solis Bio Dyne 100 bp ladder was included in each agarose run, visualized and documented by UV light illumination Gel documentation system (UVP Photo Doc) U.K.

Table (2): Oligonucleotide primers sequences were used for characterization of Salmonella isolates

Gene	Location	Sequence	Amplified product	Reference
***InvE*/A (F)**	SPI-1	TGCCTACAAGCATGAAATGG	450 bp	Sánchez-Jiménez et al , 2010
***InvE*/A (R)**		AAACTGGACCACGGTGACAA		
***ssa*Q (F)**	SPI-2	GAATAGCGAATGAAGAGCGTCC	677 bp	Soto *et al.*, 2006
***ssa*Q (R)**		CATCGTGTTATCCTCTGTCAGC		
***mgt*C (F)**	SPI-3	TGACTATCAATGCTCCAGTGAAT	655 bp	Sánchez-Jiménez et al , 2010
***mgt*C(R)**		ATTTACTGGCCGCTATGCTGTTG		
***spi4*R(F)**	SPI-4	GATATTTATCAGTCTATAACAGC	1269 bp	Sánchez-Jiménez et al , 2010
***spi4*R(R)**		ATTCTCATCCAGATTTGATGTTG		
***sop*B(F)**	SPI-5	GATGTGATTAATGAAGAAATGCC	1170 bp	Soto *et al.*, 2006
***sop*B(R)**		GCAAACCATAAAAACTACACTCA		

Table (3): Antimicrobial resistance profile of *E. coli* strains isolated from diarrheic calves.

NO	*E. coli* strains	Antimicrobial resistance profile	MAR index
1	O86:K59	AMP, AMC, CZ, C, SXT, TE, GEN, K, AZT, CIP.	0.833
2	O128:K-	AMP, AMC, CZ, TE, GEN, K, AZT.	0.583
3	O55:K59	AMP, AMC, CZ, C, SXT, TE, K, AT, AZT.	0.750
4	O55:K59	AMP, AMC, CZ, C, SXT, TE, GEN, K, AT, AZT, CIP	0.917
5	O86:K61	AMP, AMC, CZ, C, SXT, TE, GEN, K, AT, AZT, CIP	0.917
6	O119:K58	AMP, AMC, CZ, C, SXT, TE, GEN, K, AZT.	0.750
7	O08:K61	AMP, AMC, CZ, C, SXT, TE, K, AZT, CIP	0.750
8	O126:K71	AMP, AMC, CZ, C, SXT, TE, GEN, K, AZT	0.750

Ampicillin (AMP), Amoxicillin/clavulanic (AMC), Cefazolin (CZ), Chloramphenicol (C), Sulfamethoxazole/Trimethoprim (SXT), Tetracycline (TE), Gentamycin (GEN), Kanamycin (K), Azithromycin (AT), Aztreonam (AZT), Nitrofurantoin (F), Ciprofloxacin (CIP).
MAR index: Multi antibiotic resistant index

Table (4): Antimicrobial resistance profile of Salmonella strains isolated from diarrheic calves.

NO	*Salmonella* strains	Antimicrobial resistance profile	MAR index
1	S. Typhimurium	CZ, C, GEN, K, AT, AZT	0.500
2	S. Typhimurium	AMP, AMC, CZ, C, TE, GEN, K, AT, AZT, CIP.	0.833
3	S. Anatum	AMP, AMC, CZ, C, TE, GEN, K, AZT.	0.667
4	S. Anatum	AMP, AMC, CZ, C, TE, GEN, K, AT, AZT, CIP.	0.833
5	S. Anatum	AMP, AMC, CZ, C, SXT, TE, GEN, K, AZT, CIP.	0.833
6	S. Anatum	AMP, AMC, CZ, C, TE, GEN, K, AZT, CIP.	0.750
7	S. Florida	AMP, AMC, CZ, C, SXT, TE, GEN, K, AT, AZT, F, CIP.	1.0

Ampicillin (AMP), Amoxicillin/clavulanic (AMC), Cefazolin (CZ), Chloramphenicol (C), Sulfamethoxazole/Trimethoprim (SXT), Tetracycline (TE), Gentamycin (GEN), Kanamycin (K), Azithromycin (AT), Aztreonam (AZT), Nitrofurantoin (F), Ciprofloxacin (CIP).
MAR index: Multi antibiotic resistant index

Results

Bacterial isolation and biochemical testing

Bacterial isolation and biochemical tests revealed that out of 50 samples, there are 62 % (31\50) E. *coli* isolates and 14% (7\50) salmonella isolates. salmonella isolates were isolated from samples that also *E. coli* were isolated from it, while E. *coli* colonies were detected in 48% of samples without detection of salmonella on it.

Serotyping

The results of *E. coli* serotyping revealed that 7 different serotypes of *E. coli* and the results of serotyping of salmonella revealed only 3 serotypes. *E. coli* serotypes were as (1\10) 10% O86:K59, (1\10) 10% O128:K-, (2\10) 20% O55:K59, (1\10) 10% O86:K61, (1\10) 10% O119:K58, (1\10) 10% O08:K61, (1\10) 10% O126:K71 and (1\10) 10% untypeable with the available antisera and 1 sample was not identified as *E. coli*. While, Salmonella isolates serotypes were (2\7) 28.6% Salmonella Typhimurium 1,4,[5],12:i:1,2., and (4\7) 57.1% Salmonella Anatum

3,{10}{15}{15134}:e,h:1,6 and (1\7) 14.3% Salmonella Florida [1],6,14[25]:d:1,7.

Antimicrobial susceptibility Testing

Antibiotic susceptibility test which was done to *E. coli* and Salmonella isolates showed multiple multidrug resistance patterns and the most significant pattern of all isolates is Salmonella Florida which was resistant to all the tested antibiotics. Figure (1) highlights the percentage of the resistance, intermediate sensitivity and sensitivity *E. coli* isolates. All tested *E. coli* isolates were resistant to ampicillin, amoxicillin/clavulanic, aefazolin and aztreonam. Whereas Figure (2) shows the sensitivity of salmonella isolates of 12 antimicrobials, depicting that all Salmonella serovars are resistant to cefazolin, chloramphenicol, gentamycin, kanamycin and aztreonam. The antimicrobial resistance profiles for each serotype are shown in tables (3 and 4).

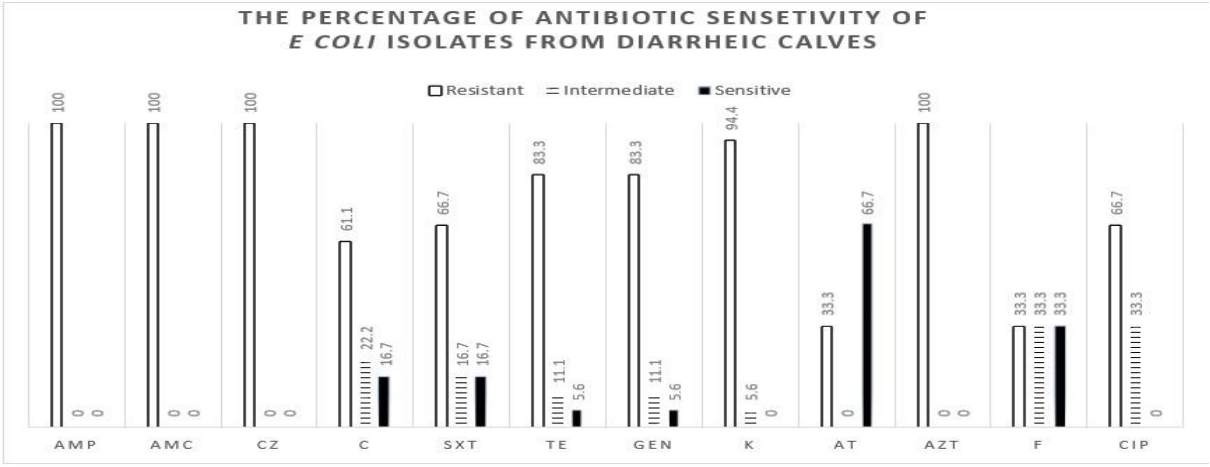

Figure (1). Results of antimicrobial sensitivity of *E. coli* isolates
Ampicillin (AMP), Amoxicillin/clavulanic (AMC), Cefazolin (CZ), Chloramphenicol (C), Sulfamethoxazole/Trimethoprim (SXT), Tetracycline (TE), Gentamycin (GEN), Kanamycin (K), Azithromycin (AT), Aztreonam (AZT), Nitrofurantoin (F), Ciprofloxacin (CIP).

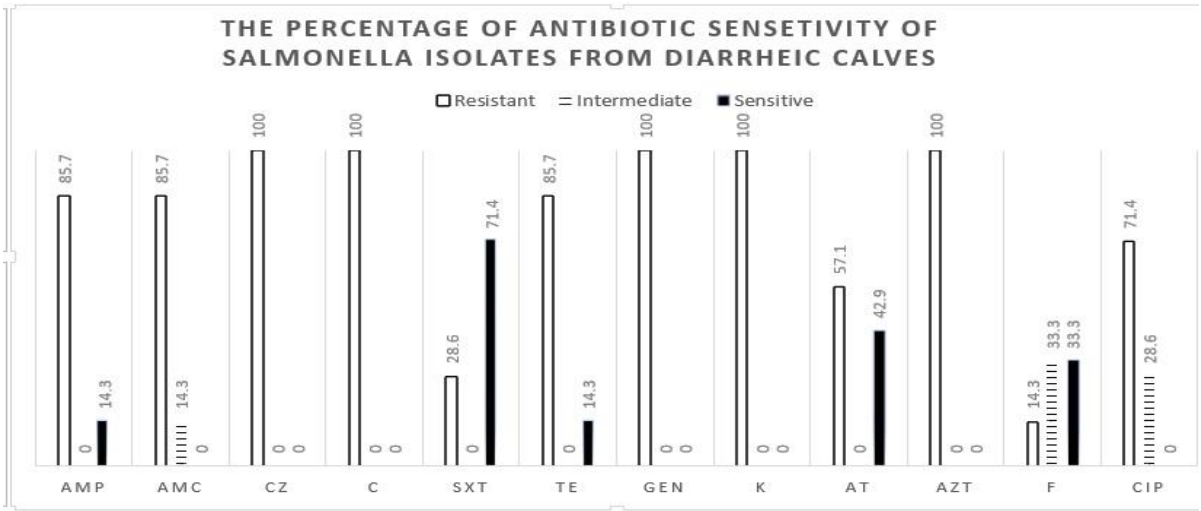

Figure (2): Results of antimicrobial sensitivity of Salmonella isolates.
Ampicillin (AMP), Amoxicillin/clavulanic (AMC), Cefazolin (CZ), Chloramphenicol (C), Sulfamethoxazole/Trimethoprim (SXT), Tetracycline (TE), Gentamycin (GEN), Kanamycin (K), Azithromycin (AT), Aztreonam (AZT), Nitrofurantoin (F), Ciprofloxacin (CIP).

Molecular characterization and identification.

Firstly, all *E. coli* isolates which were tested for *eaeA* gene were positive as shown in figure (3). Secondly, *stx1* and *stx2* genes were detected in 16.7 and 11.1% respectively, as shown in figure (4). On the other hand, all 5 Salmonella pathogenicity islands genes were detected in 71.4% of isolates, 14.3% possess only SPI-1, SPI-2 and SPI-5 and 14.3% possess SPI-2, SPI-3, SPI-4 and SPI-5 as shown in figure (5, 6 and 7).

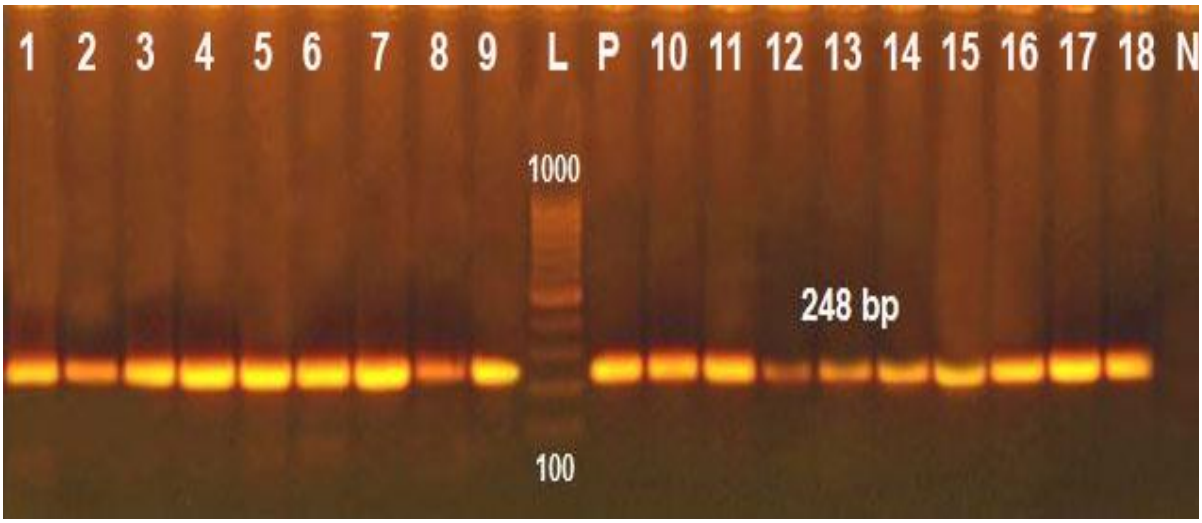

Figure (3): Result of PCR to detect *eaeA* gene of *E. coli* obtained from diarrheic calves by 1.5% agarose gel electrophoresis. Lanes 1-18: Positive *E. coli* strains for *eaeA* gene 248 bp, Lane L: GeneRuler 100 bp DNA ladder (Lane P: Positive control. Lane N: Negative control.

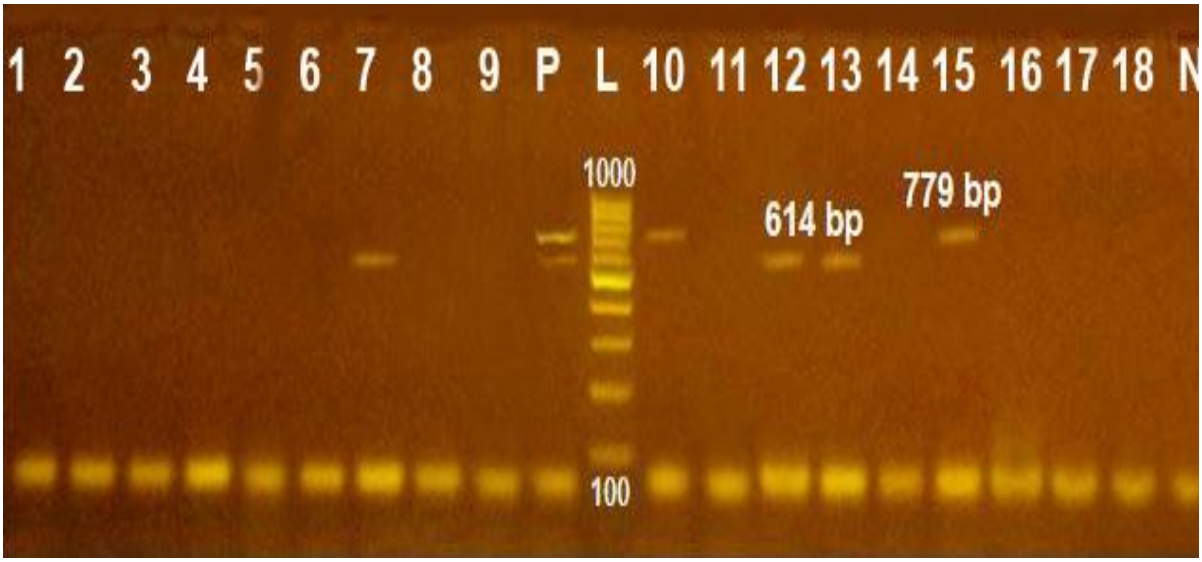

Figure (4): Result of PCR to detect *stx1* and *stx2* genes of *E. coli* obtained from diarrheic calves by 1.5% agarose gel electrophoresis. Lanes 1, 2, 3, 4, 5, 6, 8, 9, 11, 14, 16, 17 and 18: Negative *E. coli* strains for *stx1* 614 bp and *stx2* 779 bp., Lanes 7, 12 and 13: Positive *E. coli* strains for *stx1* 614 bp., Lanes 10 and 15: Positive *E. coli* strains for *stx2* 779 bp., Lane L: GeneRuler 100 bp DNA ladder Lane P: positive control., Lane N: Negative control.

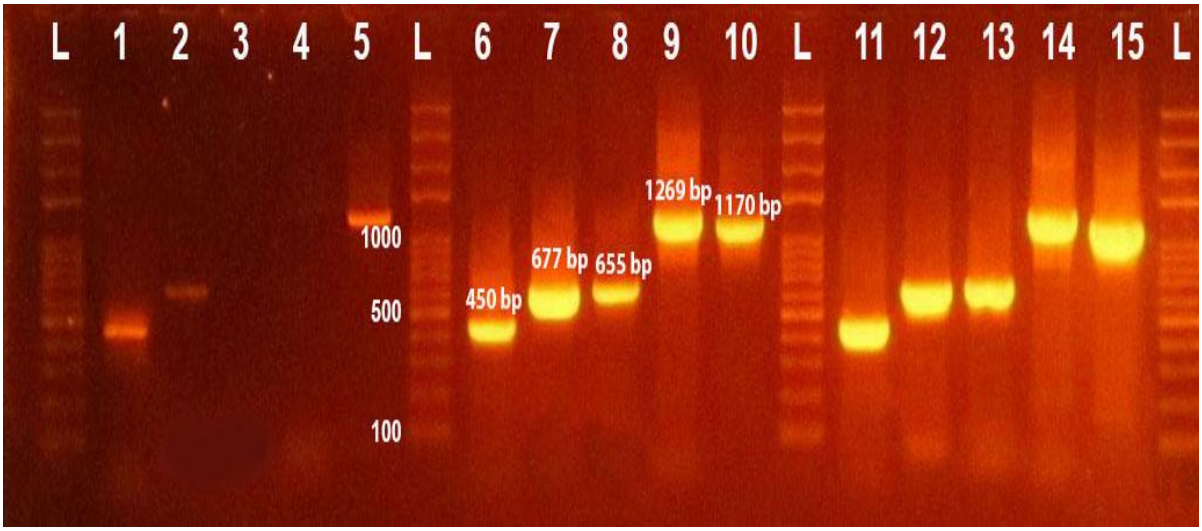

Figure (5): Results of PCR products of five pathogenicity island genes (SP-1 (450 bp), SP-2 (677 bp), SP-3 (655 bp), SP-4 (1269 bp) and SP-5 (1170 bp)) of salmonella serotypes isolated from diarrheic calves, by 1.5 % agarose gel electrophoresis. Lane L: 100 bp DNA ladder, Lanes (1-5): Pathogenicity islands of Salmonella Florida (10), Lanes (6-10): Pathogenicity islands of Salmonella Anatum (25) Lanes (11-15): Pathogenicity islands of Salmonella Anatum (39).

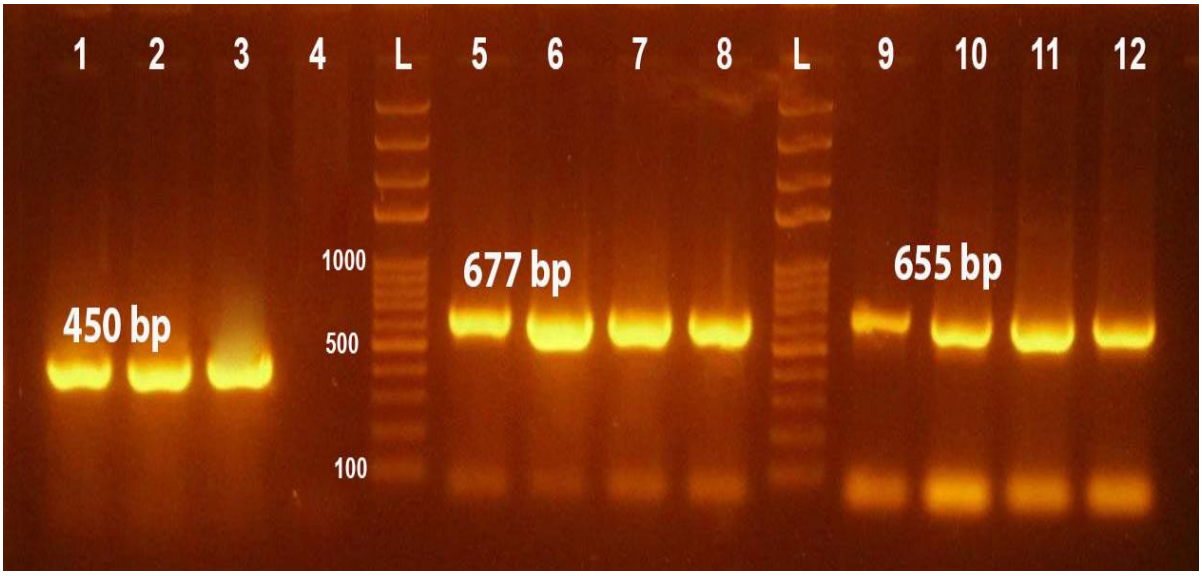

Figure (6): PCR results of 3 genes (SP-1 (450 bp), SP-2 (677 bp), SP-3 (655 bp) of Salmonella Anatum and Salmonella Typhimurium serotypes isolated from diarrheic calves, by 1.5% agarose gel electrophoresis . Lane L: 100 bp DNA ladder (Solis BioDyne, Estonia), Lanes 1-3: Positive results for SP-1 (450 bp) of Salmonella Anatum (41), Salmonella Anatum (42) and Salmonella Typhimurium (43). Lane 4: Negative result for SP-1 (450 bp) of Salmonella Typhimurium (50). Lanes 5-8: Positive results for SP-2 (677 bp) of Salmonella Anatum (41), Salmonella Anatum (42), Salmonella Typhimurium (43) and Salmonella Typhimurium (50). Lanes 9-12: Positive results for SP-3 (655 bp) of Salmonella Anatum (41), Salmonella Anatum (42), Salmonella Typhimurium (43) and Salmonella Typhimurium (50).

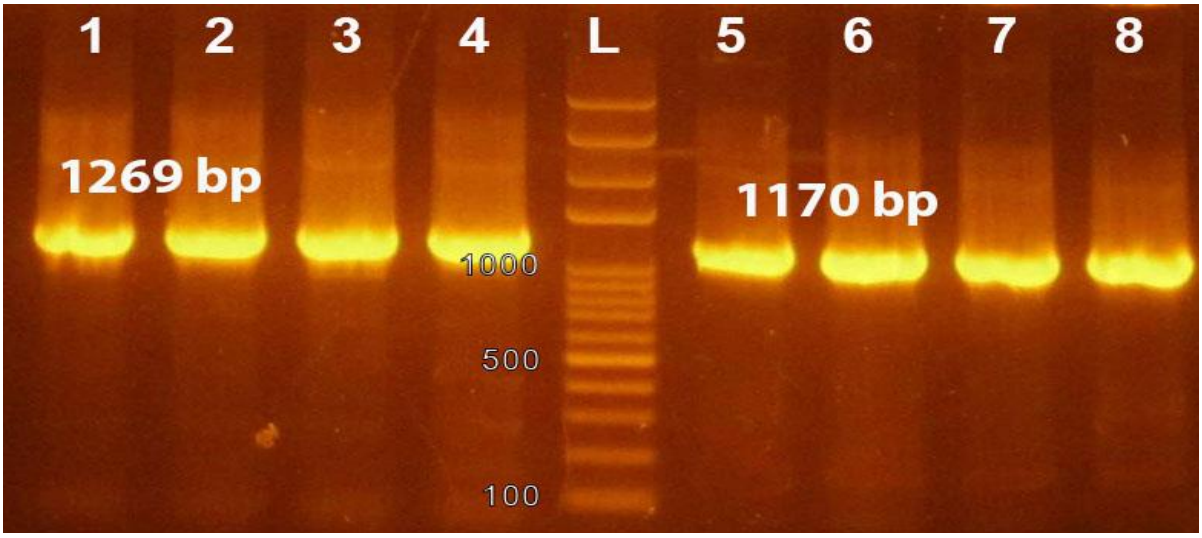

Figure (7): PCR result of 2 genes SP-4 (1269 bp) and SP-5 (1170 bp) of Salmonella Anatum and Salmonella Typhimurium serotypes isolated from diarrheic calves by 1.5% agarose gel electrophoresis. Lane L: 100 bp DNA ladder (Solis BioDyne, Estonia), Lanes 1-4: Positive results for SP-4 (1269 bp) Salmonella Anatum (41), Salmonella Anatum (42), Salmonella Typhimurium (43) and Salmonella Typhimurium (50). Lanes 5-8: Positive results for SP-5 (1170 bp) of Salmonella Anatum (41), Salmonella Anatum (42), Salmonella Typhimurium (43) and Salmonella Typhimurium (50).

Discussion

Diarrhea continues to be a pressing economic problem for cattle producers not only in Egypt but also in the world (Farid et al., 2001 and Ibrahim, 2007). *E. coli* and Salmonella as significant bacterial agents, with Rotavirus and Coronavirus are the most predominant causes of calf diarrhea (Foster and Smith, 2009). This study reveals that the percentage of *E. coli* and Salmonella are 62 and 14% respectively. The occurrence of *E. coli* is in agreement with the results of Osman et al, (2013) which was detected *E. coli* by 63.6%, higher than (El-Tawab et al, 2017) who reported *E. coli* by 47% and lower than (El-Seedy et al, 2016) who isolated *E. coli* by 75.6%. The incidence of Salmonella is within the same range of other studies in Egypt, such as; (Gharieb et al, 2015):16.25%, lower than (Mousa et al, 2010) 45.53% and higher than (Younis et al, 2009):4.09%. The variation in the results may be due to geographical location, weather, and management protocols.

Serological tests results unveil eight serovars for *E. coli*; O86:K59, O128: K-, O55:K59(2), O86:K61, O119:K58, O08:K61, O126:K71. O-Serogroups O8, O55, O86, O119, O126 and O128 have shown frequency in most previous studies that concerned calf diarrhea. For example, O8, O55 and O126 were among results of (Maher et al 2017), whereas O119 and O126 were the most prevalent serotypes with (El-Seedy et al, 2016). According to (Nataro &Kaper, 1998), O55, O86, O119, O126 and O128 were recognized as EPEC. On the other hand, O08 and O128 serogroups belong to ETEC, in addition to that, some EPEC serogroups identified as EHEC, such as O55, O119, O126 and O128 (Stenutz et al, 2006). Several publications linked possession of *eaeA* and *stx* to the pathotype of *E. coli*. According to (Kolenda, et al, 2015), serovars that are *eaeA*- positive and *stx*- positive are EHEC, while those which possess *eaeA* solely are EPEC and serovars which are positive to either *stx1* or *stx2* and negative to *eaeA* are

STEC, and this presumption largely correlates with our results.

Concerning Salmonella serotyping, the test resulted in 3 serovars; Salmonella Typhimurium, Salmonella Anatum and Salmonella Florida with a percentage of 57.1, 28.6 and 14.3% respectively. These outcomes may not coincide with most studies in Egypt, however, Salmonella Typhimurium is always one of the most common serovars in most previous studies, such as (Youssef and El-Haig 2012) and (El-Tawab et al, 2017). In respect of Salmonella Anatum which is the most prevalent serovar in the current study. It came after Salmonella Typhimurium according to (Zahran et al, 2014). Salmonella Anatum and Salmonella Typhimurium are the most noteworthy serovars because they are frequently isolated from cattle lymphatic system (Gragg et al, 2013), which implies that they may contaminate meat during the slaughter or inspection process then to consumers, causing serious health problems.

In this current study, despite its powerful molecular and antimicrobial resistance profile, Salmonella Florida is the least prevalent serovar. Nevertheless, Salmonella Florida has not been reported or isolated from diarrheic calves in previous studies in Egypt or around the world.

Molecular detection of virulence genes shows some significant findings. Starting with *E. coli*, all tested isolates are positive for *eaeA* gene, 16.7% *stx1* and 11.1% *stx2*. *eaeA* gene codes for intimin, which plays an important role in the adherence of bacteria to intestinal epithelial cells. therefore, it results in severe damage to the adjacent microvilli (Ateba et al, 2014). According to the results of PCR of *E. coli* positive only for eaeA gene , there is around 13 *E.coli* are EPEC and there is 5 *E.coli* isolates were positive for *eaeA* with *stx1*and/or *stx2* so it is classified as EHEC. The results of *eaeA* gene is higher than other recent studies in Egypt such as; (Mousa et al, 2021) and (Ezzat et al, 2023), which are relatively higher than most studies in Egypt. Salmonella molecular identification of Salmonella pathogenicity islands (1-5) virulence genes shows variable results; the majority of isolates possess all of the 5 genes with a percentage of 71.4%, while SPI-1, SPI-2 and SPI-5 detected in 14.3% and SPI-2, SPI-3, SPI-4 and SPI-5 were positive in 14.3%. Notably, from the molecular profile of these serovars, they presumably are implicated in causing calf diarrhea. It is known that SPI-1 and SPI-5 are involved in the type III secretion system, and are primarily responsible for calf diarrhea caused by Salmonella (Tsolis et al.1999), while SP-2 is concerned with the second type III secretion system and is responsible for the survival of the organism intracellularly (Ochman et al., 1996).

Antimicrobial susceptibility testing revealed several multi-drug resistant profiles. Tables (3) and (4) show that all serovars are multi-drug resistant and the markedly significant serovars are *E. coli* O55:K59, O86:K61, which are resistant to 11 drugs out of 12 and Salmonella Florida which is resistant to all of the 12 selected antibiotics. Antibiotic resistance is multifactorial and complex and constitutes a major problem in treatment of calf diarrhea. Perhaps, one of the most critical causes of antibiotic resistance in Egypt is the administration of antibiotics without doing the appropriate laboratory tests. Another reason for this is some farms adopt prophylactic antibiotic administration from the first day of birth. Here comes the role of veterinarians to ensure responsible antibiotic use on-farm, even though they do

not directly administrate the medicine and considering the ideal way of the choice of antibiotics is running culture and sensitivity testing, which ensure the best practice (BVA, 2019). In addition, with increasing the rate of multi-drug resistant bacteria, applying the preventive measures and vaccination are the best way to reduce the disease (Pereira et al, 2017)

Conclusion

E. coli and Salmonella keep the predominance of being the major bacterial causes of calves diarrhea and it is worthy of note that developing of multi-drug resistant strains makes treatment of these cases is a real struggle in the meantime. In addition, these pathogens are infectious to human and might transfer the antibiotic resistance genes to human via food chain.

References

Acha, S. J., Kühn, I., Jonsson, P., Mbazima, G., Katouli, M., & Möllby, R., 2004. Studies on calf diarrhea in Mozambique: prevalence of bacterial pathogens. Acta Veterinaria Scandinavica, 45(1), 1-10.

Ateba, C. N., & Mbewe, M., 2014. Genotypic characterization of Escherichia coli O157: H7 isolates from different sources in the north-west province, South Africa, using enterobacterial repetitive intergenic consensus PCR analysis. International journal of molecular sciences, 15(6), 9735-9747.

Bisi-Johnson, M.A.; Obi, C.L.; Vasaikar, S.D.; Baba, K.A. and Hattori, T., 2011: Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens 2011, 3:9.

British Veterinary Association., 2019. BVA Policy Position on the Responsible Use of Antimicrobials in Food Producing Animals. London: British Veterinary Association. https://www.bva.co.uk/media/1161/bva-policy-position-on-the-responsible-use-of-antimicrobials-in-food-producing-animals-1.pdf

Cho, Y. I., & Yoon, K. J., 2014. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. Journal of veterinary science, 15(1), 1-17.

Cho, Y. I., Kim, W. I., Liu, S., Kinyon, J. M., & Yoon, K. J. 2010. Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. Journal of Veterinary Diagnostic Investigation, 22(4), 509-517.

Clinical Laboratory Standard Institute, 2018. M100 Performance Standards for Antimicrobial Susceptibility Testing, 28th edition.

Costa, L. F., Paixão, T. A., Tsolis, R. M., Bäumler, A. J., & Santos, R. L., 2012. Salmonellosis in cattle: advantages of being an experimental model. Research in veterinary science, 93(1), 1-6.

Davies R., 2008. Salmonellosis In: Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris. 2:1267-83

Dipineto, L.; Santaniello, A.; Fontanella, M.; Lagos, K.; Fioretti, A. and Menna, L.F., 2006. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Letters in Applied Microbiology 43, 2006. 293–295.

Diwakar, R. P., Joshi, N., Joshi, R. K., & Yadav, V., 2014. Isolation and Antibiogram of Enterobacteria

associated with Bovine calf Diarrhea. Proteus, 100(04), 100-00.

El-Seedy, F. R., Abed, A. H., Yanni, H. A., & Abd El-Rahman, S. A. A., 2016. Prevalence of Salmonella and E. coli in neonatal diarrheic calves. Beni-Suef University journal of basic and applied sciences, 5(1), 45-51.

El-Tawab, A., Ashraf, A., Hofy, F. I., Mohamed, S. R., & Salim, R. A., 2017. Bacteriological and molecular studies on some bacterial agents from neonatal calf diarrhea. Benha Veterinary Medical Journal, 33(2), 465-475.

European Food Safety Authority, & European Centre for Disease Prevention and Control., 2021. The European Union one health 2019 zoonoses report. Efsa Journal, 19(2), e06406.

Ezzat, M., Hassanin, A. A. I., Mahmoud, A. E., & Mohamed, S., 2023. International Journal of Veterinary Science. Int J Vet Sci, 12(2), 161-168.

Farid, A.; Eid, G.E.; Abdel-Mawla, Y.R. and Nagat, A.S. 2001: Evaluation of the efficacy of Escherichia coli (K99) vaccine on the incidence of Escherichia coli and immunity in buffaloes. Vet. Med. J., Giza, 49(3): 385-399.

Ferrari, R. G., Rosario, D. K., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E., & Conte-Junior, C. A. 2019. Worldwide epidemiology of Salmonella serovars in animal-based foods: a meta-analysis. Applied and environmental microbiology, 85(14), e00591-19.

Foster, D.M., Smith, G.W., 2009. Pathophysiology of diarrhea in calves, Veterinary Clinics of North America: Food Animal Practice, 25, 13–36.

Gharieb, R. M., Fawzi, E. M., Attia, N. E., & Bayoumi, Y. H. 2015. Calf diarrhea in Sharkia province, Egypt: diagnosis; prevalence, virulence profiles and zoonotic potential of the causal bacterial agents. International Journal of Agriculture Science and Veterinary Medicine, 3, 71-87.

Gragg, S. E., Loneragan, G. H., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., ... & Brashears, M. M. 2013. Substantial within-animal diversity of Salmonella isolates from lymph nodes, feces, and hides of cattle at slaughter. Applied and Environmental Microbiology, 79(15), 4744-4750.

Ibrahim, E.D. 2007: Studies on microbial causes of diarrhea in calves. M.V.Sc. Thesis, Fac. Vet. Med., Kafr El-Sheikh Univ.

ISO, 6579, 2002. Microbiology: General guidance on methods for the detection of Salmonella. 4th Edn., International Organization for Standardization, Geneva, Switzerland.

Kolenda, R., Burdukiewicz, M., & Schierack, P. 2015. A systematic review and meta-analysis of the epidemiology of pathogenic Escherichia coli of calves and the role of calves as reservoirs for human pathogenic E. coli. Frontiers in cellular and infection microbiology, 5, 23.

Maher abdel-hakeem mohamed, g. H. A. D. A., wael abd al-azeem, m. O. H. A. M. E. D., sultan, s., & mustafa said, a. H. M. E. D. 2017. Study on virulence factors of escherichia coli isolated from calves suffer from diarrhea. Assiut Veterinary Medical Journal, 63(154), 113-121.

Molossi, F. A., Cecco, B. S. D., Henker, L. C., Vargas, T. P., Lorenzett, M. P., Bianchi, M. V., ... & Pavarini, S. P. 2021. Epidemiological and pathological aspects of salmonellosis in cattle in southern Brazil. Ciência Rural, 51.

Mousa, W. S., & Shama, U. H. A. 2021. Prevalence, antimicrobial resistance and substantial virulence-associated genes of Escherichia coli isolated from colibacillosis in neonatal calves in Egypt. Journal of microbiology, biotechnology and food sciences, 2021, 1145-1150.

Moussa, I. M., Ashgan, M. H., Mohamed, M. S., Mohamed, K. H. F., & Al-Doss, A. A. 2010. Rapid detection of Salmonella species in newborne calves by polymerase chain reaction. International Journal of Genetics and Molecular Biology, 2(4), 062-066.

Nataro JP & Kaper JB., 1998. Diarrheagenic Escherichia coli. Clin Microbiol Rev 11: 142–201.

Ochman H, Soncini FC, Solomon F, Groisman EA., 1996. Identification of a pathogenicity island required for Salmonella survival in host cells. Proc. Natl. Acad. Sci. 93:7800-7804.

Osman, K. M., Mustafa, A. M., Elhariri, M., & Abdelhamed, G. S., 2013. The distribution of Escherichia coli serovars, virulence genes, gene association and combinations and virulence genes encoding serotypes in pathogenic E. coli recovered from diarrhoeic calves, sheep and goat. Transboundary and emerging diseases, 60(1), 69-78.

Pakbin, B., Akhondzadeh Basti, A., Khanjari, A., Azimi, L., & Karimi, A., 2020. Differentiation of stx1A gene for detection of Escherichia coli serotype O157: H7 and Shigella dysenteriae type 1 in food samples using high resolution melting curve analysis. Food Science & Nutrition, 8(7), 3665-3672.

Pereira, R. V., Progar, A. L. A., & Moore, D. A., 2017. Dairy calf treatment for diarrhea: are the drugs we use effective? Washington State University Extension. https://pubs.extension.wsu.edu/download/sample/3499

Radostits OM, Gay CC, Hinchcliff KW, Constable PD., 2007. Veterinary Medicine: A textbook of diseases of cattle, horses, sheep, pigs and goats.10th Ed., Elsevier Scientific Publications, Saunders p 2160.

Sanchez-Jimenez, M.M., N. Cardona-Castro, N. Canu, S. Uzzau and S. Rubino, 2010. Distribution of pathogenicity islands among Colombian isolates of Salmonella. J. Infect. Dev. Countries, 4: 555-559.

Smith GW., 2009. Treatment of calf diarrhea: oral fluid therapy. Vet.Clin. North Am. Food Anim. Pract. 25:55-72.

Soto, S.M., I. Rodriguez, M.R. Rodicio, J. Vila and M.C. Mendoza, 2006. Detection of virulence determinants in clinical strains of Salmonella enterica serovar Enteritidis and mapping on macrorestriction profiles. J. Med. Microbiol., 55: 365-373.

Stenutz, R., Weintraub, A., & Widmalm, G., 2006. The structures of Escherichia coli O-polysaccharide antigens. FEMS microbiology reviews, 30(3), 382-403.

Svensson C, Lundborg K, Emanuelson U, Olsson SO., 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level

risk factors for infectious diseases. Prev. Vet. Med. 58(3): 179-197.

Tsolis RM, Adams LG, Ficht TA, Baumler AJ., 1999. Contribution of Salmonella typhimurium virulence factors to diarrheal disease in calves. Infect. Immun. 67:4879-4885.

Varnam, A.H. and Evans, M.G., 1991: Food borne pathogens. Wolfe Publ. Ltd., England.

Wei Xj, Wang WW, Dong Z, Cheng FS, Zhou XZ, Li B and Zhang JY., 2021. Detection of infectious agents causing neonatal calf diarrhea on two large dairy farms in Yangxin County, Shandong Province, China. Frontiers in Veterinary Science 7: 589126

World Health Organization., 2015. World health statistics 2015. World Health Organization. https://apps.who.int/iris/handle/10665/170250

Younis, E. E., Ahmed, A. M., El-Khodery, S. A., Osman, S. A., & El-Naker, Y. F., 2009. Molecular screening and risk factors of enterotoxigenic Escherichia coli and Salmonella spp. in diarrheic neonatal calves in Egypt. Research in veterinary science, 87(3), 373-379.

Youssef, A. I., & El-Haig, M. M., 2012. Herd problems and occupational zoonoses of Salmonella enterica serovars Typhimurium and Enteritidis infection in diarrheic cattle and buffalo calves in Egypt. Human and Veterinary Medicine, 4(3), 118-123.

Zahran, R., & El-Behiry, A., 2014. Prevalence, molecular identification and virulence attributes of Salmonella serovars isolated from feces of diarrheic cow and buffalo-calves. Int J Curr Microbiol App Sci, 3(11), 9-27.