EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 13
494-505
Year: 2019

MOLECULAR DETECTION OF SOME INFECTIOUS CAUSES OF DIARRHEA IN DESERT SHEEP

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Article Received on 23/10/2019 **Article Revised on 12/11/2019** **Article Accepted on 15/11/2019**

ABSTRACT
Diarrhea is a serious problem in sheep, causing great economic losses due to poor growth rates, veterinary costs and may lead to death, so, the present work was planned to investigate the prevalence of some parasitic, bacterial and viral enteropathogens causes diarrhea in sheep between February 2018 to March 2019 in Marriott district - Alexandria Governorate using PCR assay which detected genes (*ITS*, *18S rRNA*, *NP*, *S*, *5'UTR*, *phoA*, *invA*, *16S rRNA*, *23S rRNA*, *16S rRNA* and *alpha toxin*) of Eimeria, *Cryptosporidium*, *PPR virus*, *RVF virus*, *Ovine Corona virus*, *E. coli*, *Salmonella*, *L. monocytogenes*, *Campylobacter*, *Y. enterocolitica*, *C. perfringens* respectively. The molecular detection showed that, the most common bacterial enteropathogens caused diarrhea in desert sheep was *C. perfringens* in percentage of (95.62 %) followed by *Y. enterocolitica* in percentage of (94.73%), while, *E. coli* (91.25%), *Salmonella* (89.37%), *Campylobacter* (83.12%) and *L. monocytogenes* (0%). Regarding, the most common parastic enteropathogen was Eimeria in percentage of (58.75%) followed by *Cryptosporidium* in percentage of (35%), while there was no detection of viral infections, all of the percentage was detected according to the total number of samples (160 rectal samples). In conclusion, PCR has been demonstrated to be a very specific and sensitive method for the detection of parasites and bacterial to save effort, rapid, economic and sensitive tool for accurate detection of the pathogenic organisms. On the other hand, we report high prevalence of bacterial and parasitic pathogens in desert sheep, which are of public health and veterinary importance. This work provides valuable data on the prevalence of parasitic and bacterial enteropathogens in desert sheep and that will be useful to develop control measures for these pathogens in desert area.

KEYWORDS: PCR, diarrhea, *Bacteria*, Eimeria, *Cryptosporidium.*

INTRODUCTION
Sheep are the most important animal that most people depend on their meat and wool, especially in desert areas and their meat is one of favorite meats **(Shabana *et al.*, 2017),** so diseases that affect sheep are the focus of attention in which, the most important diseases are diarrhea which causes significant economic loss as it affects the growth rate and may cause death, especially in young ages as well as high prices of medicines and need for specialized veterinary care **(Weiss and Navas, 2005).** The causative factors and diarrhea epidemic have been extensively studied worldwide; however, few studies have been conducted on sheep in desert areas that deal with bacteria, parasites and viruses simultaneously.

The etiology of diarrhea is complicated because many pathogens cause it. The most important enteropathogens accompanied with diarrhea in livestock include: Escherichia coli, *Campylobacter*, Salmonella species and cryptosporidium either singly or in combination **(De la Fuente, *et al.*, 2002).** Also some pathogens are incriminated in enteric diseases including: *Clostridium perfringens*, Klebsiella, Proteus and Eimeria species. Sheep from all ages and breeds are highly susceptible to Eimeria infection, lambs from three weeks to five months of age are most highly affected by outbreaks of coccidial infection, while the remain of the flock might act as carriers **(Rehman, 2011).**

Coccidiosis is clinically characterized by diarrhea which can be hemorrhagic in adult sheep **(Walaa, *et al.*, 2018).** In the subclinical form, lambs have watery diarrhea with clumps of mucous and occasional changes in the color of feces vary from yellow or brown with impairment in growth is the main sign, and decrease in milk production in dairy goats has also been recorded **(Zhao, 2012).**

Eimeria diagnosis usually is based on morphological detection of the oocysts by light microscope. That method involves several short comings including time

and effort. It depends mainly on the skill and experience of the examiner. Recently, modern molecular techniques have been deployed for Eimeria identification including PCR amplification technique **(Khodakaram, *et al*., 2013).**

Cryptosporidium is a protozoan parasite of worldwide distribution, with infection reported in wild and domestic species including, birds, reptiles, fish and mammals. It can cause diarrhea and. affected animals are often active, alert, and nursing. The diarrhea is usually very liquid and yellow. Cryptosporidial infections of livestock have an important economic impact to farmers due to high morbidity and may be high mortality rates among farm animals. **(Ryan et al., 2014**).

Escherichia coli is a Gram-negative, motile, facultative anaerobes, non-spore forming Enterobacteriaceae. Most E. coli strains are present in gastrointestinal tract flora, but some strains have virulence factors that enable them to cause diarrhea in neonatal farm animals **(Nguyen, *et al*., 2005).**

Infection with Salmonella is primarily an enteric infection which has two forms, clinical and subclinical. An animal may have a latent infection whereby the pathogen remains dormant in the lymph nodes and may be shed in the feces **(Acha and Szyfres, 2001).**

Campylobacter, these bacteria cause diarrhea and can be present in environments such as soil, water and food, most likely as result of contact with contaminant sources like animal feces and aborted tissues **(Broman, *et al*., 2002 and Pitkanen, 2013).**

Y. enterocolitica has a considerable clinical importance, it can infect many animal species causing sailorrhea, diarrhea, and loss of weight and the clinical symptoms mainly consist of sailorrhea, diarrhea, and loss of weight (**Skurnik, M. and Toivonen, S. 2011)**. *Besides*, **Fàbrega, and Vila, (2012)** said that**,** sheep were considered a major source of infection with this pathogen.

Clostridium perfringens produces enteric diseases, called enterotoxemia in sheep. It can be a normal inhabitant of the intestine of most animal species, but when the intestinal environment is altered by sudden changes in diet or other factors, *C. perfringens* proliferates and produces potent toxins that act locally or are absorbed into the general circulation with usually devastating effects on the host **(Francisco and Glenn, 2008).**

Bovine coronavirus (BCoV) is a cosidrable a causative agent of diarrhea in cattle and small ruminants **(Muñoz et al., 1996).** Coronaviruses are more commonly identified in neonatal and to a lower extent in adults, and infected adult animals do not always show symptoms, clinically and subclinically infected animals display virus shedding and are a possibility sources of infection **(Ozmen et al., 2006).**

Peste des petits ruminants (PPR) is an acute or subacute viral disease of small ruminants, particularly in goats, The disease is characterized by high fever, necrotic stomatitis, and catarrhal inflammation of the ocular and nasal mucosae, pneumonia, diarrhea and death **(Islam et al 2012).**

There are few studies that investigated the incidence of viral, parasites and bacterial enteropathogens in sheep in desert areas. Therefore, our study is used to determine their prevalence to permit the comparison of data with other epidemiological studies by using modern molecular techniques polymerase chain reaction (PCR). PCR has been demonstrated to be a very specific and sensitive method for the detection viral, parasites and bacterial to save effort, rapid, economic and sensitive tool for accurate detection of the pathogenic organisms.

MATERIAL AND METHODS

Animals

One hundred and sixty diarrheic Barki sheep with a variety of clinical signs ages from one month to 2 years were collected from Marriott district-Alexandria Governorate which away from Alexandria about 35 km. Sheep are raised mainly for their milk and/or meat and were mostly grazing belong to private owners. All sheep were originated from northwestern coast approximately 500 km west of Cairo.

Study area and samples collection

This study was performed from February 2018 to March 2019 in Marriott district- Alexandria Governorate for identifying of bacterial, parasites and viral enteropathogens among diarrheic sheep. A total of 160 rectal samples were collected. Sheep fecal samples were collected from the rectum of each animal using an individual disposable latex glove. Each sample was placed in a plastic specimen cup with a screw- on lid, labeled and transported on ice box, and transport to the laboratory as early as possible. Demographic data of the animals such as sex, age, and source were recorded. The age vary from 1month to 2 years.

DNA extraction (for bacterial and protozoan agents)

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

RNA extraction (for viral agents)

RNA extraction from samples was performed using the QIAampviral RNA Mini kit (Qiagen, Germany, GmbH). Briefly, 140 µl of the sample suspension was incubated with 560 µl of AVL lysis buffer and 5.6 µl of carrier RNA at room temperature for 10 min. After incubation, 560 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 60 µl of elution buffer provided in the kit.

Oligonucleotide Primers. Primers used were supplied from **Metabion (Germany)** are listed in table (1.,2).

PCR amplification (for bacterial and protozoan agents)

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmolconcentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Appliedbiosystem 2720 thermal cycler.

PCR amplification(for viral agents)

Primers were utilized in a 25- µl reaction containing 12.5 µl of Quantitect probe rt-PCR buffer **(QIAgen, Gmbh)**, 1 µl of each primer of 20 pmol concentration, 0.25 µl of rt-enzyme 4.25 µl of water, and 6 µl of template. The reaction was performed in an Appliedbiosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bpand 100 bp plus ladders (Qiagen, Germany, GmbH)and generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Primers Sequences, Target Genes, Amplicon Sizes and Cycling Conditions For Bacterial And Protozoan Agents.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
E. coli phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Hu *et al.*,2011**
Salmonella invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Oliveira*et al.*, 2003**
L. monocytogenes 16S rRNA	ggACCgggg CTA ATA CCg AAT gAT AA TTC ATgTAggCgAgTTgCAgC CTA	1200	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 1 min.	72˚C 10 min.	**Kumar et al., 2015**
Campylobacter 23S rRNA	TATACCGGTAAGGAGTGCTGGAG ATCAATTAACCTTCGAGCACCG	650	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Wang*et al.*, 2002**
Y. enterocolitica 16S rRNA	AAT ACC GCA TAA CGT CTT CG CTT CTT CTG CGA GTA ACG TC	330	94˚C 5 min.	94˚C 30 sec.	62˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Wanne*et al.*, 2001**
C. perfringens alpha toxin	GTTGATAGCGCAGGACATGTTAAG CATGTAGTCATCTGTTCCAGCATC	402	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Yoo*et al.*, 1997**
Eimeria ITS	GCAAAAGTCGTAACACGGTTTCC CTGCAATTCACAATGCGTATCG	500	94˚C 5 min.	94˚C 30 sec.	56˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Verma*et al.*, 2017**
Cryptosporidium 18S rRNA	CCACATCTAAGGAAGGCAGC ATGGATGCATCAGTGTAGCG	1056	94˚C 5 min.	94˚C 30 sec.	53˚C 40 sec.	72˚C 1 min.	72˚C 12 min.	**Jellison*et al.*, 2002**

Table (2): Primers Sequences, Target Genes, Amplicon Sizes and Cycling Conditions For Viral Agents.

Target gene	Primers sequences	Amplified segment (bp)	Reverse transcription	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
PPR NP gene	TCTCGAAATCGCCTCACAGACTG CCTCCTCCTGGTCCTCCAGAATCT	351	50˚C 30 min.	94˚C 15 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Kgotlele*et al.*, 2014**
RVF S gene	CCTTAACCTCTAATCAAC TATCATGGATTACTTTCC	810		94˚C 15 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**OIE, 2014**
Ovine Corona (Border disease) 5'UTR gene	TCGTGGTGAGATCCCTGAG GCAGAGATTTTTTATACTAGCCTATRC	225		94˚C 15 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Liu*et al.*, 2019**

RESULTS

The investigated sheep showed different degree of diarrhea with a variety of clinical signs such as abdominal pain, anorexia, progressive weakness, depression. Nervous symptoms were observed in some animals. The demographic characteristics of sheep with diarrhea were detected by polymerase chain reaction (PCR) for the presence of selected parasite, viral and bacterial enteropathogens. The demographic characteristics of the examined animals are summarized in Table 3.

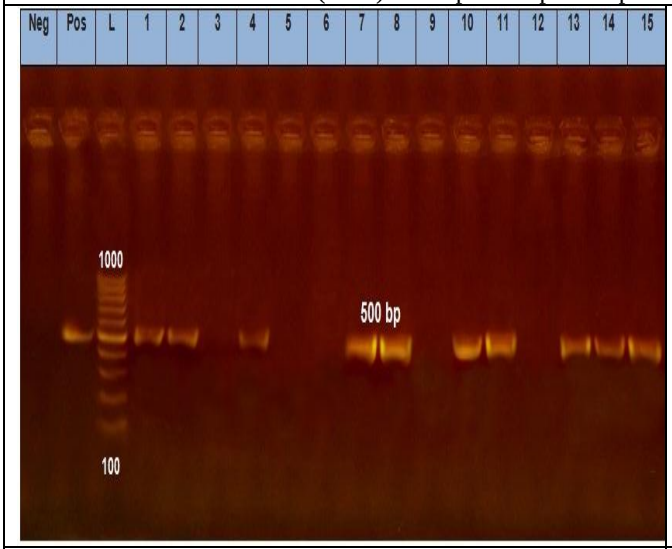
Table 3: Detection of Parasite, Viral and Bacterial Enteropathogens By Polymerase Chain Reaction.

	Number of positive	% to total number of fecal samples (160)
Eimeria	94	58.75
Cryptosporidium)	56	35
PPR virus	0	0%
RVF virus	0	0%
Ovine Corona (virus	0	0%
E. coli	146	91.25
Salmonella	143	89.37
L. monocytogenes	0	0%
Campylobacter	133	83.12
Y. enterocolitica	151	94.73
C. perfringens	153	95.62

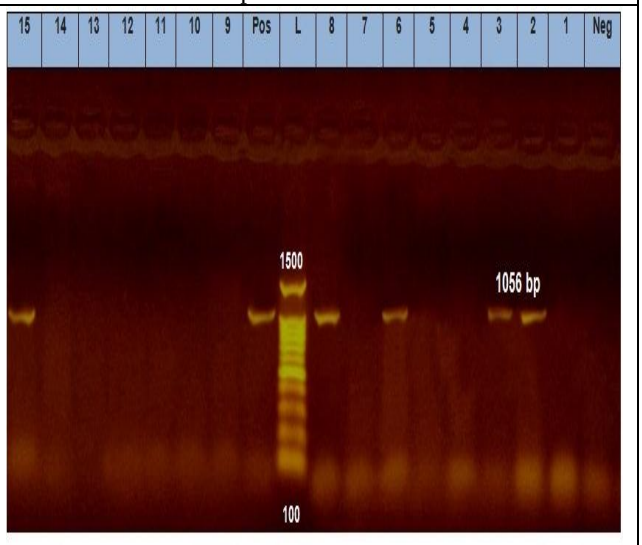
PCR with Species Specific Primers For The Detection Each of The Parasite *Eimeria, Cryptosporidium*

Molecular detection of the Eimeria 94 out of 160 were positive (58.75%) as shown in (Fig 1) the samples yielded positive bands and were amplified at the size of nearly 500bp, Molecular detection of the *Cryptosporidium* 56 out of 160 were positive (35%) as shown in (Fig 2) the samples yielded positive bands and were amplified at the size of nearly 1056 bp.

(PCR) with species specific primers for the detection of parasite



(Fig 1) *Eimeria*. PCR amplification of *Eimeria*. DNA from different samples; M, Molecular weight marker showed 100bp-1000 bp DNA ladder the type code is shown above the lanes, lanes1,2,4, 7,8,10,11,13,14,15 represents the positive product of *Eimeria* at 500bp, lanes 3,5,6, 9,12 represents the negative.

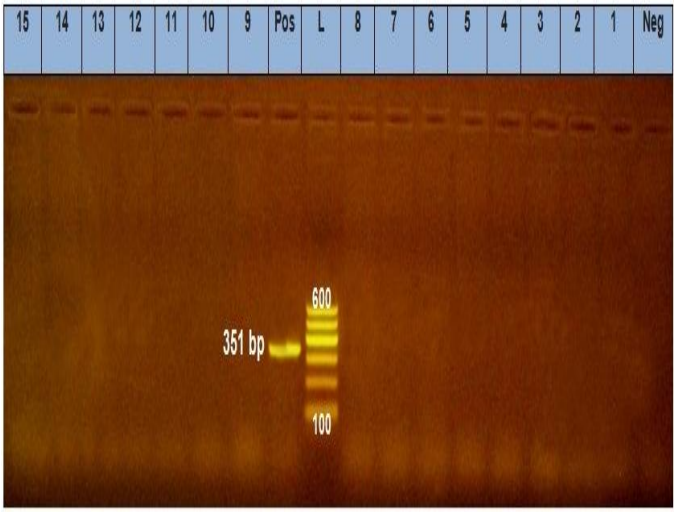


(Fig2) *Cryptosporidium* PCR amplification of *Cryptosporidium* DNA from different samples; M, Molecular weight marker showed 100bp-1500 bp DNA ladder the type code is shown above the lanes, lanes 2,3, 6,8,15 represents the positive product of *Cryptosporidium* at1056, lanes 1,4,5,7, 9,10,11,12,13,14 represents the negative.

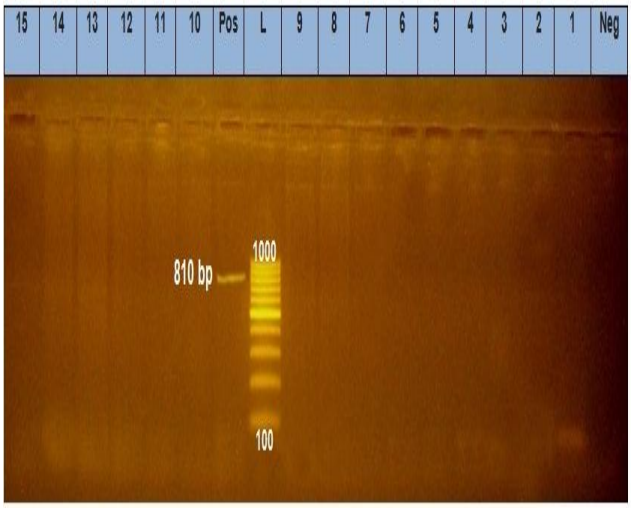
PCR with species specific primers for the detection of each of the virus

By using of Polymerase chain reaction (PCR) with species specific primers for the detection each of the virus (*PPR NP* gene, *RVF S* gene, *Ovine Corona (Border disease) 5'UTR* gene*),* molecular detection of the virus samples all samples were negative (0%) as shown in (Fig 3,4,5) the positive bands were amplified at the size of nearly 351bp 810 bp and 225 bp respectively.

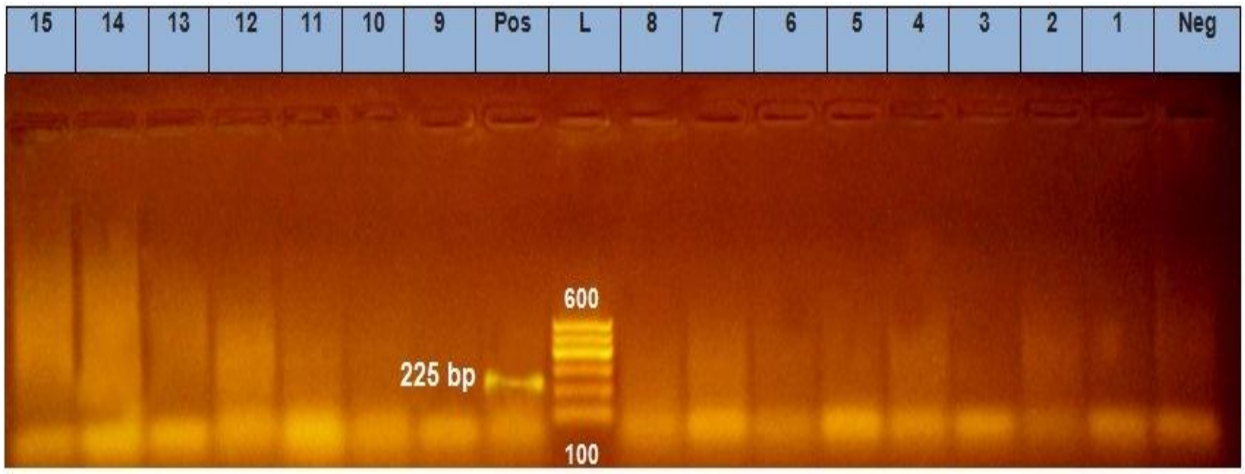
(PCR) with species specific primers for the detection of virus



(Fig 3) *PPR NP* gene. PCR amplification of PPR, DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, all samples were negative, positive sample be at 351bp



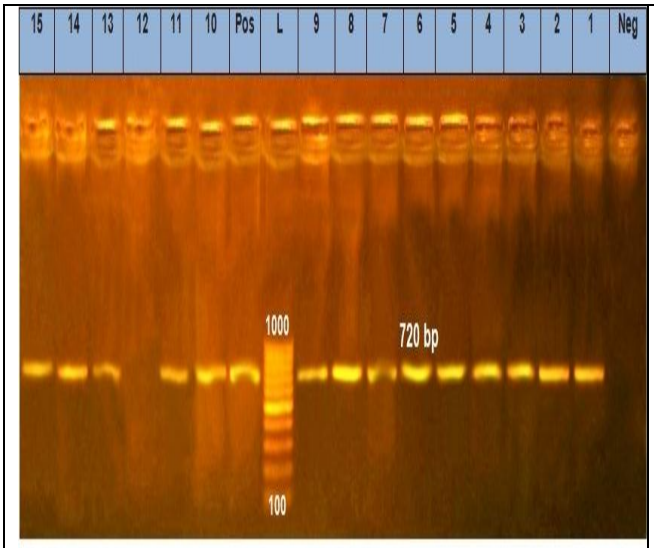
(Fig 4) *RVF S* gene. PCR amplification of *RVF S* gene, DNA from different samples; M, Molecular weight marker showed 100bp-1000 bp DNA ladder the type code is shown above the lanes, all samples were negative, positive sample be at 810 bp



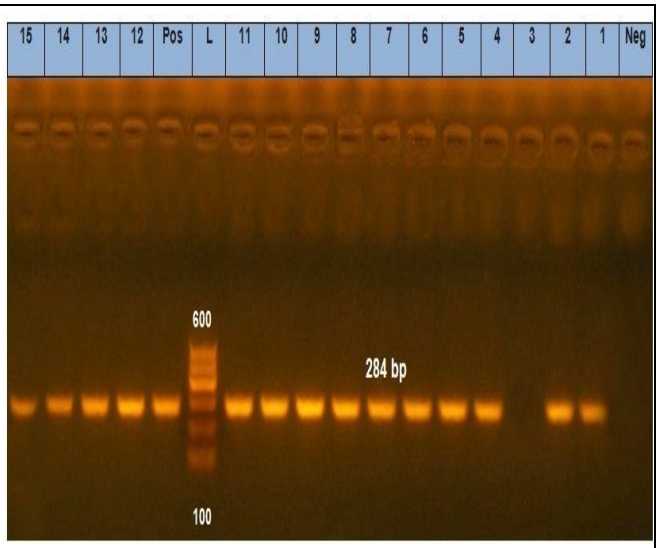
(Fig5*): Ovine Corona (Border disease) 5'UTR* gene PCR amplification of *Ovine Corona,* DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, all samples were negative, positive sample be at 225 bp.

PCR with Species Specific Primers for The Detection of Each of The Bacteria

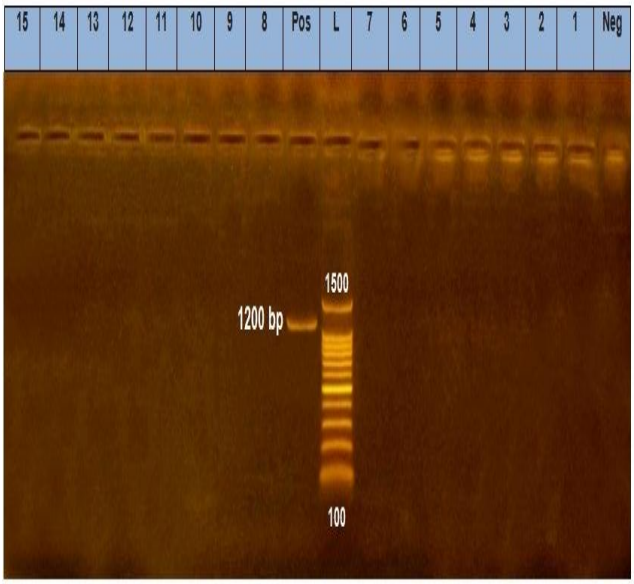
By using PCR with species specific primers for detection each of bacteria, the molecular detection of the *E. coli* 146 out of 160 were positive (91.25%) as shown in (Fig 6), the samples yielded positive bands and were amplified at the size of nearly 720bp, molecular detection of the *Salmonella* 143 out of 160 were positive (89.37%) as shown in (Fig 7) the samples yielded positive bands and were amplified at the size of nearly 284bp, molecular detection of the *L. monocytogenes* 0 out of 160 were positive (0%) as shown in (Fig 8) the positive bands were amplified at the size of nearly 1200 bp, molecular detection of the *Campylobacter* 133 out of 160 were positive (83.12%) as shown in (Fig 9) the samples yielded positive bands and were amplified at the size of nearly 650bp, molecular detection of the *Y. enterocolitica* 151 out of 160 were positive (94.73%) as shown in (Fig 10) the samples yielded positive bands and were amplified at the size of nearly 330bp and molecular detection of the *C. perfringens* 153 out of 160 were positive (95.62%) as shown in (Fig 10) the samples yielded positive bands and were amplified at the size of nearly 402bp.



(Fig 6) *E. coli*. PCR amplification of *E. coli*. DNA from different samples; M, Molecular weight marker showed 100bp-1000 bp DNA ladder the type code is shown above the lanes, lanes1,2,3,4,5,6, 7,8,9,10,11,1 3,14,15 represents the positive product of *E. coli* at 720bp, lane12 represents the negative.



(Fig 7). PCR amplification of *Salmonella* DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, lanes1,2, 4,5,6, 7,8,9,10,11,1 2,13,14,15 represents the positive product of *Salmonella* at 284 bp, lane 3 represents the negative.



(Fig 8) *L. monocytogenes*. PCR amplification of *L. monocytogenes*, DNA from different samples; M, Molecular weight marker showed 100bp-1500 bp DNA ladder the type code is shown above the lanes, all samples were negative, positive sample be at 1200bp

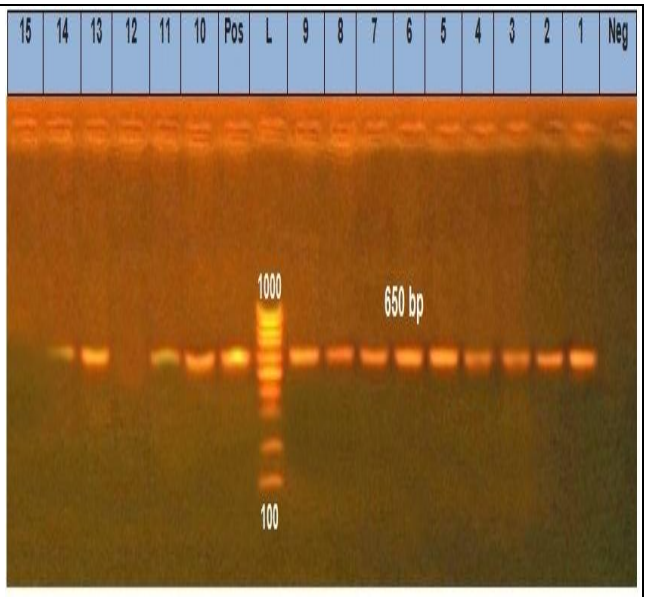


Fig 9) *Campylobacter*. PCR amplification of *Campylobacter*. DNA from different samples; M, Molecular weight marker showed 100bp-1000 bp DNA ladder the type code is shown above the lanes, all samples positive at at 650bp, except lanes 12 and 15 represents the negative samples

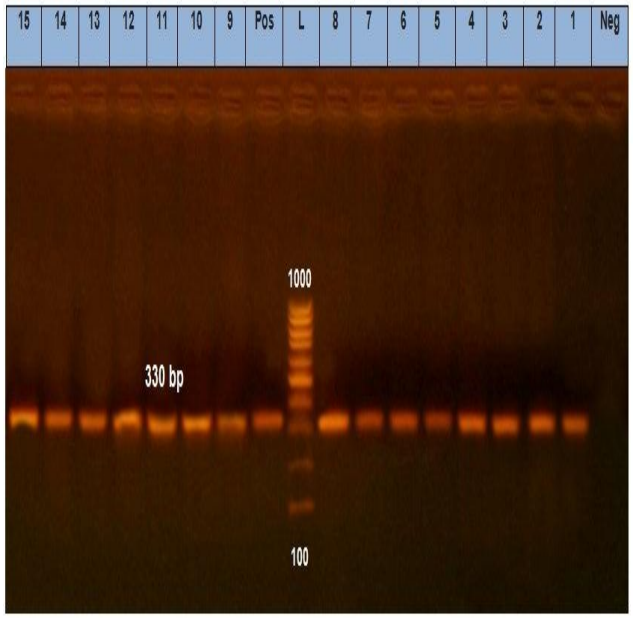


Fig 10): *Y. enterocolitica*. PCR amplification of *Y. enterocolitica* DNA from different samples; M, Molecular weight marker showed 100bp-1000 bp DNA ladder the type code is shown above the lanes, all samples were positive at 330bp

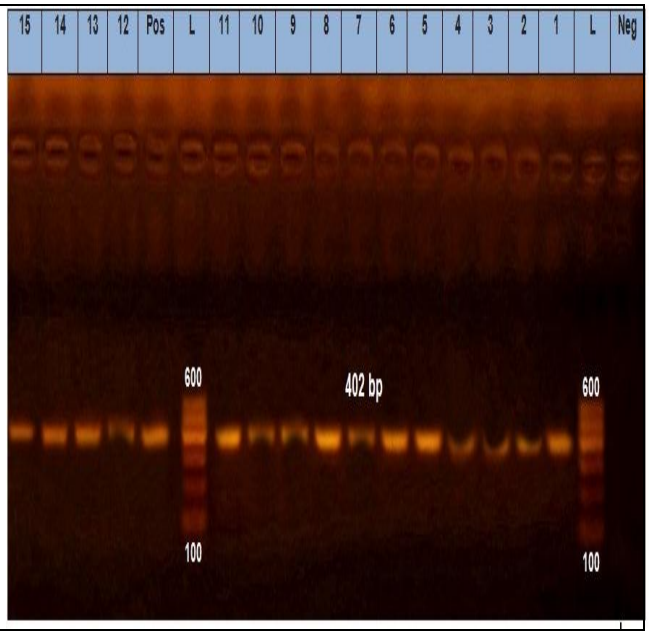


***Fig. 11: C. perfringens* PCR amplification of *Y. enterocolitica* DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, all samples were positive at 402 bp**

Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in Animal health research institute, Egypt

DISCUSSION

The present work was designed to determine the prevalence of parasitic, viral and bacterial enteropathogens in diarrheic sheep in Marriott district - Alexandria Governorate. The enteropathogens which caused diarrhea in desert sheep was *C. perfringens* (95.62 %), which the most common bacteria was causing diarrhea, followed by *Y. enterocolitica* (94.73%), while E. *coli* (91.25%), *Salmonella* (89.37%), *Campylobacter* (83.12%) and *L. monocytogenes* (0%) (To the total no. of samples) as shown in Table (3).

Results regarding, the most common parasitic enteropathogens causing diarrhea was Eimeria (58.75%) followed by *Cryptosporidium* (35%) (To the total no. of samples) as shown in Table (3).

On the other hand, there was no detection of viral infections causing diarrhea in this work as shown in Table (3) Fig. (3, 4 and 5).

Regardings, the most common bacterial pathogen causing diarrhea was *C. perfringens* Table (3) and Fig. (11) (Which induced enterotoxaemia in sheep) were detected in a high percentage (95.62 %), this similar with **El- Idrissi and Ward, (1992)** and **Fayez *et al*., 2013),** who said that enterotoxaemia is one of the most frequently occurring diseases of sheep worldwide and the reports from different countries around the world have reported prevalence rates of it ranging between 24.13% and 100%. Also, the result similar with **Elsify, *et al*., (2016)**, in detection of *C. perfringens* from diseased sheep but in differ percentage.

In the current study, *Y. enterocolitica* (94.73%), Table (3) and Fig. (10) was detected in diarrheic sheep, where as **Fàbrega, and Vila, (2012)** said that**,** sheep were considered a major source of infection with this pathogen and the result of this work similar with **Karin *et al*., (2012)** and **Ban and Mohammed (2018),** who detected same organism from fecal samples of sheep but differ in percentage. The variation in the incidence of *Y. enterocolitica* refers to different factors might influence the rate of infection as geographical locations, weather, ages, seasons, management and techniques of isolation **(Falcao, et al., (2006)** and **Bharathy, *et al*., 2015).**

The current finding revealed that, the percentages of each of E. coli and *Salmonella* Table (3) and Fig. (6 and 7 respectively) was (91.25%) and (89.37%) respectively and this disagreement with **Shabana *et al*., (2017)** who isolated *E. coli* and *Salmonella* species from diarrheic sheep in low percentages (34.7%) and (3.6%) respectively. On the other hand, **Shabana *et al*., (2017)** found that, E. *coli* was the most common bacteria causing diarrhea in diarrheic sheep and this disagreement with this work in that, the most common bacterium causing diarrhea in sheep was *C. perfringens*, besides, those authors can detected viral infections from diarrheic sheep but there are no viral infections were detected in our study.

Regardings, *Campylobacter was* detected in (83.12%) Table (3) and Fig. (9). This result was higher than that obtained by **Garcla *et al.*, (2009)** who detected 64% (51 positives/80 animals) of *Campylobacter spp* in fecal samples of sheep and lower than that obtain by the same authors who detect *Campylobacter* in percentage of 95% (76 positives/80 animals) from wool of the same sheep. This high rate of *Campylobacter* may be domestic animals constitutes a large reservoir of *Campylobacter* and it is possible to find a high rate of infection in clinically healthy animals **(Garcla *et al.*, 2009)**.

Regardings, in this study, there was no detection of *L. monocytogenes* (0%) as shown in Fig. (8), this may attributed to that, small-ruminant are characterized by a single or a few subtypes that cause disease in small ruminants **(Nightingale, *et al*., 2004)**.

Our result reveled that, Eimeria was detected in diarrheic sheep in percentage of (58.75%), Table (3) Fig. (1). this consistent with result of **Walaa *et al*., (2018).**

Regarding, in this work *Cryptosporidium* was detected in percentage of (35%) Table (3) and Fig. (2), this result agreed with **Fasihi and Fotohi, (2008)** who recorded that, the prevalence of *Cryptosporidium* infection was reported in between 4% to 85% in sheep worldwide.

Our result reveled that, *Cryptosporidium* was detected in percentage of (35%), while, **Jamal *et al.*, (2014)** recorded low percentage (11.3%) of the same parasite in sheep. This differentiation may be due the difference methods of investigations which were conducted on the prevalence of *Cryptosporidium* in sheep based on microscopy and this go in hand with **Silva *et al., (*2011)** who reported that, *Cryptosporidium* utilizing of microscopic observation of oocysts alone is not sufficient for identification.

During the course of a bacterial and parasitic infection, the rapid identification of the causative agents is necessary for the determination of effective treatment options, thus, this study proved that, molecular detection of the parasitic and bacterial enterpathogens is a valid tool for diagnosis and detection of these pathogens and PCR has been demonstrated to be a very specific and sensitive method for the detection of parasites and bacterial to save effort, rapid, economic and sensitive tool for accurate detection of the pathogenic organisms. This coinsist with **Anna, *et al.*, (2009) who** concluded that, the PCR assay rapidly provides reliable data, which can guide optimal antimicrobial treatment decisions in a timely manner.

Variation in detection of all detected causes of diarrhea in this study, high, low or similar with other authors may be attributed to using different sample types, methods of isolation and identification, seasonal variation, the environment where the animals graze and grow degree of stress, system of management, immunostatus of the animals and consistent source of infection. In the area of investigation, the sheep were reared together with camel, goats and cattle; this mixing is a major factor favoring transmission and spreading of diseases.

CONCLUSION AND RECOMMENDATION

We report a high prevalence of parasitic and bacterial enteropathogens among diarrheic sheep in the area of investigation, therefore, there is need to strengthen the control strategies to minimize the burden of these pathogens and their risk to cause zoonotic infections and food borne illness.

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