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Some Studies on *E-Coli* Mastitis in Cattle and Buffaloes

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Key Words:
***E.coli*, Cattle, Buffaloes, Serological identification, PCR**

ABSTRACT:

The aim of this study is to identify the role of *E.coli* in bovine mastitis, its virulence factors and antimicrobial sensitivity. Three hundred and sixty lactating cows and buffaloes were examined clinically and by CMT, 210 were suffering from clinical mastitis with percentage of 62.7% and 39 (20.4%) *E.coli* isolates were detected in clinical cases of mastitis, 90 cases were suffering from subclinical mastitis and 6 (6.8%) *E.coli* isolates were detected from these samples. The percentage of *E.coli* in cows was 31 (21.1%) while in buffaloes 8 (18.3%) isolates. Serotyping of *E.coli* revealed that O55 (30%), O111 (15%), O124 (15%), O119 (10%), O114 (10%), O26 (5%), O157 (10%) and O44 (5%). That's showing that O55, O111 and O124 were the most serotypes causing mastitis. PCR identification of TraT gene revealed 5 cases were positive and give positive reaction at 307 bp. and for eaeA gene revealed 6 cases and give positive reaction at 384 bp. The antimicrobial sensitivity indicated that the most effective antibiotics were lincospectine (56.6%), danofloxacin (56.6%), enrofloxacin (40%) and ceftifure (40%), while the lowest effective antibiotics were oxytetracycline and ampicillin.

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1. INTRODUCTION

Mastitis is considered the most costly disease in dairy herds due to discarded milk and lowered milk production for approximately 80% of costs associated with mastitis are treatment costs, veterinary fees, labor costs, early culling and death (Miller *et al*., 1993).

E.coli is one of the most frequently isolated pathogens from clinical infections, it was more severe than the other bacterial causes and it tended to be more severe in early lactation and during the housing period, resulting in inflammation that ranges from sub acute to per-acute. Necrosis of the mammary epithelium occurs during severe, naturally occurring clinical *E.coli* mastitis, as well as during severe experimental *E. coli* mastitis (Bradley and Green, 2001).

Escherichia coli is considered an environmental pathogen and one of the most important causes of bovine clinical mastitis, which is mostly observed in the early lactation period and in high-producing cows with low somatic cell counts (Hogan and larry,2003).

Strains of *E. coli* are traditionally characterized by serological identification of somatic O, flagellar H, capsular K, and fimbrial F antigens (Quin et al. 1998; Gyles, C.L, 1993).Differentiation of pathogenic strains from normal flora strains depends on the identification of virulence characteristics. *E. coli* strains can further be classified according to the presence of virulence factors such as enterotoxigenic *E. coli* (ETEC), attaching and effacing *E.coli* (AEEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and Shiga toxin-producing *E. coli* (STEC or VTEC) (Franck et al. 1998;Nagy et al. 1999). Virulence factors associated with strains of *E. coli* include adhesions, toxins, cell wall, capsule production, and serum resistance (Gyles, C.L, 1993) so the aim of this study was to study prevalence of *E.coli* mastitis, serotyping and detection of some virulence factors, and to study the antimicrobial sensitivity of *E.coli* isolated from mastitic cows and buffaloes.

2. MATERIAL AND METHODS

A total of 300 animals were included in this study, 210 (155cows and 55 buffaloes) animals suffering from clinical mastitis and 90 (72 cows and 18 buffaloes) were suffering from subclinical mastitis.
210 animals were raised in dairy farms and 90 animals were raised individually in farmer's houses belonging to Behira governorate. Animals were subjected to clinical examination, California mastitis test and milk samples were collected form all cases.

Samples:

Milk samples were collected under hygienic condition and each milk sample was subjected to California mastitis test (CMT) **(Schalm et al.,**

1971). The positive cases subjected to bacteriological examination.

Clinical examination

Systemic and local signs were recorded for each case. Systemic signs include general conditions, heart rates rectal temperature and appetite. Local signs include swelling, heat and pain in udder. Appearance of the milk was also recorded for each case.

California mastitis test:

Each milk sample was subjected to California mastitis test (CMT) **(Schalm et al., 1971)**. According the visible reaction of the CMT, the results were classified into four scores: 0 = negative or traces (no changes in consistency), 1 = slightly positive (+), 2 = positive (++) and 3 = highly positive (+++), Scores 1, 2 and 3 depend on the degree of gelation that was indicated by gelatinous mass**.**

The positive reaction was subjected to bacteriological examination.

Bacteriological examination of milk samples: (Quinn et al., 2002)

Samples from clinical and subclinical cases were incubated aerobically at 37ć for 18-24 hrs ,then centrifuged at 3000 rpm for 20 minutes .The cream and supernatant fluid were discarded, the sediment was streaked on Nutrient agar, MacConkey agar and EMB agar.

The inoculated plates were incubated aerobically at 37ć for 18-24hrs and examined for bacterial growth. Biochemical identification was done according to **Quinn et al. (1994).**

Serological identification of *E.coli:*

***E.coli* serotyping**

Serotyping of *E-coli* isolates was performed according to **Edwards and Ewing (1972)** using 8 polyvalent and 43 monovalent o anti sera for isolated *E-coli.* K99 pilus antigen detected by slide agglutination test using ready made international trading diagnostic serum.

Detection of *E.coli* virulence factors by PCR:

The oligonucleotide primers for PCR were synthetized according to **Wen-jie et al. (2008)** and **Kaipainen et al. (2002)** to detect nucleotide sequence of eaeA gene and TraT gene respectively. The primer sequence for TraT gene is GATGGCTGAACCGTGGTTATG, CACACGGGTCTGGTATTTATGC5 and for eaea gene is GACCCGGCACAAGCATAAGC, CCACCTGCAGCAACAAGAGG. From pure cultures, DNA was extracted by phenol-chloroform method according to **Sambrook et al. (1989)**. Preparation of PCR Master Mix according to **Emerald Amp** GT PCR master mix **(Takara)** Code No. RR310A kit, a 25 µl of master mix was prepared by adding 12.5 µl of Emerald Amp GT PCR master mix (2x premix), 4.5 µl PCR grade water, 1 µl forward primer (20 pmol), 1 µl reverse primer (20 pmol) and 6 µl template DNA.

3. RESULTS and DISCUSSION

Table (1): The percentage of *E.coli* mastitis among cattle and buffaloes

Farm	Clinically mastitic cases	Quarter affected by *E.coli*				Total clinical *E .coli* cases	
		Hind		Fore			
	No.	No.	%	No.	%	No.	%
Farm raised animals 120	Cows 105	16	15.2%	9	8.6%	25	33.8%
	Buffaloes 15	3	20%	1	6.7%	4	26.6%
Individually raised cases 90	50 cows	4	8%	2	4%	6	12%
	40buffaloes	2	5%	2	5%	4	10%
Total	155 cows	20	40.9%	11	22.4%	31	20%
	55buffaloes	5	12.5%	3	5.8%	8	14.5%

Table (2): Percentage of *E.coli* subclinical mastitis:

No. of affected animals	Cows	Buffaloes	*E.coli* isolates			
			No		%	
			Cattle	Buffaloes	Cattle	Buffaloes
Farm animals (55)	50	5	1	1	1.8%	1.8%
Individually raised cases (35)	22	13	3	1	8.5%	2.8%
Total (90)	72	18	4	2	5.2%	2.3%
	90		6		6.7%	

Table (3): Effect of the age on the incidence of *E.coli* mastitis

Age	**Type of animal**	**Total mastitic cases**	**%**	***E.coli* cases**	**%**
3-4years	**Cows**	**25**	**19.8**	**2**	**1.6**
	Buffaloes	**15**	**22.7**	**-**	**-**
4-5years	**Cows**	**38**	**44.7**	**4**	**4.7**
	Buffaloes	**22**	**40**	**1**	**1.8**
5-6years	**Cows**	**33**	**58.9**	**6**	**10.7**
	Buffaloes	**8**	**27.6**	**2**	**6.8**
6-7years	**Cows**	**32**	**63.5**	**9**	**17.3**
	Buffaloes	**3**	**9.4**	**1**	**3.1**
>7years	**Cows**	**27**	**65.9**	**10**	**24.4**
	Buffaloes	**7**	**26.9**	**4**	**15.4**
Total		**210**	**37.9**	**39**	**8.6**

Table (4): Effect of period of lactation on the incidence of *E.coli* mastitis

Stage of lactation (months)	**Total No.**	**Totoal mastitic cases**		***E.coli* mastitic cases**	
		No.	**%**	**No.**	**%**
1-2 months	110	90	81.8	20	18.2
4-6 months	170	100	58.8	14	8.2
7-9 months	80	20	25	5	6.3
Total	360	210	55.2	39	10.8

Table (5): The relationship between the virulence genes and serotypes of *E.coli* in relation to

the incidence of clinical and subclinical mastitis

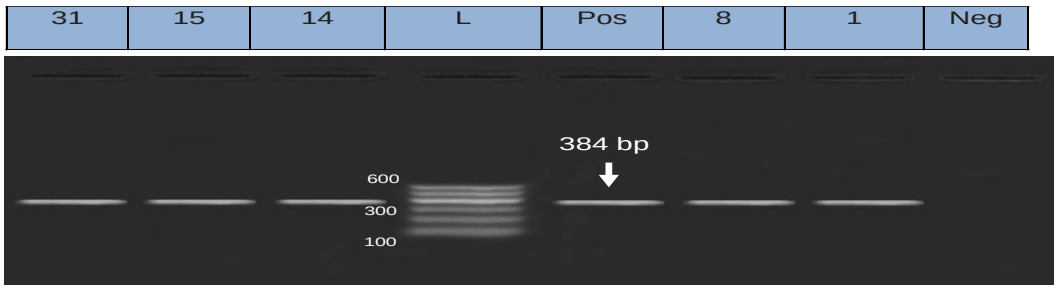
	No.	Clinical	Sub_cilincal	%
TraT gene	5	4	1	25%
eaeA gene	6	2	4	30%
O55	6	5	1	30%
O111	3	3	-	15%
O124	3	1	2	15%
O119	2	-	2	10%
O114	2	1	1	10%
O26	1	1	-	5%
O24	2	2	-	10%
O44	1	-	1	5%

The percentage calculated according to the total number of examined samples (20)

Table (6): Serotypes of *E. coli* isolated from clinical mastitis

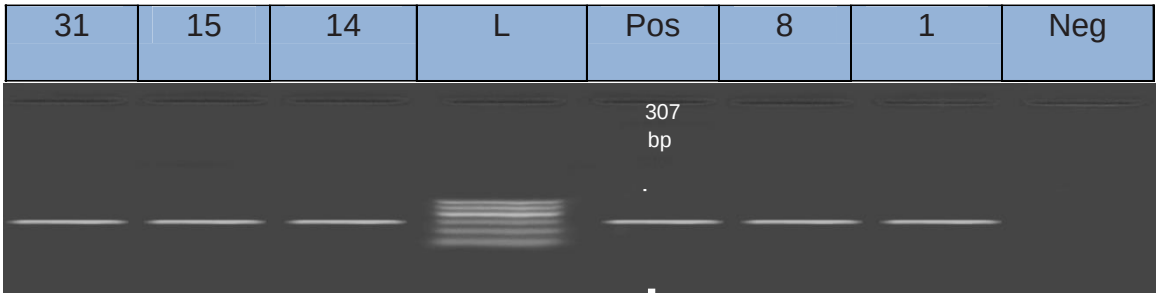
Total No. of clinical *E. coli* isolates.	Serogroups	No.	%	K99
20	O55	6	30%	+
	O111	3	15%	+
	O124	3	15%	-
	O119	2	10%	+
	O114	2	10%	-
	O26	1	5%	-
	O157	2	10%	+
	O44	1	5%	-
	Total	20	100%	

Detection of eaeA gene in *E.coli* isolated from cases of mastitis:



(20) *E.coli* isolates were examined for the presence of eaeA gene by PCR and the gene was found in 6samples in a size 384 bp.

Detection of TraT gene in *E.coli* isolated from cases of mastitis:



(20) *E.coli* isolates were examined for the presence of TraT gene by PCR and the gene was found in 5samples in a size 307 bp.

Table (7): Antimicrobial sensitivity of *E.coli* from mastitic cases

Type of the antibiotic	Number			%		
	S	M	R	S	M	R
Enrofloxacin 10%	12	10	8	40	33.3	26.6
Amoxicillin and clavulonate 10%	7	15	8	23.3	50	26.6
Lincospectin 33.33%	17	9	4	56.6	43.3	13.3
Florphenocol 10%	9	7	14	43.3	23.3	46.6
Danofloxacin 10%	17	10	3	56.6	33.3	10
Ampicillin 10%	-	-	30	-	-	100
Ceftifure 2.5%	12	10	8	40	33.3	26.6
Oxytetracycline 5%	-	-	30	-	-	100
Marbofloxacin 10%	15	9	6	50	30	20

S= Susceptible M= Moderately susceptible R= Resistant

Mastitis in dairy cows is a serious problem as it is an economically devastating disease causing immense economic losses in the dairy industry in Egypt Seleim et al. (2002) and the worldwide costliest production disease in dairy herds Miller et al. (1993).

From the results presented in table (1) out of 210 (155 cows and 55) buffaloes were mastitic and from them 31 (20.6%) cows and 8 (14.5%) buffaloes were suffering from *E.coli* mastitis and the total percentage of *E.coli* in cows and buffaloes was 35.1% and this nearly agrees with salwa et al. (2011) .Similar results were observed by Aziz (2002) and Bradley and Green (2001) .We also found that hind quarters are the mostly affected 53.4% than the fore quarters 28.3% and this nearly similar to Salwa et al.(2011), this result also nearly similar to the incidence which recorded by Wadhwa et al. (1996). It was theorized that rear quarters were more susceptible to mastitis than front quarters because they produce more milk and more exposed to chills and other environmental effects or that their teats being lower, more exposed to dirt and trauma and also due to higher contamination by urine and feces.

As shown in table (2) out of 90 cases were subclinical and from them found that 6 (6.7%) cases were infected by *E.coli.* this result is lower than the results of Abdel-rady and Sayed (2009) and khaled A. et al (2010) who found that the incidence of *E.coli* subclinical mastitis in buffaloes was (23.7%) and they attributed their results to the presence of some characteristics in buffaloes that may contribute to greater risk of mastitis such as more pendulous udder and longer teats. Besides, Krishnaswamy et al. (1965) stated that teat sphincter of buffaloes have smoother muscular fiber in such a way it constitutes a better barrier to microorganism invasion than cow's teat sphincter.

Evidence from earlier studies has shown that young cows are more resistant than older cows due to their more alert defence mechanisms Mehrzad et al. (2002b) and this agree with our study in table (3) as we found that the cows over 7 years more susceptible to *E.coli* mastitis by apercentage (24.4%) and in buffaloes (15.4%), while in earlier ages as in 4-5 years *E.coli* mastitis in cows was (4.7%) and in buffaloes was (1.8%).

Cows are known to be most susceptible to coliform mastitis during the puerperal period Hogan and Smith (2003). There are several reasons for this phenomenon; at this stage, the metabolic state of the cow does not favour efficient defence, and stress around parturition, which is related to high cortisol levels, interferes with cellular defence Breazile (1988). Tanja Lehtolainen (2004) in his field data showed an increased incidence during the post-partum period, as *E. coli* mastitis was most common in early lactation (0-21 days PP), decreasing towards the dry period and this data agree with our results shown in table (4) in the first 1 and 2 months post parturation the percent of *E.coli* mastitis (18.2%) higher than 4-5 months (8.2%) and the little occurance of *E.coli* mastitis is toward the dry period (6.3%). These results could be attributed to the decrease in the amount of milk production towards the end of lactation and to the developing immunity which developed along the course of lactation.

It appeared in table (5) that TraT gene (25%) mostly isolated from clinical cases with severe clinical signs (high temperature and severe inflammation in the udder) and this may be due to its combination with complement inducing serum resistance and this agree with previous studies of Barrow and hill (1989) and Nemeth et al. (1991).And also come in agreement with Tanja Lehtolainen (2004) and Kaipainen et al. (2002). In thier study, found that the virulence factor most frequently detected, with 38% of all isolates being TraT-positive. TraT is supposed to play an important role in the serum resistance of bacteria (Sukupolvi and O´Connors, 1990).

Also in table (5) eaeA gene mostly isolated from subclinical cases or clinical cases with mild clinical signs as this gene responsible for attachement so it mostly responsible for recurrent infections and this finding come in agreement with Salwa et al. (2011) who found that most serogroups isolated from recurrent infections were positive to eaeA gene and also this come in agreement with Kobori et al. (2004).

Also table (5) showed the distribution of *E.coli* serogroups according to somatic "O" antigen and capsular (K99) antigen. The most prevalent *E.coli* serogroups from mastitic cases were O55 (30%), O111 (15%), O124 (15%), O119 (10%), O114 (10%), O26 (5%), O157 (10%) and O44 (5%) and this result is in agreement with Salwa et al. (2011) and Aziz (2002), Correa and Marin (2002), Lipman et al. (1996).

Also it's clear from Table (6) *E.coli* strains O55, O111, O119 and O157 were given positive results with slide agglutination test for detection K99 pilus, while O124, O114, O26 and O44 give negative results,this result agree with Galone and Le-Roux

(2001) who found the strains O111 and O119 isolated from mastitic cow contain K99 antigen.

Antimicrobial sensitivity patterns are an important component of the dicision making process in determining appropriate antimicrobial therapy against bacterial infection of the mammary gland.In our study we found the most effective antimicrobials affecting *E.coli* isolated from mastitis as shown in table (7) were lincospectine (56.6%), danofloxacin (56.6%), Florphenocol (43.3%) ceftifure (40%) and enrofloxacin (40%). While, the least effective antibiotics were oxytetracycline, ampicillin as the *E.coli* was completely resistsnt to them.The penicillin clavulonate was effective by apercent of (26.6%) and this come in agreement with Hassan (2003).

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