INCIDENCE OF *YERSINIA ENTEROCOLITICA* IN FARM ANIMALS AT DAKAHLIA AND KAFR EL-SHEIKH GOVERNORATES

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ABSTRACT

Up to date little is known about incidence of Yersinia enterocolitica in farm animals in Egypt therefore this study was carried out. In this study, 200 samples were collected from diseased animals suffered from diarrhea including cows, buffaloes, sheep and goats were obtained from private and governorate farms at Dakahlia and Kafr El-Sheikh governorates. A total of 23 Y. enterocolitica strains were recovered. The prevalence rates were 14%, 14%, 10% and 8% in cows, sheep, Buffaloes and goats respectively. Y. enterocolitica isolates were biotyped, 21 isolates out of 23 were belonging to biotype 2 while,only 2 stains were belonging to biotype 1. Serotyping by using the available monovalent antisera, Y.enterocolitica were 19, 2, 2 strains belonging to O: 3, O: 5 and O: 9 serovars. Serotyping and biotypiny among different isolates revealed that 12 isolates O: 3/2, 4 isolates O: 5/2, 2 isolates O: 9/1 while 2 isolatesO: 9/2. As regard to the virulence tests, 13 out of tested strains were stable toxin strains, and 17 out of 23 strains were CRMOX producer positive but guinea pig conjunctivitis (Sereny test) was found to be negative for all examined serotypes. The PCR product was visualized after agarose gel electrophoresis. One amplification band of 591 bpwas observed when Y. enterocolitica colonies were used as template for the PCR. The serobiovars of Y. enterocolitica strains showing positive for virF gene were O: 3/2(2) and O: 9/2(1).

INTRODUCTION

Y. enterocolitica is a Gram-negative, facultative anaerobic bacillus belonging to the family Enterobacteriaceae. This organism has a world wide distribution and wide spread in nature ***(Morris and Feeley, 1976)***. Because *Y. enterocolitica* grows slowly on ordinary laboratory media, it is easily overgrown by other bacteria ***(Feng and Weagant, 1994).*** The most widely used method is the cold enrichment, which requires an incubation period up to 3 weeks, followed by subculturing onto CIN agar and biochemical confirmation ***(Thisted-Lambertz et al., 1996; Bhaduri et al., 1997 and Hoorfar and Holmvig, 1999).*** There are over 54 O serotypes of *Y. enterocolitica* but only a few of these have been implicated in human or animal diseases ***(Bercovier et al., 1980; Kay et al., 1983 and Schiemann and Devenish, 1982).*** Forty to fifty megadalton plasmid has been described as responsible for the virulence of *Y. enterocolitica* ***(Gemski et al., 1980)***. Strains which posses this plasmid exhibit properties, calcium dependancy ***(Gemski et al., 1980)*** and congo red absorption ***(Prpic et al., 1983).*** A congo red- magnesium oxalate agar medium was developed to detect expression of virulence-associated calcium dependency and congo red absorption in *Y. enterocolitica* ***(Riley and Toma, 1989).*** Virulence of *Y. enterocolitica* was measured by sereny test or mouse lethality test. It was dependant on the presence of 42±1.1 megadalton plasmid. PCR methods have proved to be very useful in further classifying *Y. enterocolitica* as virulence that attributed to *Yersinia* virulence plasmid (PYV) containing *virF* gene which also imparted calcium dependency on the strain. This gene is present only in pathogenic strain.

An initial problem with serological studies on *Y. enterocolitica* was the cross-reaction detected between *Y. enterocolitica* O: 9 serotype and *Brucella abortus* ***(Ahvonen and Sievers, 1969)***. *Brucella* and *Y. enterocolitica* O: 9 have antigenic determinants in common causing cross-reaction which interfere strongly in *Brucella* serology **(Staak et al., 2000)**. So the present work was carried out for:

1) Prevalence of *Y. enterocolitica* strains from diseased animals suffered from diarrhea including cows, buffaloes, sheep and goats.
2) Biotyping of the isolated strains.
3) Serotyping of the isolated strains.
4) Virulence tests for the isolated *Y. enterocolitica* strains including:
   a- Enterotoxin assay.
   b- Invasiveness assay.
   c- Congo red magnesium oxalate platting.
   d- Esculin hydrolysis.
   e- Salicin fermentation.
   f- Pryrazinamidase production.
5) Polymerase chain reaction technique for detection of *virulence factor (virF)* gene in isolated *Y. enterocolitica* strain.
6) Sensitivity test for the isolated strains against most common antibiotics.

MATERIAL AND METHODS

1. MATERIALS:

1.1. Samples for isolation:

200 samples were collected from diseased animals suffered from diarrhea including cows, buffaloes, sheep and goats (50 fecal samples for each species) were obtained from private and governorate farms at Dakahlia and Kafrelsheikh governorate. Samples were collected from May 2007 to December 2008.

1.2. Reference strains of *Y. enterocolitica* O: 3 and O: 9:

They were kindly supplied by Bacteriology Department Animal Health Research, Institute, Dokki, Giza. It used as a positive control strain for PCR.

1.3. Culture media used:

1.3.1. Media for isolation of *Y. enterocolitica* :

1.3.1.1. Solid media:

- Cefsulodin-irgasan-novpbiocin(C.I.N) medium ***(Schiemann, 1979)*:** It was prepared by addition of *Yersinia* Selective Agar base (Oxoid CM653) to *Yersinia* Selective Supplement (Oxoid,SR109).

- MacConkey's agar medium (Oxoid).

- Nutrient agar (Oxoid).

1.3.1.2. Semisolid media:

-Semisolid Nutrient agar (Oxoid).

1.3.2. Media for biochemical identification of *Y. enterocolitica*:

All media was prepared according to ***MacFaddin (1976)*** and ***Bailey and Scott (1986)***.

- Triple sugar iron agar (TSI) (Oxoid).
- Simmon's citrate (Oxoid).
- Urea agar base (Oxoid)
- Glucose phosphate broth (Oxoid).
- Nitrate broth (Difco).
- Oxidation fermentation (OF) basal medium (Bio-Merieux).
- Peptone water 1% (Oxoid).
- Aesculin broth ***(Farmer et al., 1992):***
- Sugar fermentation (Oxoid).

1.3.3. Media used for virulence tests (Pai and Mors, 1978).

- Media for production and testing of enterotoxin.
- Brain heart infusion broth (Oxoid): used for Sereny test.
- Tryptone soya agar (Oxoid): used for Sereny test.
- 5% sheep blood agar used for CRMOX.
- Congo red magnesium oxalate agar (CRMOX) medium ***(Riley and Toma, 1989).***
- Aesculin agar: used for aesculin hydrolysis.
- Phenol red peptone water containing 0.5% of salicin: used for salicin fermentation test.
- Media used for pyrazinamidase test ***(Kandolo and Wsuiters, 1985).***

1.4. Reagents and Solutions:

- 3%hydrogen peroxide solution, used for catalase test.
- 1% tetramethyl-P-phenyliamine dihydrochloride solution used for oxidase test.
- 40% urea solution (Oxoid, SR20) used for urease test.
- 0.02% methyl red solution used for methylred test.
- Kovac's reagent, for indole test.
- 10% lactose for OF test.
- 0.8% sulfanilic acid in 5N acetic acid and 0.5% alpha naphthylamine in 5N acetic acid used for nitrate reducyion test.
- Paraffin oil for decarboxylase and OF tests.
- 5% alpha naphthol in absolute ethyl alcohol and 40% potassium hydroxide for voges proskauer test.
- 1% wt/vol freshly prepared ferrous ammonium sulphate(aqueous) solution: used for pyrazinamidase test.

1.5. Antibacterial discs used for antibacterial sensitivity test:

12 Antibacterial discs from oxoid containing the following chemotherapeutics:

Amikacin (30 µg), ampicillin (10 µg), Chloramphenicol (30 µg), ofloxacin (10 µg), streptomycin (10 µg), neomycin(10 µg), gentamycin (10 µg), erythromycin (15 µg), tetracycline (30 µg), cefotaxime (30 µg), colistine sulphate (10 µg), and sulphamethoxazol-trimethoprim (1.25+5.75 µg),

2. Methods:

2.1. Collection of samples:

200 fecal samples from diarrheic animals and received in aseptic bag to lab immediately.

2.2. Isolation of *Y. enterocolitica*:

2.2.1. Direct plating: *(Nilehn, 1969; Schiemann, 1979 and Fredriksson-Ahomaa et al., 1999)*.

Fecal swabs were streaked directly onto CIN and MacConkey's agar plates and incubated at 30°C for 18-24h. Lactose negative colonies on MacConkey's agar were streaked onto CIN agar and incubated at 30°C for 18-24h.

2.2.2 Cold enrichment: *(Adesiyun et al., 1992 and Fredriksson-Ahomaa et al., 1999)*.

One loopful of fecal sample was incubated into 5 ml sterile phosphate buffered saline (pH7.6) tubes. The tubes were enriched at 4°C (in a refrigerator) for one month and subcultured weekly at intervals one, two, three and four weeks onto CIN and MacConkey's agar which were incubated at 30°C for 18-24h. Lactose negative colonies on MacConkey's agar were streaked onto CIN agar and incubated at 30°C for 18-24h. Suspected colonies were picked up and subcultured onto nutrient agar Plates to confirm its purity, and then transferred (as well as reference strain) to nutrient slopes for maintenance till they were screened.

2.3. Identification of the *Y. enterocolitica* isolates *(Nilehn, 1969a and Pianetti et al.,1990):*

2.3.1. Morphological and Culture examination.

Pure cultures were prepared from all suspected colonies, shape, size, type of colonies either lactose fomenter or non lactose fomenter onto MacConkey's agar.

2.3.2. Biochemical identification: *(Bercovier and Mollaret, 1984)*:

The suspected colonies were biochemically identified using the following tests:

Oxidase test, Catalase test, sugar fermentation test, Hydrogen sulphide production test using TSI agar, Urease test, Citrate utilization test (at 25 and 37°C), Indole production test, Methyl red test (at 25 and 37°C), Voges proskauer (at 25 and 37°C), Nitrate reduction test, Oxidation fermentation test and Aesculin hydrolysis test.

2.4. Biotyping of *Y. enterocolitica* isolates (Nilehn, 1969a):

Biotyping according to results of biochemical tests as shown in table 1:

Table (1): Biotyping of *Y. enterocolitica* using different biochemical tests.

Biochemical reactions	Biovars				
	1	2	3	4	5
Salicin(acid)	+	-	-	-	-
Indole	+	-	-	-	-
Lactose (O-F)	+	+	-	-	-
Xylose(acid)	+	+	+	-	-
Nitrate reduction	+	+	+	+	-
Trehalose(acid)	+	+	+	+	-
Voges proskauer	+	+	+	+	-
Sorbose(acid)	+	+	+	+	-
Sorbitol(acid)	+	+	+	+	-
Sucrose(acid)	+	+	+	+	-

+: Positive -: Negative

2.5. Serotyping of the isolates *(Winblad at al., 1966 and Winblad, 1967):*

The isolates were serotyped using the available monovalent antisera; O: 3, O: 5, O: 8 and O: 9 by slide agglutination test**.**

2.5.1. Virulence test:

Reference strain was used as a positive control as well as all *Y.enterocolitica* isolates.

2.5.2. Enterotoxin assay:

The ability to produce heat stable enterotoxin was assayed by the infant mouse test ***(Pai and Mors, 1978 and Robins-Browne et al. 1993).***

2.5.3. Infant mouse assay *(Pai and Mors, 1978 and Schiemann and Devenish, 1982):*

0.1 ml of culture filtrate of identified *Y.enterocolitica* strain was introduced through abdominal wall into milk filled stomach of each three mice which were 1 – 2 days old. After 4 hours, the mice were killed and the entire intestine was removed. The ratio between the intestine and the remaining body weight were calculated. A ratio greater than 0.083 was taken as positive for enterotoxin.

2.5.4. Invasiveness assay:

Test was done in guinea pig eye model " Sereny test" according to ***Schiemann and Devenish, 1982 and Kay et al., 1983.***

2.5.5. Congo red magnesium oxalate (CRMOX):

It was used for detection of pathogenic *Y. enterocolitica* strain ***(Riley and Toma, 1989)*.**

Tested strains was first grown on blood agar plates (5%sheep blood) at 22°C for 18h and then plated onto CRMOX medium and incubated for 24hrs at 36°C. Strains were CRMOX negative if only large colorless colonies were present.

Positive strains (CRMOX) always produce small red colonies.

2.5.6. Aesculin hydrolysis (Farmer et al.,1992):

All *Y. enterocolitica* isolates were inoculated into aesculin broth and incubated at 25°C . results recorded daily up to 7 days. Strains that hydrolysis aesculin show blackening of the media.

2.6 PCR for detection of *vir F* gene(Bhaduri et al.,1997):

Ten *Y.enterocolitica* isolates were evaluated for the presence of *vir F* gene with PCR assay. All selected isolates showing the virulence characteristics. The PCR technique was carried out at Biotechnology Lab., Fac.Vet.Med ., Cairo University.

Field Y.enterocolitica strains and DNA extraction method *(Nakajima et al.1992):*

A suspension of *Y.enterocolitica* isolates having 108 CFU/ml was prepared I TE buffer by adjusting its turbidity with McFarland standards. 10 µl of this suspension was put onto thin walled microtubes and was placed onto boiling water for 10 min followed by plunging into an ice bath. One µl of supernatant was used directly for amplification without any further processing.

Primer selection and preparation *(Bhaduri et al.,1997)*:

The *vir F* gene from Y.enterocolitica has been used. PCR was designed to amplify a 591-bp fragment (nucleotide region 430-1020) of the *vir F* gene encoded by plasmid PYV.

Oligonucleotide primer pair used were 5'- TCA TGG CAG AAC AGT CAG-3' and 5'-ACT CAT CTT ACC ATT AAG AAG-3' for amplification of *vir F*.

(PCR) DNA amplification procedure:

DNA samples were amplified in a total of 50 µl of amplification mixture.

All samples were overlaid with 50 µl of mineral oil, PCR was performed on a PTC-100 programmable thermal controller.

PCR cycling protocol *(Bhaduri et al., 1997)*:

Amplification cycles were as follows: initial denaturation step of 5 mm at 95°C followed by 30 cycles of 94°C for 30 sec (Denaturation) 60 °C for 30 sec (Annealing) and 70°C for 1 min (Extension). A positive and Negative control (no template) were included in each PCR run.

Analysis of PCR product using Agarose Gel Electrophoresis:

1.5% agarose gel was prepared in TAE IX buffer in the microwave after complete dissolving of the agarose in the buffe. Ethidium bromide was added in 0.5 mg/ml and mixed thoroughly. The gel left to become warm at room temperature. The tray was prepared and the comb 0.5-1 mm was placed above the tray in a suitable distance.

2.7. in Vitro antibacterial sensitivity test by disk diffusion technique according to Finegold and Martin, 1982. As shown in table2.

Table (2): Antibacterial sensitivity in relation to the zone of inhibition interpreted by the manufacturing company (Oxoid).

Antibacterial drug	Disc content	Diameter of inhibition zone	
		Resistant	Sensitive
Amikacin	30 µg	14 – 16	16 or more
Ampicillin	10 µg	13 or less	14 or more
Cefotaxime	30 µg	15 or less	16 or more
Chloramphenicol	30 µg	17 or less	18 or more
Ofloxacin	10 µg	15 or less	16 or more
Streptomycin	10 µg	14 or less	15 or more
Neomycin	30 µg	16 or less	17 or more
Gentamycin	10 µg	14 or less	15 or more
Erythromycin	15 µg	17 or less	18 or more
Tetracycline	30 µg	18 or less	19 or more
Colistine sulphate	10 µg	8 or less	11 or more
Trimethoprim- sulphamethoxazol	1.25+ 5.75 µg	15 or less	16 or more

RESULTS

3.1. Prevalence rate of *Y. enterocolitica* in the examined animal species:

Table(3): The prevalence rate of *Y. enterocolitica* in diseased animals suffered from diarrhea.

Animal species	Type of samples	No. of cases	Positive ones	
			No.	%
Cows	Fecal	50	7	14
Buffaloes	Fecal	50	5	10
Sheep	Fecal	50	7	14
Goats	Fecal	50	4	8
Total		**200**	**23**	**11.5**

Table (4): Comparison between direct plating and cold enrichment techniques for isolation of *Y. enterocolitica* from 200 samples collected from different animals.

Animal species	Type of samples	No. of samples	Positive by direct plating		Positive by enrichment	
			No	%	No	%
Cows	Fecal	50	-	-	**7**	**14**
Buffaloes	Fecal	50	**1**	**2**	**5**	**10**
Sheep	Fecal	50	-	-	**7**	**14**
Goats	Fecal	50	-	-	**4**	**8**
Total		**200**	**1**	**2**	**23**	**11.5**

3.2. Identification of *Y. enterocolitica* isolates (23) morphologically, colonial characters, motility and Biochemically:

Gram stained films showed small coccoid, gram negative bacilli, non sporulated, non capsulated and arranged singly or in short chains or heaps.On CIN agar *Y.enterocolitica* colonies were approximately 0.5-2mm in diameter after 18-24hr at 30°C. they were characterized by dark red center surrounded by an outer translucent zone" Bulls eye". On MacConkey agar, colonies were pale non lactose fermenter, pinpoint or flat colonies, translucent and 1mm in diameter after 24hr at 30°C.On nutrient agar purified colonies were circular, smooth, low convex, 1-2mm in diameter, translucent, with glistening surface and entire or slightly crenated edge after 24-48hours at 25°C. All isolates were found to be motile at 25°C and non motile at 37°C. Biotyping depending on reactions against different biochemical tests.

Table (5): Biovars of *Y. enterocolitica* strains isolated from the examined animal species:

[Percentage Of biotypes were calculated in relation to number of isolates (23)]

Animal species	**Type of samples**	**No. of samples**	**No. of isolates**	Biotype			
				No.1	%	No.2	%
Cows	Fecal	50	7	1	14.3	6	85.7
Buffaloes	Fecal	50	5	-	-	5	100
Sheep	Fecal	50	7	1	14.3	6	85.7
Goats	Fecal	50	4	-	-	4	100
Total		**200**	**23**	**2**	**8.9**	**21**	**91.1**

3.2. Identification of *Y. enterocolitica* isolates (23) serologically:

Using the available monovalent antisere O: 3, O: 5, O: 8 and O: 9 by slide agglutination test.

Table (6): Serotypes of *Y. enterocolitica* strains isolated from different examined animal species:

Type of samples	**No. of isolates**	**Serovars**							
		O: 3		**O: 5**		**O: 9**		**O: 8**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Fecal	23	15	82.6	4	8.6	4	8.6	0	0

3.3. Distribution of *Y. enterocolitica* serotypes and Biotypes in different examined samples:

Table (7): Serotyping and biotyping of *Y. enterocolitica* strains among examined animal species:

Animal species	Type of samples	No. of isolates	Serobiovars			
			O: 3/2	O: 3/2	O: 9/1	O: 9/2
Cows	Fecal	7	**5**	**-**	**1**	**1**
Buffaloes	Fecal	5	**3**	**1**	**-**	**1**
Sheep	Fecal	7	**4**	**2**	**1**	**-**
Goats	Fecal	4	**3**	**1**	**-**	**-**
Total		23	**15**	**4**	**2**	**2**

3.4. Virulence tests:

Table (8): Relation between virulence factors and *Y. enterocolitica* serobiovars.

Virulence test	*Y. enterocolitica* serobiovars								Total	
	O: 3/2 (15)		O: 5/2(4)		O: 9/1(2)		O: 9/2(2)			
	No.	%	No.	%	No.	%	No.	%	No.	%
ST	11	73.3	2	50	0	0	2	100	15	65.2
CRMOX	13	86.6	4	100	0	0	2	100	19	82.6
GPC	0	0	0	0	0	0	0	0	0	0
AH	0	0	0	0	2	100	0	0	2	8.7
SF	0	0	0	0	2	100	0	0ne:	2	8.7
PP	0	0	0	0	2	100	0	0	2	8.7

ST: Heat stable enterotoxin production. CRMOX: Congo red magnesium oxalate.

GPC: Guinea pig conjunctivitis(sereny test). AH: Aesculin hydrolysis

SF: Salicin fermentation PP: Pyrazinamidase production

3.5. Results of PCR for detection of *vir F* gene:

Ten selected *Y. enterocolitica* isolates of serotype O: 3/2 (8 isolates), O: 9/2(2 isolates) were subjected toPCR technique together with reference strain.

As shown in photo (1), the PCR product visualized after agarose gel electrophoresis was specific for the virulent gene of *Y. enterocolitica* (*vir F*), lane 3,4 (field strains) and lane 2 (reference strain).

One amplified band of 591-bp was observed when the DNA of *Y. enterocolitica* colonies were used as template for PCR. The serobiovar of *Y. enterocolitica* strains showing positive for *vir F* gene were O: 3/2 (lane 3) and O: 9/2 (lane 4) while the lane 2 was the positive control(serobiovar O: 3/2).

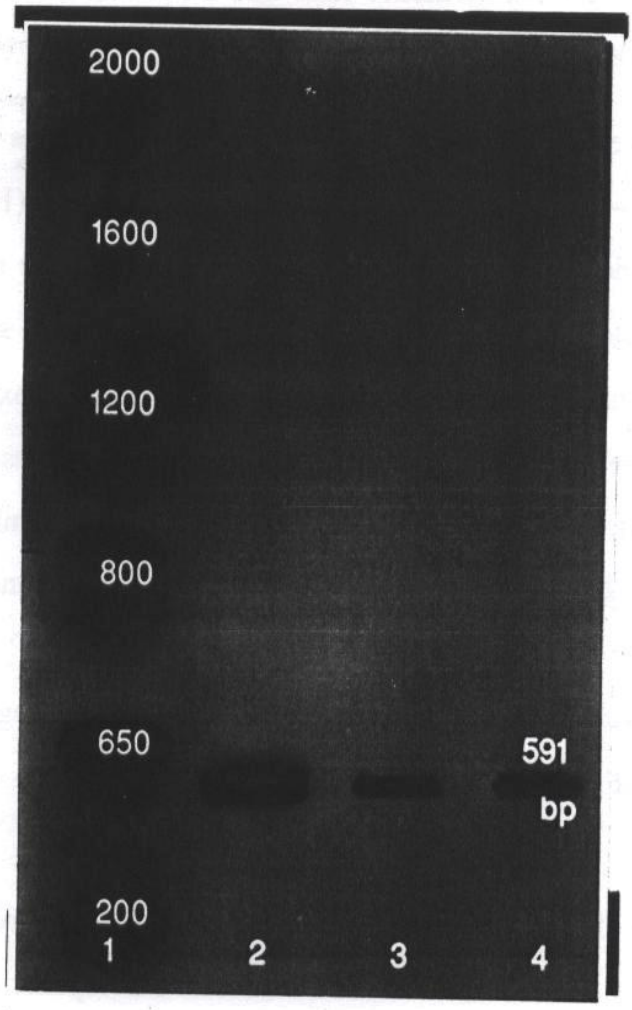

Photo (1): Result of PCR assay

Lane 2: Reference strain (Control O:3/2).

Lanes 3: Field strain (O:3/2)

Lanes 4: Field strain (O:9/2)

3.6. Correlation between virulence tests and PCR results for the presence of *vir F* gene:

Table (9): Virulence characteristics and PCR results for the presence of *vir F* gene of Y. *enterocolitica* isolates.

Phenotype tests and virulence gene	O: 3/2	O: 9/2	O: 3/2	O: 5/2	O: 9/2	O: 3/2	O: 5/2	Total positive
ST	+	+	+	+	+	-	-	8 (80%)
CRMOX	+	+	+	+	+	+	+	10(100%)
GPC	-	-	-	-	-	-	-	0 (0%)
AH	-	-	-	-	-	-	-	0 (0%)
SF	-	-	-	-	-	-	-	0 (0%)
PP	-	-	-	-	-	-	-	0 (0%)
virFgene	+	+	-	-	-	-	-	3 (30%)

ST: Heat stable enterotoxin production. CRMOX: Congo red magnesium oxalate.

GPC: Guinea pig conjunctivitis (sereny test). AH: Aesculin hydrolysis

SF: Salicin fermentation PP: Pyrazinamidase production

3.7. Results of antibiotic sensitivity tests of *Y. enterocolitica* isolates:

Table (10): Sensitivity tests of *Y. enterocolitica* isolates against different antibiotics.

Chemotheraputic agent	Sensitive		Resistant	
	No.	%	No.	%
Amikacin	23	100	-	0.0
Ampicillin	0.0	0.0	23	100
Cefotaxime	20	78.6	3	21.4
Chloramphenicol	15	53.6	8	46.4
Colistin sulphate	10	35.7	13	64.2
Erythromycin	2	7.1	21	92.9
Gentamycin	23	100	-	0.0
Neomycin	15	53.6	8	46.4
Ofloxacin	18	71.4	5	28.5
Tetracycline	16	57.1	7	42.9
Streptomycin	14	50	9	50
Trimethoprim- sulphamethoxazol	13	42.9	10	42.9

DISCUSSION

Y. enterocolitica is capable of causing a variety of diseases in both animals and human; gastroenteritis, septicaemia, terminal ileitis closely resembling appendicitis ***(Ahovonen, 1972 and Mittal and Tizarcl, 1981).*** In the present study , the prevalence rates of *Y. enterocolitica* recovered from 200 samples collected from diseased animals suffered from diarrhea were 14%, 10%, 14% and 8% in cows, buffaloes, sheep and goats respectively. Meanwhile, the same findings were recorded in Egypt by ***Tanios (1994)*** who revealed that 34 animals carrying *Y. enterocolitica* with prevalence rate of 3.2% but for examined animals (2.5%) in cows, (3%) in buffaloes and (3%) in sheep. In another study ***Ahmed (1998)*** found that *Y. enterocolitica* have a role for inducing enteric disease in cattle, sheep and goats. It was isolated from 16 samples (8%) from buffaloes, 4 samples (2%) from cows, 3 samples (1.5%) from goats.Nearly similar results were recorded by ***Ahvonen et al., (1973) and Alonso et al.,(1979).*** The rates of isolation of *Y. enterocolitica* have varied widely within and between countries possibly because of true differences in the prevalence of *Y. enterocolitica* infection. However, the isolation technique has been reported to have a significant effect on the rates obtained ***(Okoroafor et al., 1988 and Ahmed, 2003).***

Our study referred that one sample (0.5%) was positive to *Y. enterocolitica* by direct plating technique and 23 samples (11.5%) were positive by cold enrichment method. Our findings agreed with ***Davey et al., (1983) and Zheng (1987)*** who recorded that cold enrichment technique increase the frequency of isolation of *Y. enterocolitica*.As well as ***Thisted-Lambertz et al.,(1996) , Bhaduri et al., (1997), Hoorfar and***

Holmvig (1999) stated that most widely used method was the cold enrichment with an incubation period up to 3 weeks followed by subculturing onto CIN agar. ***Greenwood and Hooper (1988), Fukushima and Gomyoda(1986) and Hussein et al., (2001),*** concluded that selective enrichment with Lurria-Bertani- Bile salts Irgasan(LBBSI) produced the highest recovery rate (63%) of *Y. enterocolitica* isolates, when compared with cold enrichment (52%) and direct plating on CIN agar alone (43%).

The biochemical variability within this group encouraged the development of different biotyping schemes ***(Nilehn, 1969b, Knapp and Thai, 1973, Brenner et al., 1977 and Winbhul, 1967).*** According to ***Nilehn (1969a)***, the biotyping of *Y. enterocolitica* were done using different biochemical tests including Aesculin hydrolysis, Salicin fermentation, Indole, Lactose (O-F), Xylose, Nitrate reduction, Trehalose, Ornithine decarboxylase, Voges proskauer, Sorbose, Sorbitol and Sucrose fermentation. Through this study the majority of strains (21) were biotype 2. While only 2 stains were biotype 1. In both cows and sheep, 6 isolates were biotype 2 and only one was biotype1, meanwhile all strains isolated from buffaloes and goats were of biotype2. The same result of biotyping were recorded by ***Fukushima et all (1993), Tanios (1994), Zamora et al., (1997), Filetici et al., (2000) and Ahmed (2003)*** who isolated 26 *Y. enterocolitica* strains from cattle, sheep and goats. They biotyped into biotype 1 (24 isolates) and biotype 2 (2 isolates).

As regard to seological identification by using the available monovalent antisera O: 3, O: 5, O:8 and O: 9 and by slide agglutination test; the result of serotyping of the 23 *Y. enterocolitica* strains were as the

following: 15, 4 and 4 strains were serotyped O: 3, O: 5 and O:9 serovars respectively, serotype O: 3 was the most prevalent serotype recovered in the present study. These results were agree with ***Pedersen (1979), Christensen (1980), Tauxe et al., (1987) and Bottone (1997)*** concluded that O: 3 serotype was one of the important human gastrointestinal pathogens. On the other hand, ***Ahmed (2003) and Falcao et al., (2004)*** isolated *Y. enterocolitica* serotype O: 3 (92.5%) and O: 9 (7.69%) from examined cattle, sheep. The distribution of *Y. enterocolitica* strains in different animal species were four different bioserovars of *Y. enterocolitica* . O: 3/2 serobiovar was the most prevalent one (65.2%). The other serobiovars recovered were O: 5/2, O: 9/1 and O: 9/2 with a prevalence rates of 17.4%, 8.7% and 8.7% respectively.These results go parallel with that obtained by ***Corbel et al.,(1990), Fantasia et al.,(1993), Zheng and Xie (1996) and Ahmed(2003)*** identified serobiovars O: 3/2 and O: 9/1 in 92.3% and 7.69% respectively in examined cattle, sheep and goats.The presence of the gene *vir F* (virulence factor) confirms the presence of virulence plasmid, which is essential for bacterial pathogenesis ***(Cornelis, 1994).***In the present study, 23 *Y. enterocolitica* strains of different bioserovars were examined for seven different virulence tests, Heat stable enterotoxin(ST) was produced by 62.5% of examined strains, regarding toCRMOX test, all isolates of O: 5/2 and O: 9/2 were positive, but negative for O:9/1, Sereny test was found to be negative for all examined serotypes and the organism can't be reisolated from the animals 7 days after inoculation. There was 100% agreement between Aesculin hydrolysis, Salicin fermentation and Pyrazinamidase production for the examined *Y. enterocolitica* strains.

The combined use of CRMOX, Sal/Esc and Pyz tests provides a method for accurately differentiating between pathogenic and non pathogenic *Y. enterocolitica* strains. Also, our results revealed that the isolated Y. enterocolitica can be able to produce heat stable enterotoxin, showed an agreement with those reported by ***Pai and Mors (1978), Delor et al., (1990), Delor and Cornell's (1992) and Robins-Browne et al., (1993)*** investigated 28 clinical isolates of *Y. enterocolitica* for their abilities to produce heat stable enterotoxin, while the same findings were reported by ***Bin Kun et al., (1994)*** who studied acute yersniosis in sheep due to *Y. enterocolitica* toxin, that all isolates producing thermostable toxins capable of producing bleeding from capillaries, myocardial degeneration and death.

In regard to sereny test, ***Brewer and Corbel (1983)*** agree with the results obtained that *Y. enterocolitica* isolates from abortion or enteritis cases of cattle and lambs can't produce any sign of local or general disturbance after conjunctival instillation and the organism can't be reisolated from the animals 7 days after instillation.

The first PCR method used for detection of pathogenic *Y. enterocolitica* was developed by ***Wren and Tabaqchali 1990***. Pathogenic strains of *Y. enterocolitica* are characterized by a chromosomally encoded ability to invade cultured mammalian cells and the presence of a virulence plasmid, PYV, that may facilitate bacterial survival within host tissues ***(Miller and Falkow, 1988 and Isberg, 1990).*** In the present study, ten selected *Y. enterocolitica* isolates of serotype O: 3/2 and O: 9/2 were subjected to PCR technique together with reference strain. It was specific for the virulent gene of *Y. enterocolitica* (*vir F*). Lane 3 and 4

(field strains) and Lane 2 (reference strain). One amplified band of 591 bp was observed when the DNA of *Y. enterocolitica* colonies were used as template for PCR. The serobiovars of *Y. enterocolitica* strains showing positive for *vir F* gene were O: 3/2 (Lane3) and O:9/2 (Lane4). While the lane 2 was the positive control (serobiovar O: 3/2).These results were agree with that obtained by ***Kappariid (1991)*** who stated that the best characterized virulence factor for *Y. enterocolitica* encoded by the *vir F* gene mapped to a 70 Kbp plasmid.

In the present work the DNA of *Y. enterocolitica* was extracted by simple boiling for colonies of the organism for 10 min, then followed by cooling. The same method was used by ***Sritharan and Barker (1991) and Kocagoz et al., (1993)*** for DNA preparation.They suggested that, the preparation of DNA by just boiling rather than enzymatic lysis followed by phenol-chloroform extraction was better foe amplification with PCR, on the other hand, ***Thisted lambertz et al., (1996)*** described that PCR method provided results within 24h when nested PCR was used. Besides increasing the sensitivity, the PCR also ensures higher specificity. Several measures have been introduced in order to prevent such contamination. Many blank reactions must be involved in the PCR assay to confirm contamination free operation during the test. Another problem was that PCR didn't differentiate between viable and non viable bacteria. The unstable nature of virulence plasmid ***(Bhaduri et al.,1991 and Kwaga and Iverseng, 1991)*** complicated the isolation of plasmid bearing virulent *Y. enterocolitica.* The overgrowth of virulent cells eventually leading to completely avirulent culture ***(Toora et al., 1994).***

The correlation between the virulence tests and PCR results for the presence of *vir F* gene. From the tested 10 *Y. enterocolitica* isolates, 3 strains of serobiotypes O:3/2(2) and O:9/2(1) were positive virulence in all virulence characteristics including heat stable enterotoxin production (ST) and Congo red magnesium oxalate (CRMOX). While they were negative for Sereny test (GPC), Aesculin hydrolysis (AH), Salicin fermentation (SF) and Pyrazinamidase production (pp), they were also positive for the presence of *virF* gene.

On the other hand,three isolates of serobiovar O:3/2 were positive for virulence characteristics of ST and CRMOX but negative for GPC, AH,SF and PP, while non of them possessed the *vir F*-gene. 2 strains of serobiovars O:5/2 and O:9/2 showed positive results for all the virulence tests except for GPC,SF and PP were negative and vir F gene was not identified in any of them. One isolate of serobiotype O:3/2 was CRMOX+ and it was negative for ST, GPC, AH,SF, PP, and not possessed vir F gene.At the same time one isolate of bioserotype O:5/2 was positive only for CRMOX test and negative for other examined virulence tests and/also it was negative for *vir F* gene. These findings were agree with ***Bhaduri et al., (1997)*** who found that virulent plasmid-bearing clones of *Y. enterocolitica* (YEP+) were recovered from both artificially contaminated group pork and naturally contaminated tongues expressed plasmid-associated virulence characteristics, including colonial morphology (appearance of small sized colonies of 1.13 mm),CV binding (appearance of dark violet colony) L or low calcium medium (appearance of pin point colonies 0.36 mm), CR uptake (appearance of red pin point colonies). The mouse virulence test of

YEP+ isolates was positive for all examined samples,also they confirmed CR+ colonies of *Y. enterocolitica* by multiplex PCR. Using the chromosomal ail gene (attachment-invasion locus) and *vir F* gene (transcriptional activator for the expression of plasmid encoded outer membrane protein yop.51) from the virulence plasmid. The primer pairs were used (5'-TCA TGG CAG AAC AGC AGTGAG-3' and 5'-ACT CATCTT ACC ATT AAG AAG-3') for detection of the *vir F* gene (430- to 1,020-nucleotide region) amplified a 591-bp product from the virulence plasmid.The authors suggested that the irgasan suppress the growth of pure cultures YEP+ when addaed at the onest of growth and didn't when added after the lag phase, thus they eliminated it from initial enrichment medium. It was found that addition of igrasan at 24h have the best recovery of YEP+ colonies. This timing may reduce the inhibitory effect of the antibiotic. They also added, both CIN and MacConkey's agar were used for presumptive isolation YEP+ strains.CIN agar didn't prove to be effective in isolating colonies that were eventually shown to be YEP+ strain due to antibiotic content of CIN agar may diminish the viability of YEP+ strains. On the other hand, MacConkey's agar gave consistently higher number of presumptive which were eventually shown to be YEP+ strains. 10% of presumptive *Y. enterocolitica* from MacConkey's agar was found to comprise YEP+ strains.

In the present work, although the examined virulence characteristics, the low number of *Y. enterocolitica* isolates (3 out of 10) possessed the *virF* gene were obtained. This may be related to using of irgasan which was added to the CIN medium at initial step of isolation of strains from examined samples and not after the lag phase as described

by ***Bhaduri et al., (1997)*** and this may lead to lose of plasmid. Moreover, culturing at 37°C and prolonged storage of *Y. enterocolitica* can result in loss of the virulence plasmid or these isolates may lost its virulence plasmid during boiling for preparation of DNA for PCR as mentioned by ***Kaneko et al., (1995).***

In Egypt, ***Gad El-Said et al., (1996)*** examined blood samples from apparently healthy cattle, buffaloes and sheep for agglutination. 43% reacted strongly for antibodies of serovars O: 3 and/ or O: 9. 41% were positive for *Y. enterocolitica* after elimination of 8 sera samples which gave cross reactivity with B.abortus. Antibody prevalence was the highest in buffaloes and sheep (46% for each), while cattle 37% were positive. That's in agreement to great extent with our above results. Tha same results were observed by ***Hurvell and Lindberg (1973), Mittal and Tizard (1981) and Chen et al., (1983).***

Our obtained results showed that PCR was the most accurate rapid and a simple assay for detection of *Y. enterocolitica* and save time consumed for diagnosis, these findings were agree with ***Zheng et al., (2008), Lambertz et al., (2008) and Zheng et al.,(2007)*** who reported that the detection of *Y. enterocolitica* is higher in PCR than culture method.

Concerning sensitivity test of the isolated *Y. enterocolitica* strains, reveal that amikacin and Gentamycin were the most effective antibiotics against all tested strains followed by cefotaxime and ofloxacin. These findings were in agreement with ***Baumgartnera et al., (2007) and Zheng et al., (2008)*** who proved that amikacin, gentamycin and cefotaxime were highly effective against *Y. enterocolitica* isolated followed by ofloxacin.

REFERENCES

- ***Adesiyun A.A.; Kaminjolo J.S. and Loregnard R. (1992) :*** Frequency of isolation of *Y. enterocolitica* from livestock in Trinidad. Vet. Rec., 131 (22):516.
- ***Ahmed A.O.(1998):*** A note on relationship between *Yersinia* groups and *Brucella* groups of microorganisms. M.V.Sc. thesis (Microbiology), Faculty of Veterinary Medicine, Cairo University.
- ***Ahmed A.O (2003):*** The role of *Y.enterocolitica* in enteric diseases of cattle, sheep and goats. Ph.D.Thesis (Microbiology). Faculty of Veterinary Medicine, Cairo University.
- ***Ahovonen, P. (1972):*** Human yersiniosis in finland.I.Bacteriology and serology. Ann, Clin. Res., 4:30-38.
- ***Ahovonen P. and Sievers K.C. (1969):*** *Yersinia enterocolitica* infection associated with Brucella agglutinins. Clinical features of 24 patients. Acta Med.Scad. 185: 121-125.
- ***Ahovonen P.; Thal E. and Vasenius H.(1973):*** Occurrence of *Y. enterocolitica* in animal in Finland and Sweden. Microbiol. Immunol., 2: 135-136.
- ***Alonso J.M.; Bercovier H.; Servan J and Mollaret H.H. (1979):*** Contribution to the study of the ecology of *Y. enterocolitica* in France. Cont.Microbiol., Immunol. 5: 132-143.
- ***Bailey W.R. and Scott E.G. (1986):*** Diagnostic Microbiology. 7 th ed. C.V. Mosby Co.USA.

- ***Baumgartnera A.; Kuffer M.; Suter D.; Gemmi T. (2007):*** Antimicrobial resistance of *Y. enterocolitica*. Int. J. Food Microbiol., 115 (1): 110-114.

- ***Bercovier H. and Mollaret H.H. (1984):*** Genus XIV. *Yersinia vanloghem*. pp. 498-506. In Kreig N.R. and Halt J.G.(eds.) Bergey's Manual of Systemic Bacteriology, Vol. 1 Williams and Wilkins, Baltimore.

- ***Bercovier H.; Brenner D.J.; Ursing J.; Steigervvalt A. G.; Fanning G.R.; Alonso J.M.; Carter G.P. and Mollaret H.H. (1980):*** Characterization of *Y. enterocolitica* sensu stricto. Curr.Microbiol., 4: 201-206.

- ***Bhaduri S.; Cottrell B. and Allan R.R. (1997):*** Use of a single procedure for selective enrichment, isolation and identification of Plasmid-Bearing Virulent *Y. enterocolitica* of various serotypes from pork samples. Appl. Environ.Microbiol., 63 (5): 1657-1660.

- ***Bhaduri S.; Turner- Jones C. and Lachica R.V. (1991):*** Convenient aearose medium for the simulataneous determination of low calcium response and Congo red binding by virulent strains of *Y. enterocolitica.* J. Clin. Microbiol., 29: 2341-2344.

- ***Bin Kun H.; Sheng D.E.; Hong B.O.; Shixian G.Z. and Slee K.J. (1994):*** Yersiniosis in sheep due to *Y. enterocolitica*. Br.Vet. J., 150: 473-475.

- ***Bottone E.J. (1997):*** *Y. enterocolitica* overview and epidemiologic correlates. Microbes and Infection, 1: 323-333.

- ***Brenner D.J.; Hickman F.W.; Asbury M.A. and Steigerwalt A.G. (1977):*** Taxonomic and Nomenclature changes in *Enterobacteriaceae*. HEW Publication no. (CDC) 78-8356. US. Department of Health, Education and Welfare, Atlanta Ga.

- ***Brewer R.A. and Corbel M.J. (1983):*** Characterization of *Y. enterocolitica* strains isolated from cattle, sheep and pig in the United Kingdom. J.Hyg. 90 (3): 425-433.

- ***Chen M.G.; Zeng G.J.; Sun Y.Q.; Xia Q.L.; Zhang B.H. and Zhong J. (1983):*** Survey of antibody levels against *Y. enterocolitica* infection in pigs. Chinese J.Vet. Med., 9(12): 12-14.

- ***Christensen S.G. (1980):*** *Y. enterocolitica* in Danish pigs. J. Appl. Bacteriol. 48: 377-382.

- ***Corbel M.J.; Brewer R.A. and Hunter D. (1990):*** Characterization of *Y. enterocolitica* associated with ovine abortion. Vet.Rec.127: 526-527.

- ***Cornells G.R. (1994):*** *Yersinia* pathogenicity factors. Curr.Trop. Microbiol, Immunol.192. 243- 263.

- ***Cruickshank R.; Dugid J.P.; Marmion B.P. and Swain R.H.A. (1975):*** Medical Microbiology 12th ed. Churchill livingstone Edinburgh, London and New York.

- ***Davey G.M.; Bruce J. and Drysdale E.M. (1983):*** Isolation of *Y. enterocolitica* and related species from the feces of cows. J.Appl.Bact.55: 439-443.

- ***Delor J. and Cornell's G. (1992):*** Role of YST toxin in experimental infection of young rabbits. Infect. Immun.10: 4269-4277.

- ***Delor L.; Kaeckenbeek A.; Wauters G. and Cornelis G.R. (1990):*** Nucleotide sequence of YST. The *Y. enterocolitica* gene encoding the heat stable enterotoxin, and prevalence of the gene among pathogenic and non pathogenic *Yersinia*. Infect. Immun.58:2983-2988.

- ***Falcao J.P.; Brocchi M.; Proneaca- Modena J.L.; Acrani G.O.; Corea E.F. and Falcao D.P. (2004):*** Virulence characteristics and epidemiology of *Y. enterocolitica* and *Yersinia* other than *Y. pseudotuberculosis* and *Y. pestis* isolated from water and sewage. J.Appl. Microbiol., 96:1230-1236.

- ***Fantasia M.; Mingrone M.G.; Marini A.; Boscato U. and Crolti D. (1993):*** Characterization of *Yersinia* species isolated from cattle and pig farms. Vet. Rec, 22: 532-534.

- ***Farmer J.J.; Carter G.p.; Miller V.L.; Falkow S. and Wachsmuth I.K. (1992):*** Pyrazinamidase, CRMOX agar, Salicin fermentation-Esculin hydrolysis and D-xylose fermentation for identifying pathogenic serotypes of *Y. enterocolitica*. J. Clin. Microbiol., 30(10): 2589-2594.

- ***Feng P. and Weagant S.D. (1994):*** *Yersinia* In Food Borne Disease Handbook, Vol. 1, ed. Hui, Y.H., Gorham, J.R., Man-ell, K.D. and Oliver, D.O. pp 427-460. New York: Marcel Dekker.

- ***Finegold S. and Martin W. (1982):*** Diagnosis Microbiology. 6th Ed., The C.V. Mosby Co., St. Louis, Toranto, London.

- ***Filetici E.; Anastasio M.P.; Pourshabon M. and Fantasia M. (2000):*** Genotypic and phenotypic characteristics of *Yersinia* spp. Isolates from food and man. Food Microbiol., 17:261-267.

- ***Fredriksson-Ahomaa B.j.; Hielm S. and Korkeala H. (1999):*** Prevalence and characterization of pathogenic *Y. enterocolitica* in pig tonsils from different slaughter houses. J. food Prot., 62(2): 123-127.

- ***Fukushima H. and Gomyoda M. (1986):*** Growth of *Y. tuberculosis* and *Y. enterocolitica* biotype 3B serotype O3 inhibited on cefsulodin-Irgasan-novobiocin agar. J.Clin.Microbiol., 24(1) 116-120.

- ***Fukushima H.; Hoshina K. and Gomyoda M. ((1993):*** Selective isolation of pathogenic *Y. enterocolitica* from pork using Hela cell lines. In Proceedings, II The International Symposium of The World Association of Veterinary Food Hygienists, 24-29 October 1993, Bangkok, Thialand (WAVFH) 509-511.

- ***Gad El-Said W.A.; El-Danaf N.A.; Tanios A.I.; Shaaban A.I. and Rashed M.E. (1996):*** Seroprevalence of *Y. enterocolitica* O:3 and/or O:9 in animals in Egypt with reference to its serological cross-reactions with *Br. abortus*. Vet. Med.J. (3): 629-635.

- ***Gemski P.; Lazere J.R. and Casey T. (1980):*** Plasmid associated with pathogenicity and calcium dependency of *Y. enterocolitica*. Infect.Immun. 27: 682-685.

- ***Greenwood M.H. and Hooper W.L. (1988):*** The use of alkalinity and incubation at 9 C for improved recovery of *Yersinia* spp from feces.Epid. Inf., 101 (1): 53-58.

- ***Hoorfar J. and Holmvig B.F. (1999):*** Evaluation of culture methods for rapid screening of swine fecal samples for *Y. enterocolitica* O:3/ biotype 4. J. Vet. Med.B. 46: 189-198.

- ***Hurvell B. and Lindberg A.A. (1973):*** Serological cross reactions between different immunological studies on phenol water extracted lipopolysaccharides from *Br. abortus* and *Y. enterocolitica* type IX. Acta path. Microbiol, Scand., 81:113-119.
- ***Hussein H.M.; Fenwick S.C. and Lumsden J.S. (2001):*** A rapid and sensitive method for the detection of *Y. enterocolitica* strains from clinical samples. J.Appl. Microbiol., 33 (6): 445-449.
- ***Isberg R.R. (1990):*** Pathways for the penetration of enteroinvasive *Yersinia* into mammalian cells. Mol.Biol. Med., 7:73-82.
- ***Kandolo K. and Wauters G. (1985):*** Pyrazinamidase activity in *Y. enterocolitica* and related organisms. J. Clin.Microbiol., 21: 980-982.
- ***Kaneko S.; Ishizaki N. and Kokubo Y. (1995):*** Detection of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* from pork using Polymerase chain reaction. Contrib. Microbiol., Immunol. 13: 153-155.
- ***Kapperud G.(1991):*** *Y. enterocolitica* in food hygiene. Int.J.Food Microbiol., 12:53-66.
- ***Kay B.A.; Wachsmuth P.I.K.; Gemski P.; Feely J.C.; Quan T.J. and Brenner D.J. (1983):***Virulence and phenotype characterization of *Y. enterocolitica* isolated from humans in the United State. J.Clin.Microbiol., 17:128-138.
- ***Knapp W. and Thai E. (1973):*** Differentiation of *Y. enterocolitica* by biochemical reactions. Cont. Microbiol., Immunol.2: 10-16.
- ***Kocagoz T.; Yilmaz E.; Ozkara S.; Kocagoz S.; Hayran M.; Sachedeva M. and Chambevs H.F. (1993):*** Detection of *Mycobacterium tuberculosis* in sputum samples by polymerase chain reaction using a simplified procedure. J. Clin. Microbiol., 31(6): 1435-1438.

- ***Kwaga J.K. and Iverseng J.O. (1991):*** Laboratory investigation of virulence among strains of *Y. enterocolitica* and related species from pigs and pork products. Cand.J.Microbiol., 38: 92-97.

- ***Lambertz S.T.; Nilsson C. and Lindblad N. (2008):*** Real time PCR method for detection of pathogenic *Y. enterocolitica.* Appl. Environ.Microbiol., 74 (19): 6060-6067.

- ***MacFaddin J.F. (1976):*** Biochemical Tests for identification of Medical Bacteria. 1st Ed., Williams and Wilkins, Baltimore, USA.

- ***Miller V.I. And Falkow S. (1988):*** Evidence for two genetic loci of *Y. enterocolitica* that can promote invasion of epithelial cells. Infect. Immun. 56: 1242-1248.

- ***Mittal K. R. and Tizard I.R. (1981):*** Serological cross- reaction between *Br. abortus* and *Y. enterocolitica* O:9 serological response. Am. J.Vet. Res., 41 (10): 1607- 1610.

- ***Morris G.K. and Feeley J.C. (1976):*** *Y. enterocolitica* : A review of its role in food hygiene. Bull. WHO, 54: 79-85.

- ***Nakajima H.; Inoue M., Mori L.; Itoh K.I. and Watanabe H. (1992):*** Detection and Identification of *Y. pseudotuberculosis* and pathogenic *Y. enterocolitica* by an improved PCR method. J. Clin.Microbiol., 30: 2484-2486.

- ***Nilehn B. (1969a):*** Studies on *Y. enterocolitica* with special references to bacterial diagnosis and occurrence in human acute enteric disease. 1- Characterization of *Y. enterocolitica* strains of human origin and their relation to strain of various animal origin. Acta. Path. Microbiol., Scand. Suppl. 206: 1-48.

- ***Nilehn B. (1969b):*** Studies on *Y. enterocolitica* growth on various solid media at 37C and 25C. Acta. Path. Microbiol., Scand. 77: 685-697.

- ***Okoroafor A.A.; Adesiyun A.A. and Agobnlahor D.E. (1988):*** Prevalence and characteristics of *Y. enterocolitica* strains isolated from pigs in Jos, Nigeria. Br.Vet.J.144:131-138.

- ***Pai C.H. and Mors V. (1978):*** Production of enterotoxin by *Y. enterocolitica.* Infect. Immunol., 19: 908-911.

- ***Pedersen K.B. (1979):*** Occurrence of *Y. enterocolitica* in the throat of swine. Contrib. Microbiol., Immunol. 5: 253-256.

- ***Pianetti A.; Bruscolini F.; Baffone W.; Brandi G.; Salvagglo L.; Biffi M.R. and Albavo A. (1990):*** *Y. enterocolitica* and related species isolated in the Pesaro and Urbino area (Italy) from 1981 to 1986. J. Appl. Bact., 68: 133-137.

- ***Prpic J.K.; Robins-Browne R.M. and Davey R.B. (1983):*** Differentiation between virulent and avirulent *Y. enterocolitica* isolated by using congo red agar. J.Clin. Microbiol., 18(3): 468-490.

- ***Riley G. and Toma S. (1989):*** Detection of pathogenic *Y. enterocolitica* by using Congo Red Magnesium Oxalate agar medium. J. Clin.Microbiol., 27(1): 213-214.

- ***Robins-Browne R.M.; Takeda T.; Fasano A.; Marie A.B.; Dohi S.; Kasuga H.; Fong G.; Prado V.; Guerrant R.L. and Morris, G. (1993):*** Assessment of enterotoxin production by *Y. enterocolitica* and identification of a novel heat stable enterotoxin produced by a noninvasive *Y. enterocolitica* strain isolated from clinical material. Infect. Immun.61 (2):764-767.

- ***Schiemann D.A. (1979):*** Synthesis of selective agar medium for Y. *enterocolitica.* Cand. J. Microbiol., 25: 1298- 1304.

- ***Schiemann D.A. and Devenish J. A. (1982):*** Relationship of Hella cell infectivity to biochemical, serological and virulence characteristics of *Y. enterocolitica*. Infect. Immun., 35(2): 497-506.

- ***Staak C.; Draeger A.; Bahn P. and Nockler K. (2000):*** Contribution to the differentiation of cross-reacting antibodies in brucellosis serology. 1- Reaction with various *Yersinia* serotypes and antibody avidity. Berl. Munch. Tierarztl. Wochenschr, 113(10): 361-367.

- ***Tanios A.I. (1994):*** Carnage of *Y. enterocolitica*: A prospective study of bacteriological and serological features. Ph. D. Thesis (Microbiology), Faculty of Vet. Medicine, Cairo University.

- ***Tauxe R.V.; Vandepitte J.; Wauter G.; Martin S.M.; Goossens V.; De Mol P.; Van Noyen R.; and Thieis G (1987):*** *Y. enterocolitica* infection and pork: the missing link. Lancet, I(8542): 1129-1132.

- ***Thisted-Lambertz S.; Ballagi-Pordany A.; Nilson A.; Norberg P. and Danielsson-Tham M.L. (1996):*** A comparison between a PCR method and a conventional culture method for detecting pathogenic *Y. enterocolitica* in food. J.Appl. Microbiol., 81:303-308.

- ***Toora S.; Singh G.; Tiwari R.P. and Singh G. (1994):*** Drug resistance and lecithinase activity of *Y. enterocolitica* isolated from buffaloes milk . Int. J. Food Microbiol., 9: 167-171.

- ***Winblad S. (1967):*** Studies on serological typing of *Y. enterocolitica*. Acta Path. Microbiol., Scand., Suppl. 187: 115. Cited in Vet. Bull., 51(7): 501-505.

- ***Winblad S.; Nilehn B. and Sternby N.H. (1966):*** *Y. enterocolitica, Pasteurella X* in human enteric infections. Br.Med.J., 2: 1363-1366.

- ***Wren B.W. and Tabaqchali S. (1990):*** Detection of pathogenic *Y. enterocolitica* by PCR. Lancet 336: 693.

- ***Zamora J.; Reinhardt G.; Polette M.; Macias P. and English J. (1997):*** Isolation of *Y. enterocolitica* and *Y. kristensenii* from ovine feces. Arc. Med. Vet. 29 (2): 301-305.

- ***Zheng H.; Sun Y.; Lin S. and Mao Z. (2008):*** *Y. enterocolitica* infection in diarrheae patients. Eur.J. Clin. Microbiol. Infect. Dis.,27(8): 741-752.

- ***Zheng H.; Wang J.; Sun Y. and Jiang B. (2007):*** Clinical isolation and characterization of *Y. enterocolitica* in China. Digestion, 75 (4): 199-204.

- ***Zheng X.B. (1987):*** Isolation of *Y. enterocolitica* from feces of diarrhoeic swine. J. Appl. Bact. 62 (6): 521- 525.

- ***Zheng X.B. and Xie C. (1996):*** Note: Isolation, characterization and epidemiology of Y. *enterocolitica* from humans and animals. J. Appl. Bact. 81: 681-684.