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STUDIES ON VIRULENCE FACTORS OF *YERSINIA ENTEROCOLITICA* ISOLATED FROM DIARRHOEIC AND APPARENTLY HEALTHY COW AND BUFFALO CALVES

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SUMMARY

In the present study investigation of virulence factors of Yersinia enterocolitica was done .From 35 tested *Yersinia enterocolitica* isolates belonged to 5 serobiovars (O:8/1A, O:8/1B, O:5/1A, O:9/2;O:3/1A) isolated from diarrhoic and apparently healthy cow and buffalo calves, 28 isolates (80.00%)were positive for Congo red test and able to survive in normal calf serum.Twenty six isolates (74.29%) were able to bind with crystal violet, while 34 and 32 isolates were haemagglutination positive using bovine and guinea pig RBCs with an incidence of 97.10% and 91.43%, respectively . Twenty isolates (57.14%) were positive for autoagglutination , pyrazinamidase production and calcium dependency tests , while 24 isolates (68.57%) were able to grow in normal calf serum. Concerning HEP-2 cells penetration, enterotoxin production and Sereny test, 31, 23 and 16 isolates gave positive results with incidence of 88.57% , 56.71% and 45.71% , respectively.

INTRODUCTION

Yersinia enterocolitica is a well-established zoonotic pathogen for human and animals, causing, gastroenteritis, terminal ileitis, mesenteric adenitis and septicemia **(Bottone, 1977)**. The source of most infections is unknown but outbreaks have been attributed to the consumption of contaminated food or water. Domestic animals have been widely suspected of acting as a source of pathogenic *Yersinia enterocolitica* in several countries **(Wauters, 1979)**.

The primary step in intestinal Yersinia enterocolitica infection is the adherence of bacteria onto the gut epithelium. After adherence, colonization and multiplication of *Yersinia enterocolitica* in the intestines follow. As this takes place at body temperature, the circumstances may favour the bacteria to invade the tissues.

A wide range of experimental procedures has been applied for the determination of virulence among strains of *Yersinia enterocolitica.*

Bahduri et al. (1987) indicated that binding of crystal violet to virulent strains allows the rapid and simple differentiation of virulent and avirulent strains of *Yersinia enterocolitica.*

The use of tissue culture cell penetration as an experimental model of in-vivo invasive disease. It is consistent with the usual illness produced by

Yersinia enterocolitica **(Kay et al., 1983).**

Accordingly **Grant et al . (1998)** found that clinical isolates of *Yersinia enterocolitica* invaded HEP-2 cells to a significantly greater extent than non-clinical strains.

The suckling mouse assay is a reproducible assay system for the detection of heat stable enterotoxin .*Yersinia enterocolitica* heat-stable enterotoxin has a similar mode of action and is structurally and immunologically related to heat-stable enteotoxin of E .coli, which is responsible for profuse watery diarrhoea in humans and animals **(Walker and Gilmour,1990).**

Generally, Yersinia enterocolitica virulence is a complex and *multifactorial* phenomenon, no individual test was found as a single reliable measure of virulence.

The aim of the present work was to investigate the virulence attributes of *Yersinia enterocolitica* by performing the following tests on field isolates; congo red dye absorption assay, crystal violet binding assay, calcium dependency test, serum sensitivity test heamagglutination test, auto-agglutination test, pyrazinaminidase test , invasiveness in HEP-2 cells, enterotoxin production and Sereny test.

MATERIAL AND METHODS

A total of 35 *Yersinia enterocolitica* strains recovered from apparently healthy and diarrhoeic cow and buffalo calves by Refai (Department of Microbiology. Faculty of Vet. Med. Cairo Univeristy) were tested in the present work for their virulence factors using the following tests:

1-Congo red absorption assay **(Prpic et al.,1993)**.

2-Crystal violet binding assay **(Bahduri et al .,1987)**.

3-.Haemagglutination test **(Kapperud and Lassen,1983),** in the presence or absence of D-mannose

4-Autoaglutination test **(Laird and Cavanaugh, 1980).**

5-Calcium dependency test **(Okoroafor et al., 1988)**.

6-Pyrazinaminidase test **(Kandolo and Wauters, 1989)**.

7-Serum sensitivity test **(Pia and De-Stephano, 1982)**.

8-Invasion of HEP-2cells **(Kay et al., 1983)**.

9-Enterotoxin production **(De-Guzman et al., 1991)** using the infant mouse assay.

10-The ability of Yersinia enterocolitica isolates to cause conjunctivitis in the guinea pig eye model "Sereny test" **(Aulisio et al.,1983)**.

RESULTS AND DISCUSSION

1-Congo red (CR) binding activity

In this study, all *Yersinia enterocolitica* isolates obtained from diarrhoeic cow and buffalo calves bounded Congo red dye and developed red

colonies (CR+). Meanwhile, two isolates (22.2%) recovered from apparently healthy calves were only positive for Congo red binding activity (Table1)

These findings suggest that there is a positive association between the isolation of CR+, *Yersinia enterocolitica* and disease status. This assumption is confirmed by **Styles and Flammer (1991)**.

In this aspect, **Prpic et al. (1985)** added that Congo red was the most reliable predictor of pathogenicity for animals.Howeve , a significant number of avirulent food borne isolates of Yersinia enterocolitica were Congo red positive.

2. Crystal violet (CV) binding

In this study, out of 26, 24 isolates of Yersinia enterocolitica recovered from diarrhoeic cow and buffalo calves bounded crystal violet with an incidence of 92.30 %. On the other hand, two (22.22) *Yersinia enterocolitica* isolates recovered from apparently healthy calves were positive for crystal violet (Table2).

Mahal et al. (1994) reported that 87.5 % of clinical isolates of *Yersinia enterocolitica* bound crystal violet dye at 37°C but not at 25°C. **Borie et al. (1997)** found that 73.3 % of tested *Yersinia enterocolitica* strains were positive for crystal violet binding assay.

3. Haemagglutination

Examination of the recovered *Yersinia enterocolitica* isolates for haemagglutination(HA) and mannose resistant haemagglutination (MRHA) was done. As shown in Tables (3), among the isolates recovered from diarrhoeic cow calves 100 % and 94.44 % were positive for haemagglutination using bovine and guinea pig RBCs , respectively. While all isolates (100 %) recovered from diarrhoic buffalo calves were positive for haemagglutination using bovine and guinea pig RBCs. It is interest to note that all isolates were negative for haemagglutination using chicken and human RBCs.

These results agree with that of **Kapperud et al. (1987),** who used guinea pig RBCs as indicator for haemagglutination activity and **Aleksic (1995),** who suggested that homologous RBCs were the most appropriate for testing haemagglutination activity.

4. Autoagglutination and calcium dependency

In the present study, all autoagglutinated isolates (20/35) were Ca- dependent with an incidence of 57.14 % (Tables 4 and 5). Eighteen out of 26 isolates of Yersinia enterocolitica recovered from diarrhoeic calves were autoagglutinable and Ca dependent with an incidence of 69.23 % . On the other hand, two out of 9 isolates of *Yersinia enterocolitica* recovered from apparently healthy calves were positive for autoagglutination and Ca-dependency with an incidence of 22.22 % each. It is clear that, 13 out 21 (61.90 %) and 7 out of 14 (50.00 %) isolates from cow calves and buffalo

calves , respectively were autoagglutinable and Ca-dependent. One out of 3 (33.33 %) and 12 out of 18 (66.67 %) isolates, were positive for autoagglutinablity and Ca-dependency were collected from apparently healthy and diarrhoeic cow calves, respectively. Meanwhile, one isolate (16.67 %) and six out of 8 (75.00 %) isolates, which were positive for autoagglutination and Ca-dependent were obtained from apparently healthy and diarrhoeic buffalo calves, respectively.

In this aspect, **Pia and Destephano (1982)**, demonstrated that *Yersinia enterocolitica* strains that grow poorly at 37°C on magnesium oxalate(MOX) agar and autoagglutinate when grow at 37°C in tissue culture media were virulent and strains that have lost the property for calcium dependency and autoagglutination were avirulent.

Moreover, **Laird and Cavanaugh (1980)** reported that the production of diarrhoea was limited to strains which had the ability to autoagglutinate at 35°C in a tissue culture medium. **Prpic et al. (1985)** examined 86 clinical isolates of *Yersinia enterocolitica*, 52 of them were Ca dependent. Also they showed that all virulent strains were ca- dependent, however, not all ca- dependent strains were virulent.

In Egypt,**Gad EL-Said et al. (1997)** found that all *Yersinia enterocolitica* serovars isolated from clinically healthy cows, buffaloes, sheep and pigs were negative for autoagglutination test

5. Pyrazinamidase test

In the present study, (Table 6) revealed that eleven out 21 (52.38 %) and 9 out of 14 (64.29 %) *Yersinia enterocolitica* isolates recovered from cow calves and buffalo calves , respectively were pyrazinamidase positive. Meanwhile, 8 out of 18 (44.44 %) and 3 out of 8 (37.50 %) isolates recovered from diarrhoeic cow calves and diarrhoeic buffalo calves were pyrazinamidase positive. These findings are in contrary to that reported by **Nobel et al. (1987),** who recorded that *Yersinia enterocolitica* biotype 2 whether from human or animal pathogens were pyrazinamidase negative.Moreover, **Farmer et al. (1992)** reported that the pyrazinamidase test is widely used to differentiate potentially pathogenic *Yersinia enterocolitica* isolates from non-pathogenic ones. **Burnens et al.(1996)** found the sensitivity and specificity of pyrazinamidase test was100% to detect pathogenic *Yersinia enterocolitica.*

6. Serum sensitivity

In the present study, of Yersinia enterocolitica isolates recovered from diarrhoeic and apparently healthy calves was tested for the survival in calf serum (Table, 7). Out of 26 isolates of *Yersinia enterocolitica* recovered from diarrhoeic calves, 23 (88.96%) were able to survive in calf serum.Only five *Yersinia enterocolitica* isolates from apparently healthy calves were able to survive in serum (55.56 %). In this aspect, **Gemski et al. (1980)** showed that the susceptibility of the organisms to the bactericidal action of normal serum was related to virulence in *Yersinia enterocolitica.* Virulent strains were able to survive in fresh normal sera, whereas

their avirulent variants were readily killed. **Pia and De-stephano (1982)** observed that virulent strains of *Yersinia enterocolitica* were able to survive in fresh normal sera of humans or rabbits, whereas their avirulent variants were readily killed. at 37°C . On the other hand **Chiesa and Bottone (1983)** found that some Yersinia enterocolitica strains were resistant to serum killing even though these strains did not express other virulence plasmid markers, **Martinez (1989)** mentioned that serum resistance in *Yersinia enterocolitica* was thermoregulated, and its expression correlated well with the presence of virulence plasmid-encoded outer membrane proteins.

7. Determination of invasiveness

As shown in Table (8), 31 Yersinia enterocolitica isolates were able to invade the HEp-2 cells with an incidence of 88.57 %. All isolates of *Yersinia enterocolitica* recovered from diarrhoeic calves were able to invade HEp-2 cells after 3 hours of inoculation while 5 out of 9 (55.55 %) isolates recovered from apparently healthy calves were invasive. It is clear that, 20 out 21 (95.24 %) and 11 out of 14 (78.57 %) recovered from cow calves and buffalo calves, respectively invaded HEp-2 cells

Vesikari et al. (1981), who found a positive correlation among the presence of plasmid in isolates of *Yersinia enterocolitica,* autoagglutination, and adherence to HEp-2 cell cultures, all of these properties were lost by culturing at 37°C in the absence of calcium and they added that strains of *Yersinia enterocolitica* O:3 and O:9 carried of the plasmids showed increased invasiveness in HEp-2 cell culture model, with out invasiveness in guinea pig.

8. Enterotoxigenic activity

It is clear from Table (9) that , 23 out of *Yersinia enterocolitica* isolates (65.71 %) were positive for production of enterotoxin. Out of these isolates 21 out of 26 (80.77 %) and 2 out of 9 (22.22 %) were recovered from diarrhoeic and apparently healthy calves, respectively Forteen out 21 (66.67 %) and 9 out of 14 (64.29 %) recovered from cow calves and buffalo calves , respectively were enterotoxigenic. The incidences of enterotoxigenic isolates in diarrhoic cow and buffalo calves were 77.78% and 87.51%, respectively,while in apparently healthy , the corresponding figures were 0.0% and 33.33%, respectively.

These results are consistent with the findings of **Mors and pai (1980); Kay et al. (1983)** and **Kwaga and Iversen (1992).** Although, **Schiemann and Devenish (1982)** reported that there was no association between virulence and the ability to produce enterotoxin measured by the infant mouse assay, **Swaminathan et al. (1982)** found that the illness may occur as a result of consumption of performed *Yersinia enterocolitica* enterotoxin,

In this aspect, **Gad EL-Said et al. (1997)** examined the virulence of 34 strains of *Yersinia enterocolitica* belonging to 6 serovars isolated from clinically healthy cows, buffaloes, sheep and pigs in Egypt. They found that 21 isolates (81.8%) were positive for heat stable enterotoxin., while **Grant et al. (1998)** found thirty three strains (29.7 %) produced an enterotoxin that was reactive in infant mice. Recently Singh and Virdi (2004) found that 77.7 % of clinical isolates of Yersinia enterocolitica showed enterotoxigenicity in infant mice.

Table (1): Congo red reaction among *Yersinia enterocolitica* recovered from calves

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates tested	Positive for Congo red test		No. of isolates tested	Positive for Congo red test		No. of isolates tested	Positive for Congo red test	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	1	33.33	6	1	16.67	9	2	22.22
Diarrhoeic calves	18	18	100.00	8	8	100.00	26	26	100.00
Total	21	19	90.48	14	9	64.29	35	28	80.00

% calculated according to number of isolates.

Table (2): Crystal violet binding assay of *Yersinia enterocolitica* recovered from examined calves.

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for crystal violet test		No. of isolates	Positive for crystal violet test		No. of isolates	Positive for crystal violet test	
		No	%		No	%		No.	%
Apparently healthy calves	3	1	33.33	6	1	16.67	9	2	22.22
Diarrhoeic calves	18	16	88.89	8	8	100.00	26	24	92.30
Total	21	17	80.95	14	9	64.29	35	26	74.29

% calculated according to number of isolates.

Table(3): Heamagglutination of different erythrocytes by *Yersinia enterocolitica* isolates recovered from examined calves.

by *Yersinia enterocolitica* isolates recovered from examined calves RBCs	Types ofHA	Source of *Yersinia enterocolitica* isolates												Total (35)	
		Cow calves						Buffalo calves							
		Apparently healthy (3)		Diarrhoeic (18)		Total (21)		Apparently healthy (6)		Diarrhoeic (8)		Total (14)			
		No.	%	No.	%	No.	%	No	%	No.	%	No.	%	No.	%
Bovine	MRHA	1	33.33	14	77.78	15	71.43	2	33.33	7	87.50	9	64.29	24	68.57
	MSHA	1	33.33	4	22.22	5	23.80	4	66.67	1	12.50	5	35.71	10	28.57
	HA	2	66.67	18	100.0	20	95.23	6	100.0	8	100.0	14	100.0	34	97.14
Guinea pig	MRHA	1	33.33	12	66.67	13	61.90	2	33.33	7	87.50	9	64.29	22	62.86
	MSHA	2	66.67	5	27.78	7	33.33	2	33.33	1	12.50	3	21.43	10	28.57
	HA	3	100.0	17	94.44	20	95.24	4	66.67	8	100.0	12	85.71	32	91.43
Chicken	MRHA	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	MSHA	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	HA	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Human	MRHA	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	MSHA	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	HA	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00

HA: Positive haemagglutination activity. MRHA: Mannose resistant haemagglutination. MSHA: Mannose sensitive haemagglutination .

Table (4): Autoagglutination of *Yersinia enterocolitica* recovered from calves.

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for autoagglutination		No. of isolates	Positive for autoagglutination		No. of isolates	Positive for autoagglutination	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	1	33.33	6	1	16.67	9	2	22.22
Diarrhoeic calves	18	12	66.67	8	6	75.00	26	18	69.23
Total	21	13	61.90	14	7	50.00	35	20	57.14

% calculated according to number of isolates.

Table (5): Calcium dependency of *Yersinia enterocolitica* recovered from calves

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for calcium dependency		No. of isolates	Positive for calcium dependency		No. of isolates	Positive for calcium dependency	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	1	33.33	6	1	16.67	9	2	22.22
Diarrhoeic calves	18	12	66.67	8	6	75.00	26	18	69.23
Total	21	13	61.90	14	7	50.00	35	20	57.14

% calculated according to number of isolates

Table (6) : Pyrazinamidase activity of *Yersinia enterocolitica* recovered from calves.

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for Pyrazinamidase activity		No. of isolates	Positive for Pyrazinamidase activity		No. of isolates	Positive for Pyrazinamidase activity	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	3	100.00	6	6	100.00	9	9	100.00
Diarrhoeic calves	18	8	44.44	8	3	37.50	26	11	42.31
Total	21	11	52.38	14	9	64.29	35	20	57.14

% calculated according to number of isolates.

Table (7): Serum sensitivity of *Yersinia enterocolitica* recovered from calves.

Animal species / Health condition	Cow calves isolates					Buffalo calves isolates					Total			
	No. of isolates	Survival in serum		Growth in serum		No. of isolates	Survival in serum		Growth in serum		No. of isolates	Survival in serum		Growth in serum	
		No	%	No	%		No.	%	No.	%		No.	%	No.	%
Apparently healthy calves	3	1	33.33	1	33.33	6	4	66.67	3	50.00	9	5	55.56	4	44.44
Diarrhoeic calves	18	16	88.89	14	77.78	8	7	87.50	6	75.00	26	23	88.46	20	76.92
Total	21	17	80.95	15	71.43	14	11	78.57	9	64.29	35	28	80.00	24	68.57

% calculated according to number of isolates.

Table (8): Invasiveness property of examined *Yersinia enterocolitica* isolates using HEp-2 cells.

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for invasiveness		No. of isolates	Positive for invasiveness		No. of isolates	Positive for invasiveness	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	2	66.67	6	3	50.00	9	5	55.55
Diarrhoeic calves	18	18	100.00	8	8	100.00	26	26	100.00
Total	21	20	95.24	14	11	78.57	35	31	88.57

% calculated according to number of isolates

Table (9) : Enterotoxigenicity of examined *Yersinia enterocolitica* isolates using infant mice assay.

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for enterotoxin		No. of isolates	Positive for enterotoxin		No. of isolates	Positive for enterotoxin	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	0	0.00	6	2	33.33	9	2	22.22
Diarrhoeic calves	18	14	77.78	8	7	87.51	26	21	80.77
Total	21	14	66.67	14	9	64.29	35	23	65.71

% calculated according to number of isolates

Table (10) : Sereny test of *Yersinia enterocolitica* isolates recovered from examined calves.

Animal species / Health condition	Cow calves isolates			Buffalo calves isolates			Total		
	No. of isolates	Positive for Sereny test		No. of isolates	Positive for Sereny test		No. of isolates	Positive for Sereny test	
		No.	%		No.	%		No.	%
Apparently healthy calves	3	0	0.00	6	0	0.00	9	0	0.00
Diarrhoeic calves	18	11	61.11	8	5	62.50	26	16	61.54
Total	21	11	52.38	14	5	35.71	35	16	45.71

% calculated according to number of isolates.

Table (11) : Virulence and pathogenicity tests of isolated *Yersinia enterocolitica* serobiovars.

Serobiovars	Number of isolates	Congo red activity		Crystal violet test		Haemagglutinin production				Autoagglutination		Calcium dependency		Pyrazinamidase		Serum sensitivity				HEp-2 cells invasion		Enterotoxin production		Sereny test	
						Bovine RBCs		G. pig RBCs								Survival		Growth							
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
O:3/1A	5	4	80.00	2	40.00	5	100.00	3	60.00	2	40.00	2	40.00	5	100.00	2	40.00	0	0.00	5	100.00	2	40.00	1	20.00
O:5/1A	7	3	42.86	3	42.86	6	85.70	7	100.00	1	14.29	1	14.29	7	100.00	3	42.86	2	28.57	3	42.86	3	42.86	0	0.00
O:8/1A	7	5	71.43	6	85.71	7	100.00	6	85.70	1	14.29	1	14.29	7	100.00	7	100.00	6	85.71	7	100.00	3	42.86	2	28.57
O:8/1B	9	9	100.00	9	100.00	9	100.00	9	100.00	9	100.00	9	100.00	0	0.00	9	100.00	9	100.00	9	100.00	9	100.00	9	100.00
O:9/2	7	7	100.00	6	85.71	7	100.00	7	100.00	7	100.00	7	100.00	1	14.29	7	100.00	7	100.00	7	100.00	6	85.71	4	57.14
Total (35)	35	28	80.00	26	71.29	34	97.10	32	91.43	20	57.14	20	57.14	20	57.14	28	80.00	24	68.57	31	88.57	23	65.71	16	45.71

% calculated according to number of isolates.

9. Pathogenicity to guinea pigs (Sereny test)

Results of testing the isolated Yersinia enterocolitica for the ability to produce conjunctivitis are illustrated in Table (10). From 35 tested strains, 16 Yersinia enterocolitica strains (45.71 %) were able to produce guinea pig conjunctivitis. Sixteen out of 26 (61.54 %) isolates of *Yersinia enterocolitica* recovered from diarrhoeic calves were able to produce guinea pig conjunctivitis. On the other hand, all *Yersinia enterocolitica* recovered from apparently healthy calves did not produce conjunctivitis in guinea pigs.

These findings agree with that of **Mors and Pia (1980)** ; **Schiemann and Devenish (1982)** and **Miliotis et al. (1990)**. On the other hand, **Gad EL-Said et al. (1997)** found that 1 isolate (2.9%) was positive for guinea pig conjunctivitis test from 34 strains of *Yersinia enterocolitica* belonging to 6 serovars isolated from clinically healthy cows, buffaloes, sheep and pigs in Egypt.

The collective data presented in Table (11) suggested that no single current assay correlated with virulence of *Yersinia enterocolitica,* This assumption is confirmed by **Kay et al. (1983)**. The data obtained indicated that most isolates from diarrhoeic calves were positive in one or more virulent tests suggesting that they might have been virulent. However it is unwise - based on data of this work ñ to conclude or relate the results of virulence assays to pathogenicity in animals or humans. This assumption conforms with conclusions drawn in a series of publications that pointed out that pathogenicity differences among Yersinias confirmed the complex nature of virulence in *Yersinia enterocolitica* **(Kay et al. , 1983 and Robins-Browne , 1993)** .

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تم فى هذا البحث إجراء الإختبارات الخاصة بعوامل الضراوة لعدد ٣٥ عترة لميكروب البرسينيا إنتيروكولتيكا المعزولة من عجول البقر والجاموس المصابة بالإسهال والسليمة ظاهرياً . وقد وجد أن ٨٠٪ من هذه العترات إيجابى لإختبار الكونجورد بينما ٧٤,٢٩٪ يمكنها أن ترتبط بالكريستال قيوليث كما أن ٢٧,١٠٪ و ٩١,٤٣٪ إيجابى الإختبار التلزن الدموى بإستخدام كرات الدم الحمراء لخنازير غنياً والأبقار .

أما بإستخدام إختبار التلزن الذاتى وإنزيم بيرازين أميداز والإعتماد على الكالسيوم . فكانت النسبة الإيجابية هى ٥٧,١٤٪ بينما نسبة ٦٨,٥٧٪ من العترات قادرة على النمو فى سيرم العجول ، أما بالنسبة لإختراق الخلايا الطلائية البشرية الأنش . بى. أى و إنتاج السم المعوى وإلتهاب قرنية عين الخنزير العينى فكانت النسبة الإيجابية هى ٨٨,٥٧٪ و ٥٦,٧١٪ و ٥٦,٧١٪ و ٤٥,٧١ على التوالى .