

Page

1. Introduction	1
2. Review of literature	5
2.1. General characters of Yersinia enterocolitica	5
2.2. Isolation and identification of Yersinia enterocolitica	6
2.3. Cold enrichement technique	10
2.4. Biochemical and serotyping characters	11
2.5. Prevalence of Yersinia enterocolitica in calves	15
2.6. Virulence and pathogenicity tests	20
2.6.1. Congo red (CR) dye absorption	20
2.6.2. Crystal violet (CV) binding	21
2.6.3. Haemagglutination (HA)	22
2.6.4. Autoagglutination	23
2.6.5. Calcium dependency	25
2.6.6. Lack of pyrazinamidase enzyme	26
2.6.7. Serum sensitivity	27
2.6.8. Invasiveness in HEp-2 cells	29
2.6.9. Enterotoxin production	30
2.6.10 . Ability to evoke conjunctivitis in guinea pigs	33
2.7. Characterization and biological properties of LPS	35
2.8. Characterization and biological properties of	
outer membrane proteins (OMPs)	39
2.9. Serological examinations of the serum	
using ELISA	42
2.10. Serological cross-reaction	45
3. Material and Methods	50
3.1.	50
Material	00
3.1.1. Samples	50
3.1.2. Media used for isolation of Yersinia	51
enterocolitica	
3.1.3. Media used for biochemical identification	
of the isolates	52

Page

3.1.4. Media used for virulence and	
pathogenicity tests	54
3.1.5. Media used for whole bacterial antigen,	
outer membrane protein (OMP) and	
Lipopolysaccharide (LPS) preparation	57
3.1.6. Reagents and chemicals	57
3.1.7. Stains	63
3.1.8. Sera used	64
3.1.9 . Diagnostic Yersinia antisera	64
3.1.10. Cells used	64
3.1.11. Laboratory animals	64
3.1.12. Reference strains	65
3.1.13. Other materials and apparatus	65
3.2. Methods	66
3.2.1. Collection of samples	66
3.2.2. Primary isolation and purification	66
3.2.3. Identification of the isolates	67
3.2.4. Biotyping of the isolates	69
3.2.5. Serotyping of the isolates	69
3.2.6. Virulence and pathogenicity tests	72
3.2.6.1. Congo Red (CR) binding	
test	72
3.2.6.2. Crystal Violet (CV) binding	
assay	72
3.2.6.3. Haemagglutination (HA) test	
	73
3.2.6.4. Autoagglutination	74
3.2.6.5. Calcium dependency for growth at	
37°C	75
3.2.6.6. Pyrazinamidase activity	76
3.2.6.7. Serum sensitivity test	76
3.2.6.8. Invasion of HEp-2 cells	//
3.2.6.9. Enterotoxin Production	78
3.2.6.10. Sereny test.	79
3.2.1. Preparation of antigens.	80
3.2.1.1. Preparation whole bacterial antigen (W.B.)	80

3.2.7.2. Preparation of outer membrane proteins	80
3.2.7.3. Extraction and purification of LPS	81
3.2.8. Polyacrylamide gel electrophoresis (SDS-	
PAGE)	83

Page

3.2.9. Production of	
antisera	87
3.2.10. Serological examination of the serum	87
4. Results	91
4.1. Prevalence of Yersinia enterocolitica	91
4.2. Comparison between direct plating and	
cold enrichment techniques for isolation of	
Yersinia enterocolitica	93
4.3. Biochemical tests of Yersinia enterocolitica isolates	95
4.4. Biotyping of isolated Yersinia	
enterocolitica	97
4.5. Serovars of isolated Yersinia enterocolitica	100
4.6. Correlation between biovars and serovars of	
isolated Yersinia	
enterocolitica	103
4.7. Virulence and pathogenicity tests	105
4.7.1. Results of Congo Red (CR) binding activity	
among Yersinia enterocolitica isolates recovered	
from examined calves	105
4.7.2. Results of Crystal violet (CV) binding activity	
among Yersinia enterocolitica recovered from	
examined calves	109
4.7.3. Haemagglutination properties of Yersinia	
enterocolitica isolates recovered from	
examined calves	113
4.7.4. Results of autoagglutination of Yersinia	
enterocolitica recovered from examined	
calves	117
4.7.5. Results of Calcium dependency of Yersinia	
enterocolitica recovered from examined	
calves	120
4.7.6. Results of Pyrazinamidase test of Yersinia	
enterocolitica recovered from examined	
calves	123

4.7.7. Results of Serum sensitivity of Yersinia	
enterocolitica recovered from examined calves	126
4.7.8. Determination of invasiveness property of	
Yersinia enterocolitica recovered from	
examined calves using HEp-2 cells	130
4.7.9. Enterotoxigenic activity of Yersinia enterocolitica	
recovered from examined calves	134
4.7.10. Results of Sereny test among Yersinia	
enterocolitica isolates recovered from	
examined calves	137

Page

4.8. Characterization and biological properties of lipopolysaccharide (LPS):	
	143
4.8.1. SDS-PAGE electrophoresis of LPS of <i>Yersinia</i>	4.40
482 SDS-PAGE electrophoresis of outer membrane	143
proteins (OMPs) of <i>Yersinia enterocolitica</i>	
isolates	146
4.9. Detection of antibodies of <i>Yersinia enterocolitica</i>	
calves by ELISA	149
4.9.1. Whole bacterium (WB) antigens	149
4.9.2. Lipopolysaccharide (LPS) antigens by ELISA	152
4.9.3. Outer membrane proteins (OMPs) antigen	155
using different antigens	158
4.10. Cross reaction between different antigens	100
of Yersinia enterocolitica and Brucella abortus	
in rabbit antisera	160
5. Discussion	162
6. Summary	193
8. Arabic summary	190

6- SUMMARY

In the present study, 358 faecal samples (230 cow calves and 128 buffalo calves) were collected from animals and subjected to bacteriological examination for the presence of *Yersinia enterocolitica*. The overall prevalence of *Yersinia enterocolitica* was 9.78 %.

Yersinia enterocolitica could be isolated from buffalo calves in a higher rate than cow calves (10.93 % Vs 9.13 %). The organisms could be isolated from apparently healthy and diarrhoeic calves in an incidence of 3.33 Vs 12.86 % and 8.57 % Vs 13.79 % in cow and buffalo calves, respectively.

The percentage of isolation by direct plating was (57.14 %) while that of cold enrichment followed by plating on CIN agar was (82.85 %).

The biotyping of the isolated Yersinia enterocolitica revealed that 19 out of 35 isolates (54.29 %) belonged to Yersinia enterocolitica biovar 1A, 9 isolates (25.71 %) to biovar 1B and 7 isolates (20.00 %) to biovar 2.

Yersinia enterocolitica isolates were serotyped into four different serovars. Most isolates 16/35 (45.71 %) belonged to serovar O:8, while 7 isolates (20.00 %) belonged to O:3 and O:9 each and the remainders 5/35 (14.28 %) belonged to serovar O:3.

The correlation between serovars and biovars of *Yersinia enterocolitica* isolated from examined calves revealed that 5 serobiovars were obtained. The most common serobiovar was O:8/1B (9 isolates; 25.71 %), followed by serobiovar O:5/1A, O:8/1A and O:9/2 (7 isolates each; 20.00 %) and the lowest serobiovar was O:3/1A with an incidence of 14.29 %.

From 35 tested Yersinia enterocolitica isolates belonged to 5 serobiovars, 28 isolates with an incidence of 80.00 % were positive for Congo red test and able to survive in normal calf serum. Twenty six isolates with an incidence of 74.29 % were able to bind with crystal violet, as well as 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 with an incidence of 57.14 % were positive for autoagglutination, pyrazinamidase production and calcium dependency tests, while 24 isolates with an incidence of 68.57 % were able to grow in normal calf serum.

Concerning HEp-2 cells invasion, enterotoxin production and Sereny test, 31, 23 and 16 isolates gave positive results with incidence of 88.57 %, 56.71 % and 45.71, respectively.

The LPS analysis of five Yersinia enterocolitica serobiovars by SDS-PAGE analysis showed that Yersinia enterocolitica LPS contained about four bands after staining with silver nitrate method which ranged from 11.485 kDa to 17.309 kDa. The OMPs analysis of five Yersinia enterocolitica by SDS-PAGE analysis showed that Yersinia enterocolitica OMPs contained about five bands after staining with silver nitrate method which ranged from 33.981 kDa to 71.342 kDa.

The use of indirect ELISA using whole bacterium and LPS antigens revealed that 10.63 % and 8.75 % of serum samples from apparently healthy calves were seropositive to *Yersinia enterocolitica* antibodies, respectively, while 26.77 % and 27.78% serum samples from diarrhoeic calves were seropositive. On the other hand, 6.25 % and 17.7 % serum samples collected from apparently healthy and diarrhoeic calves were seropositive, respectively, by using OMP as coating antigen in indirect ELISA.

In the present study sensitivity and specificity of ELISA using WB, LPS and OMPs extracted from *Yersinia enterocolitica* serobiovars were studied. The sensitivity of WB antigen, LPS antigen and OMPs antigen in detection of *Yersinia enterocolitica* antibodies was 91.43 %, 91.43 % and 97.14 %, respectively. Outer membrane proteins (OMPs) ELISA gave the highest specificity (96.59 %), followed by lipopolysaccharide (LPS) ELISA (88.54 %) and whole bacterium (WB) ELISA (88.24 %).

Strong cross-reactions were observed between Yersinia enterocolitica O:9 and Brucella abortus using WB-ELISA. These results validated the use of OMPs-ELISA as a suitable assay to differentiate clearly between Brucella abortus infections.