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PREVALENCE OF YERSINIA ENTEROCOLITICA IN MILK AND FAECES OF SOME LACTATING ANIMALS AND TYPING OF THE OBTAINED ISOLATES BY PLASMID PROFILE

(With 5 Tables and 1 Figure)

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مدى تواجد ميكروب اليارسينيا انتيروكوليتيكا في لبن وبراز بعض الحيوانات الحلابة وتصنيف العترات المعزولة باستخدام البلازميد بروفيل

إيناس البرنس ، أمل سيد محمد سيد

في الآونة الأخيرة تزايدت حالات النزلات المعوية الناتجة عن تلـــوث الألبـــان بميكـــروب اليارسينيا انتيروكوليتيكا، لذلك فقد أجريت هذه الدراسة لتحديد دور الحيوان كمصـــدر لنقـــل العدوى للإنسان ومعرفة مدى تواجده في لبن وبراز الأبقار والأغنام والماعز. فقد تم جمـــع ١٨٠ عينة من هذه الحيوانات بواقع ٦٠ عينة من كل منها (٣٠ عينة ألبان ، ٣٠ عينة براز) من عدة قرى بمحافظة أسيوط وذلك في الفترة من شهر مايو إلى أغســـطس ٢٠٠١. وقـــد أسفرت النتائج عن تلوث العينات بهذا الميكروب بنسبة ٣,٣٣% في عينات ألبان كـــل مـــن الأبقار والماعز ، بينما لم يتم عزله من لبن الأغنام. هذا بالإضافة إلى عزله من براز كـــل من الأبقار (١٣,٣٣%) ، الأغنام (١٠%) والماعز (١٠%). وتم عزل هذا الميكروب مـــن لبن وبراز حيوان واحد فقط من كل من الأبقار والماعز بنسبة ٣,٣٣%. وقد تم التأكد مـــن ضراوة عترتين من الميكروب والتي تحمل البلازميد بنسبة ٨,٣٣% لكل من ألبان الأبقـــار والماعز ، كما وجد أن ٣ (٢٥%) عترات معزولة من براز الأبقار وعترة واحدة (٨,٣٣%) معزولة من براز الأغنام كانت ضارية وحاملة للبلازميد وتم تصنيفهم إلى biotypes1B, 4. وقد دلت النتائج على أن عـــدد ١ (٨,٣٣%) ، ٢ (١٦,٦٧%) و٣ (٢٥%) عـــترات مـــن ميكروب اليارسينيا انتيروكوليتيكا والمعزولة من براز الأبقار والأغنام والماعز على التوالـــي كانت غير ضارية ولا تحمل البلازميد وصنفت إلى biotypes 1A, 4.وقد تمـــت مناقشـــة الأهمية الصحية والوبائية لميكروب اليارسينيا انتيروكوليتيكا ومصادر التلوث المختلفة ، هـــذا بالإضافة إلى الإجراءات الصحية لرفع جودة الألبان.

SUMMARY

Yersinia enterocolitica gastroenteritis in humans have been recognized with increasing frequency in recent years. One hundred and eighty random samples of milk and faeces of lactating cows, ewes and goats (60 each) constituting 30 milk samples and 30 faecal specimens were aseptically collected from different districts in Assiut governorate in the period from May to August 2001. These samples were examined for *Y. enterocolitica* isolation and typing of the obtained isolates by plasmid profile. The organism was recovered from one milk sample (3.33%) of both cows and goats, however, it failed to be detected in ewe's milk. Concerning the examined faeces of cows, ewes and goats, the prevalence of *Y. enterocolitica* was 13.33, 10 and 10%, respectively. Moreover, it has been isolated from milk and faeces of only one (3.33%) of both dairy cows and goats. In addition, out of 12 *Y.enterocolitica* isolates, 2 (16.67%) from milk samples and 10 (83.33%) from faecal samples were obtained. Whereas, 2 virulent, plasmid bearing isolates and of biotypes 1B and 4 (8.33%) were recovered from cow's and goat's milk, respectively. 3 isolates (25%) of cow's faeces and one (8.33%) of ewe's faeces proved to be virulent, plasmid bearing and of biotypes 1B and 4, respectively. While, one (8.33%), 2 (16.67%) and 3 (25%) isolates of cows, ewes and goats faecal samples were avirulent, plasmidless and of biotypes either 1A or 4. The public health hazard of *Y. enterocolitica* and the sanitary measures for improving milk quality as well as the potential source of infection were discussed.

Key words: *Prevalence, Y. enterocolitica, Cows, Ewes, Goats, Plasmid profile.*

INTRODUCTION

Yersinia enterocolitica remains a most versatile ubiquitous bacterial pathogen. It has emerged as the prototypical species capable of navigating through various host defense mechanisms to establish itself in human body and cause illness (Bottone, 1997). In the past three decades, there has been a dramatic increase in the number of isolation of *Y.enterocolitica* from humans which rivals *Salmonella* and *Campylobacter* as a cause of gastroenteritis (Cover and Aber, 1989).

Recently, its importance has been highlighted with reports of fatalities from the transfusion of yersinia infected blood, although transfusion mediated sepsis seems to be a rare event (Wilkinson *et al.*,

1991; Centers for Disease Control and Prevention, 1997 and Strobel *et al.*, 2000). The predominant disease caused by pathogenic strains of *Y. enterocolitica* is enterocolitis which accounts for two-thirds of reported cases especially in young children and characterized by fever and diarrhea which frequently accompanied by abdominal pain lasting 1-3 weeks (Marks, 1980). However, serious cases may occur with rectal bleeding and perforation of the ileum (Rabinovitz, 1987). Moreover, there may be secondary immunologically mediated complications such as arthritis, erythema nodosum and to a lesser extent Reiter's syndrome, glomerulonephritis, myocarditis, exudative pharyngitis and septicaemia, which is less common and often reported in immunosuppressed individuals after contaminated blood products transfusion (Borg *et al.*, 1992; Bottone, 1997 and Strobel *et al.*, 2000). Sometimes *Y. enterocolitica* causes a syndrome which mimics appendicitis oftenly in older children and young adults (Butler, 1998; Naktin and Beavis, 1999 and Lamps *et al.*, 2001).

Epidemiological study of human yersinia infections has been implicated water, milk and dairy products, as well as wild and domestic animals such as pigs, rodents, rabbits, sheep, goats, cattle, horses, dogs and cats as reservoirs of the organism (Swaminathan *et al.*, 1982 and Butler, 1998). Worldwide, milk has been responsible for many *Y. enterocolitica* food poisoning outbreaks as recorded by several investigators (Shayegani *et al.*, 1983; Barrett, 1986; Mingrone *et al.*, 1988; WHO, 1988; Barrett, 1989; Greenwood and Hooper, 1990 and Ackers, 1995). The organism could be isolated from raw milk in Canada (Schiemann and Toma, 1978); Australia (Hughes, 1979); Denmark (Christensen, 1981); France (Vidon and Delmas, 1981) and Alsace (Delmas and Vidon, 1982). In Egypt, the recovery rates of this microorganism were 12, 7.5, 10 and 8.8% as recorded by Moustafa *et al.* (1983); Saad and Moustafa (1989); Moustafa (1990) and Khalil *et al.* (1993), respectively. While El-Gaml (1994) could detect *Yersinia* species in 8 and 6% of the examined individual cow's milk and bulk milk, of which 3.3 and 2% were *Y. enterocolitica* , respectively. Moreover, out of 145 raw milk samples examined, 12 samples proved to harbour *Y. enterocolitica* (8.3%) as reported by Henin and Kaldas (1995). On the other hand, El-Prince and Sabreen (1998) postulated that the incidence of the organism in raw milk was 16% of which, 62.5% were virulent and 37.5% were avirulent strains.

In addition, *Y. enterocolitica* microorganisms were found to exist in the faeces of milking bovine animals which could be regarded as

potential contaminants of raw milk (Davey *et al.*, 1983 and Quaglio *et al.*, 1988). Furthermore, *Y. enterocolitica* could be isolated from faeces of apparently healthy cows in Australia (Hughes, 1979) and South West of Scotland in percentage of 50% (Davey *et al.*, 1983). While, in Egypt, El-Gaml (1994) and Tanios (1994) recorded incidences of 1 and 2.5%, respectively. Also, Tanios *et al.* (2000) pointed out that 5 samples out of 60 examined faecal matter samples (7.58%) of buffalo-calves were contaminated by *Y. enterocolitica*.

In recent years, sheep and goats breeding is gradually increased as they constitute an excellent source of income among livestocks due to their milk, meat, wool, skin and high quality manure. Although ewes and goats milks have become of growing concern in human diet allover the world, they may represent a public health hazard as they are mostly consumed raw (Cosentino and Palmas, 1997). It has been demonstrated that *Y. enterocolitica* was isolated and identified from tested goat's milk samples from retail outlets and farms in New South Wales (Jensen and Hughes, 1980 and Chubb *et al.*, 1985). In Australia, New Zealand and Norway, *Y. enterocolitica* was recovered from outbreaks of enteritis and deaths in young sheep and goats (Buddle *et al.*, 1988; Slee and Button, 1990 and Philbey *et al.*, 1991).

The present investigation was designed to spotlight on the prevalence of *Y. enterocolitica* in milk and faeces of cows, ewes and goats. In addition, plasmid profiles of the obtained strains were investigated and its epidemiological significance was discussed.

MATERIAL and METHODS

Samples collection:

One hundred and eighty random samples of milk and faecal matter of lactating cows, ewes and goats (60 each) constituting 30 milk samples and 30 faecal specimens, were aseptically collected from different districts in Assiut governorate in the period from May to August 2001. All samples were kept in an insulated ice-box and transported to the laboratory and analyzed within 2 h.

Enrichment procedure: "Cold enrichment technique" (Varnam and Evans, 1991).

One ml of each milk sample and approximately 5% of a faecal specimen by volume (Quinn *et al.*, 1994) were transferred to 10 ml of phosphate buffer saline pH 7.6 (PBS) supplemented with 2% peptone.

The inoculated enrichment broth was incubated at 4°C for 14 days (Greenwood and Hooper, 1989).

Isolation of *Yersinia enterocolitica:* (Varnam and Evans, 1991)

Loopfuls from the incubated enrichment broth were streaked onto Yersinia selective agar plates supplemented with Yersinia selective supplements: Cefsulodin-Irgasan-Novobiocin (CIN) and incubated at 28°C for 24 h. Dark red, bull eye-like colonies surrounded by transparent borders were subcultured on nutrient agar slants for further confirmation and identification.

Identification of the isolated strains:

Presumptive colonies were screened by microscopic appearance and biochemical reactions including: Kligler iron agar (KIA), urea hydrolysis and sugar fermentation (salicin and sucrose) (Schiemann and Devenish, 1982). Typical *Y. enterocolitica* strains were biotyped as recorded in the scheme of Wauters (1970).

Determination of virulence markers:

Autoagglutination and congo red uptake were used to identify plasmid containing pathogenic strains of *Y.enterocolitica* (Varnam and Evans, 1991).

Extraction of plasmid DNA:

It has been carried out in the Molecular Biology and Genetic Engineering Research Center in Assiut University. A single colony of *Y.enterocolitica* was grown to saturation in 5 ml of L-broth at 37°C for 10 h (overnight) in a shaking bath and 2 ml of each culture were transferred to 1.5 ml Eppendorf tube for plasmid extraction by using the alkaline lysis procedure as described by Woodford *et al.* (1994).

Detection of plasmid DNA by agarose gel electrophoresis:

10 µl of the extracted plasmid were mixed with 10 µl of loading buffer and the aliquots were loaded onto 0.7% agarose gel stained with ethidium bromide (0.5 µg/ml). Electrophoresis was carried out at 90 v for 2-3 h and visualized under UV transillumination (Biometra) at 320 nm and photographed (Woodford *et al.*, 1994). 2.06 to 16.210 kb supercoiled DNA ladder molecular marker (Sigma) was used as DNA size standard.

RESULTS

The obtained results are recorded in Tables 1-5 and Figure 1.

Table 1. Prevalence of *Yersinia enterocolitica* and other *Yersinia* species in milk and faeces of the examined lactating animals.

Source of samples	Positive samples		*Yersinia species*			
			Yersinia enterocolitica		Other *Yersinia species*	
	No./30	%	No./30	%	No./30	%
Milk	2	6.67	1	3.33	1	3.33
Cows: Faeces	7	23.33	4	13.33	3	10.0
Milk	2	6.67	-	-	2	6.67
Ewes: Faeces	8	26.67	3	10.0	5	16.67
Milk	9	30.0	1	3.33	8	26.67
Goats: Faeces	5	16.67	3	10.0	2	6.67
Milk	13	14.44	2	2.22	11	12.22
Total : Faeces	20	22.22	10	11.11	10	11.11

Table 2. Surveillance of *Yersinia enterocolitica* in milk and faeces of the examined lactating animals.

Animal species	Milk		Faeces		*Milk & Faeces	
	No./30	%	No./30	%	No./30	%
Cows	1	3.33	4	13.33	1	3.33
Ewes	-	-	3	10.0	-	-
Goats	1	3.33	3	10.0	1	3.33

* Milk & Faeces of the same animals.

Table 3. Biotyping of *Yersinia enterocolitica* isolates recovered from the examined samples.

Source of Samples	No. of isolates	Biotype 1				Biotype 4	
		1A		1B			
		No./12	%	No./12	%	No./12	%
Milk	1	-	-	1	8.33	-	-
Cows: Faeces	4	1	8.33	3	25.0	-	-
Milk	-	-	-	-	-	-	-
Ewes: Faeces	3	1	8.33	-	-	2	16.67
Milk	1	-	-	-	-	1	8.33
Goats: Faeces	3	-	-	-	-	3	25.0
Milk	2 (16.67%)	-	-	1	8.33	1	8.33
Total : Faeces	10 (83.33%)	2	16.67	3	25.0	5	41.67

Table 4. Virulent and avirulent strains of *Yersinia enterocolitica* recovered from the examined samples.

Source of samples		No. of isolates	Virulent strains		Avirulent strains	
			No./12	%	No./12	%
Cows:	Milk	1	1	8.33	-	-
	Faeces	4	3	25.0	1	8.33
Ewes:	Milk	-	-	-	-	-
	Faeces	3	1	8.33	2	16.67
Goats:	Milk	1	1	8.33	-	-
	Faeces	3	-	-	3	25.0
Total :	Milk	2	2	16.67	-	-
	Faeces	10	4	33.33	6	50

Table 5. Correlation between virulence of *Yersinia enterocolitica*, biotypes and plasmids.

Source of Samples		No. of isolates	Biotyp	Plasmid bearing Isolates		Biotyp	Plasmidless Isolates	
				No./12	%		No./12	%
Cows:	Milk	1	1B	1	8.33	-	-	-
	Faeces	4	1B	3	25.0	1A	1	8.33
Ewes:	Milk	-	-	-	-	-	-	-
	Faeces	3	4	1	8.33	1A,4	2	16.67
Goats:	Milk	1	4	1	8.33	-	-	-
	Faeces	3	-	-	-	4	3	25.0
Total:	Milk	2		2	16.67		-	-
	Faeces	10		4	33.33		6	50

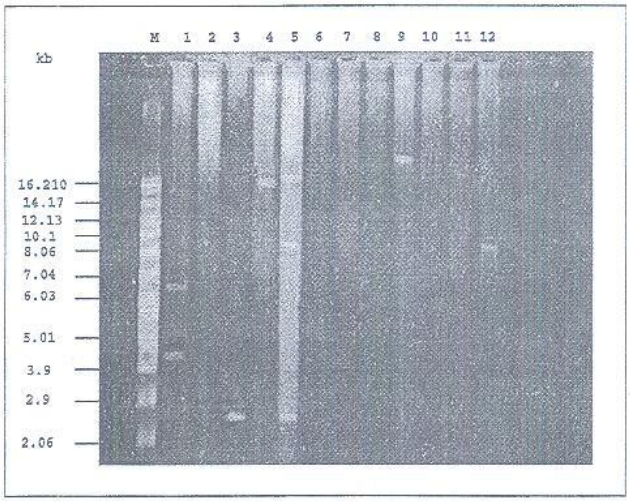

Figure 1: Plasmid profiles of *Y.enterocolitica* isolates.
M: Supercoiled DNA ladder (Sigma).
Lanes 1,3,4,5,9 and 12: Plasmid bearing *Y.enterocolitica* isolates.
Lanes 1: (6.5 and 4.2 kb), 3(2.5 kb), 4(16.2 kb), 5(16.2, 8.5 and 2.5 kb), 9(20 kb) and 12 (8.2 kb).
Lanes: 2,6,7,8,10 and 11: Plasmidless *Y.enterocolitica* isolates.

DISCUSSION

The genus *Yersinia* is a typical member of family enterobacteriaceae that contains three species which are recognized pathogens of humans, *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica. Y. enterocolitica* is a zoonotic, Gram negative bacterium capable of causing severe gastrointestinal infection (Varnam and Evans, 1991; Pritchard *et al.*, 1995 and Butler, 1998). It produces a heat stable enterotoxin that is associated with food poisoning strains in man (Quinn *et al.*, 1994) and it is a heterogenous species divided phenotypically into five biotypes, 1 (1A & 1B), 2, 3, 4 and 5, and numerous serotypes, many of them are considered environmental and nonpathogenic (Burnens *et al.*, 1996).

Results demonstrating the prevalence of *Y. enterocolitica* and other *Y.* species, in milk and faeces of cows, ewes and goats, are recorded in Table 1. The isolation rates of *Y.* species were 3.33, 6.67 and 26.67% and 10, 16.67 and 6.67% of the concerning samples and animals, respectively.

The obtained result in case of cow's milk is lower than that stated by El-Gaml (1994), but he recorded a lower incidence in case of cow's faeces (2%). The higher percentage detected in samples of cow's faeces confirm the belief that *Y.*species are common within the environment and some are considered as being opportunist pathogens in livestock and may occasionally be found in their food products, particularly raw cow's milk (Butler, 1998).

The results presented in Table 2 declared that *Y. enterocolitica* could be recovered from one milk sample (3.33%) of both examined lactating cows and goats. However, the organism failed to be detected in the examined milk samples of lactating sheep. Our findings in cow's milk are in conformity with the observations of Abdel-Hady (1993), El-Gaml (1994) and Pritchard *et al.* (1995). In contrast, higher incidences were recorded by Moustafa *et al.* (1983), Saad and Moustafa (1989), Toora *et al.* (1989), Moustafa (1990), Khalil *et al.* (1993), Henin and Kaldas (1995), Abdel-Khalek (1998) and El-Prince and Sabreen (1998). While, the work carried out by Vidon and Delmas (1981) and Delmas and Vidon (1982) indicated that the isolation rates of *Y. enterocolitica* from the examined raw milk were 81.4 and 54.5%, respectively. These differences could be attributed to several factors including seasonal variation (Delmas, 1983) as the organism being much higher (25-50%) during winter than summer (0-17%) as stated by Toora *et al.* (1989), enrichment broths and plating media used as well as the extent and sources of contamination (Schiemann and Wauters, 1992).

Most of research works have dealt with *Y. enterocolitica* in cattle milk while, few of them were directed towards sheep and goats milk. Anyhow, goat's milk is still marketed as raw milk and a high percentage is consumed by infants and others with allergies to cow's milk (Jensen and Hughes, 1980). However, it has been reported that ewe's and goat's milk could be a reservoir of several pathogens including food poisoning microorganisms (Anon, 1983, El-Leboudy and Gamal, 1994 and Cosentino and Palmas, 1997).

The presence of *Y. enterocolitica* in milk is of a particular concern because of the potential public health hazard. It is therefore of value to establish whether this organism occurs in faeces of the dairy

animals or not (Davey *et al.*, 1983). *Y. enterocolitica* has been recovered frequently from sheep and act as a significant cause of enteritis and mortality among goats (Buddle *et al.*,1988 and Slee and Button ,1990). Concerning the examined faeces of cows, ewes and goats, it is evident from Table 2 that prevalence of *Y.enterocolitica* of the corresponding animals was 13.33, 10 and 10%, respectively. Similarly, Hughes (1979) could isolate this bacterium from faeces of healthy cows. Higher levels were estimated by Davey *et al.* (1983) while, Adesiyun *et al.* (1992), El-Gaml (1994), Tanios (1994) and Tanios *et al.* (2000) recorded lower percentages. In the contrary, there is no case of faecal contamination with *Y. enterocolitica* in examined bovine faeces in Italy as estimated by Quaglio *et al.* (1988). On the other hand, the recovery rate of *Y. enterocolitica* from the examined ewe's and goat's faecal matter was found to be 10%. Philbey *et al.* (1991) demonstrated yersinia infection in 4% of Australian sheep with diarrhoea, ill thrift or mortality. However, earlier studies elsewhere on sheep and goats have failed to isolate *Y. enterocolitica* (Adesiyun *et al.*, 1992). Controversely, Butler (1998) suggested that, the environmental stress could have interacted with yersinia infection to result in clinical disease, or could be concomitant infection with nematodes and coccidian which are common in infected flocks.

Because *Y.enterocolitica* is not known to cause mastitis in animals, most contamination of milk is thought to occur through contact with faeces or polluted water (Schiemann, 1989) and thus explains the ability to isolate the organism from both milk and faeces of the same animal from one (3.33%) of cows and goats as shown in Table 2.

It was apparent that *Y. enterocolitica* biotype 4 was the most frequent biotype detected in the examined milk and faecal samples of different animals representing 50% of the isolates followed by biotype 1B (33.33%) then biotype 1A (16.67%), whereas, 16.67% of ewe's faeces, 8.33% of goat's milk and 25% of goat's faeces were belonged to Wauters' biotype 4 as shown in Table 3. The same biotype was isolated from raw milk as recorded by Khalil *et al.* (1993). On the other hand, isolates recovered from cow's milk (8.33%) and cow's faeces (25%) were belonged to Wauters' biotype 1B. However, biotype 1A constituted two isolates recovered from both cow's faeces and ewe's faeces in a percentage of 8.33%. Schiemann (1978) reported that biotype 1 is the third most common strain isolated from man in Canada. Moreover, Vidon and Delmas (1981) demonstrated the isolation of biotype 1 from raw milk and also, Wauters (1981) considered that *Y. enterocolitica*

biotype 1 (excluding serotype 0:8) and the related species are ubiquitous and largely lacking in clinical significance. Furthermore, a majority of their isolates was found to belong to biotype 1 and 4 (Swaminathan *et al.*, 1982).

The data in Table 4 revealed that, one (8.33%) virulent *Y. enterocolitica* was isolated from each of the examined cow's and goat's milk using congo red uptake and autoagglutination test. In contrast, Pritchard *et al.*(1995) indicated that none of the isolates of this bacteria were pathogenic. Lower rate (3.33%) of virulence was stated by Abdel-Hady *et al.* (1995) however, relatively higher incidence (62.5%) of examined raw milk samples was detected by El-Prince and Sabreen (1998). It is also apparent from Table 4 that 3 (25%) isolates of cow's faeces and one (8.33%) isolate of ewe's faeces proved to be virulent, while one (8.33%), 2(16.67%) and 3 (25%) isolates of examined cow's, ewe's and goat's faecal samples were avirulent, respectively. Virulence of *Y. enterocolitica* results from a complex interplay between a series of temperature-controlled plasmid borne and chromosomal genes (Cornelis, 1994 and Robins-Browne, 1997). The chromosomal gene encoding a low molecular weight, heat stable enterotoxin produced by several species of diarrheagenic bacteria and hence is a useful marker of potential virulence (Yoshimura *et al.*, 1987).

It was investigated that, out of 12 isolates of *Y. enterocolitica,* 6 (50%) harboured one to three plasmids ranging in size from 2.5 to 20 kb (Figure 1) and they were found to express plasmid associated virulence markers and belonged to Wauters' biotypes 1B and 4. On the other hand, the remaining 6 (50%) were plasmidless and belonged to Wauters' biotype 1A and 4 (Table 5). It has been reported that virulent enteropathogenic strains of *Y. enterocolitica* belonged to biotypes 1B, 2 through 5 and are concerned in human infection (Ibrahim *et al.*, 1997). On the other hand, although biotype 1A is commonly regarded as avirulent strains which are ubiquitous and lack virulence determinants of invasive isolates, it has been incriminated in human infection sporadically (Burnens *et al.*, 1996). The obtained strains carrying plasmids were grouped into 6 plasmid profiles (Figure 1). One isolate of *Y.enterocolitica* (8.33%) recovered from cow's milk, carried two plasmids (6.5 and 4.2 kb), however, three isolates (25%) obtained from cow's faeces harboured plasmids, two of which carried one plasmid (2.5 and 16.2 kb) and the other isolate harboured three plasmids (16.2, 8.5 and 2.5 kb). Also, the isolates of ewe's faeces and goat's milk harboured

one plasmid each of 8.2 kb and 20 kb, respectively as illustrated in Figure 1.

The virulence plasmids of yersinias are involved with a number of temperature-regulated phenotypes, including autoagglutination (Laird and Cavanaugh, 1980), serum resistance (Martinez, 1983); production of V and W antigens (Perry and Brubaker, 1983), expression of certain outer membrane proteins (Bolin *et al.*, 1988) and low calcium response (Snellings *et al.*, 2001). It has been suggested that the organism is primed for virulence and invasion during growth outside the mammalian body at lower temperature and only an initial burst of this activity is required to establish an infection (Maurelli, 1989). Subsequent steps in pathogenesis may then require down-regulation of the genes for adherence and internalization (Isberg, 1989).

The results of this study indicates that *Y. enterocolitica* occurred in a higher frequency in faeces of lactating animals, although it has been isolated from raw milk which is considered as a common factor in sporadic outbreaks. Additionally, the virulent *Y. enterocolitica* isolates obtained from cows, ewes and goats were unlikely to be related and were highly diverse. Careful sanitary procedures coupled with the application of hazard analysis of critical control points (HACCP) programs to dairy farms during milk production as well as good personal hygiene should be adopted for improving it's microbiological safety.

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