PATHOLOGIE INFECTIEUSE

Prevalence of Salmonella, Listeria monocytogenes, Campylobacter spp., Yersinia enterocolitica and Cryptosporidium spp. in bulk milk, cows' faeces and effluents of dairy farms in Trinidad

A.A. Adesiyun ^{1*} L.A. Webb ¹ H. Romain ² J.S. Kaminjolo ¹

Key words

Salmonella - Listeria monocytogenes - Campylobacter - Yersinia entero-colitica - Cryptosporidium - Dairy cow - Milk - Faeces - Waste water - Dairy farm - Contamination - Trinidad and Tobago.

Summary

The prevalence of Listeria, Salmonella, Campylobacter, Yersinia and Cryptosporidium species in bulk milk, faeces of dairy cows and effluents of milking parlours from dairy farms in Trinidad was investigated. Of the 177 bulk milk samples studied, 3 (1.7 %), 3 (1.7 %) and 2 (1.1 %) were positive for L. monocytogenes, Salmonella spp. and Y. enterocolitica, respectively, but were all negative for Campylobacter and Cryptosporidium species. From 333 faecal samples tested, L. monocytogenes, Campylobacter spp. and Cryptosporidium spp. were detected in 11 (3.3 %), 19 (5.7 %) and 7 (2.1 %), respectively, which were significantly (P < 0.05; χ^2) higher than the detection rate (0 %) for Yersinia spp. Salmonella spp. were isolated from 3 (0.9 %) of the faecal samples. From 168 effluent samples cultured, Campylobacter spp. were isolated from 7 (4.2 %) samples while Salmonella was recovered from only 1(0.6 %) sample with no sample positive for either Yersinia or Listeria. The difference was significant (P < 0.05; χ^2). A total of 19 (73.1 %) of 26 Campylobacter isolates exhibited resistance to one or more of the six antimicrobial agents tested. All 14 (100 %) isolates of L. monocytogenes were resistant to at least one agent while all Salmonella and Yersinia enterocolitica strains were susceptible to all antimicrobial agents used. It was concluded that the five enteropathogens tested were present on dairy farms in Trinidad and the potential for milk-borne diseases, due to these pathogens, exists for consumers, emphasizing the need for good sanitary practices.

■ INTRODUCTION

Milk from dairy animals, particularly cattle, has been responsible for several outbreaks of infections and intoxications in human consumers worldwide (22, 26, 39). Most of these epidemics have been associated with the consumption of raw milk although pasteurized milk has also been implicated (20, 42).

Pathogens in milk may be aetiological agents for subclinical or clinical mastitis (1, 38), part of the normal flora of the skin of dairy animals or skin and anterior nares of their human handlers (35). Dairy cows are also carriers of several enteropathogens in their gut (41).

In Trinidad and Tobago, *Campylobacter*, *Salmonella*, *Yersinia* and *Listeria* species have been isolated from livestock (5), raw meat

^{1.} School of Veterinary Medicine, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad, West Indies

^{2.} Veterinary Public Health Unit, Ministry of Health, Port of Spain, Trinidad,

^{*} Correspondence: same as in 1. - E-mail: biodun@medsci.uwi.tt

Enteric pathogens on Trinidadian dairy farms

(2) and ready-to-eat foods (4) while *Cryptosporidium* oocysts have been detected in diarrhoeic livestock (5). Also, verocytotoxigenic *Escherichia coli* and enterotoxigenic *Staphylococcus aureus* have been recovered from raw cows' milk sampled at the levels of the processing plant (3) and collection centres (8). To date, information is unavailable on the prevalence of enteropathogens in bulk milk and dairy cows at the farm level.

The study was conducted to determine the prevalence of *Campylobacter*, *Listeria*, *Yersinia*, *Salmonella* and *Cryptosporidium* species in bulk milk, faeces of dairy cows and effluents of milking parlours and to characterize the enteropathogens isolated.

■ MATERIALS AND METHODS

Study design

Dairy farms supplying bulk milk to the eight collection centres (IC, 5C, 5H, 2G, 2C, 2B, 6H and 3G), where the microbial quality of milk was earlier determined (8), were investigated. With the aid of a computer print out of the list of dairy farms in these milking areas, every other farm was randomly selected to be involved in the study. On every farm selected, 20 % of lactating cows was to be sampled.

Source, type and collection of samples

Bulk milk was pooled from the churns containing the morning's yield of each selected farm. Approximately, 100 ml of bulk milk samples were collected per dairy farm. In practice, a bulk milk sample originated from each dairy farm studied (table I). The median number of lactating dairy cows per farm in each milking centre during the visits is shown in brackets as follows: IC (9), 5C (4), 5H (2), 2G (5), 2C (2), 2B (12), 6H (2) and 3G (3). Overall, a total of 177 dairy farms across 8 milking centres were studied with a median number of lactating dairy cows per farm of 4. Faeces, or rectal swabs in cases where faeces could not be collected, were obtained from the rectum with the use of gloves and immediately

put into sterile wide-mouthed plastic containers. Effluents were collected, from the most distal point of the milking parlour or shed, into wide-mouthed sterile containers. Approximately, 100 ml of effluent were collected.

Isolation and identification of microorganisms

To detect *Listeria* spp. in 1 g of faeces or 25 ml of bulk milk or effluent, the faecal and milk or effluent samples were enriched in 9 ml and 225 ml, respectively, of nutrient broth (Difco, USA) at 4°C for 6 weeks. Subcultures were made onto *Listeria* selective media with Oxford suplement (Oxoid, UK) forthnightly. Inoculated plates were incubated at 37°C in 5 % CO₂ in a CO₂ incubator (Forma Scientific, USA) for 24-48 h. Typical black colonies were Gram-stained and tested for catalase and oxidase activities. Colonies that were Gram-positive short rods were inoculated into triple sugar iron (TSI) agar slants, urea slants and motility medium. Isolates with acid/acid, reaction without gas in TSI, urease-positive and typical umbrella-shaped motility were subjected to further biochemical tests (32).

Salmonella spp. were detected in either 1 g of faeces and 25 ml each of bulk milk or effluent which were inoculated into 9 ml of selenite cystine (SC) broth and 225 ml of lactose broth (LB), respectively, and incubated overnight at 42°C. Enriched faecal samples were inoculated onto xylose lysine desoxycholate (XLD) agar and incubated at 37°C for 24 h. One millilitre each of preenriched bulk milk and effluent in LB was inoculated into 10 ml each of selenite cystine and tetrathionate broth and incubated overnight at 37°C. Typical pink colonies with black centres on XLD and colonies with characteristic silvery appearance on BS plates were subjected to biochemical tests using standard methods (32). Serologic identification was initially carried out using the slide agglutination test with the polyvalent antiserum A-I α Vi. Confirmation of Salmonella isolates and complete serological typing was kindly done at the Caribbean Epidemiology Centre (CAREC), Port of Spain, Trinidad.

For the isolation of *Campylobacter* spp., a loopful of samples (faeces, bulk milk or effluent) was inoculated directly onto

Table I

Prevalence of species of *Listeria*, *Salmonella*, *Campylobacter*, *Yersinia* and *Cryptosporidium* in bulk milk

		Number (%) of samples positive							
Milking Centre	Num. of bulk milk samples tested	Listeria spp.*	Salmonella spp.**	Campylobacter spp.	Yersinia spp.***	Cryptosporidium			
IC	43	0 (0.0)	3 (7.0)	0 (0.0)	1 (2.3)	0 (0.0)			
5C	35	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
5H	33	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
2G	16	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
2C	15	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
2B	14	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)			
6H	11	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
3G	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
Total	177	3 (1.7)	3 (1.7)	0 (0.0)	2 (1.1)	0 (0.0)			

- * Made up of L. monocytogenes
- ** All isolates were S. agona
- *** Both isolates were Yersinia enterocolitica, serotype 0:3, biotype 4

Revue Élev. Méd. vét. Pays trop., 1996, 49 (4) : 303-309

Campylobacter blood-free agar with CCDA supplement (Oxoid, UK). Inoculated plates were incubated in a CO₂ incubator (Forma Scientific Inc., USA) with 8-10 % CO₂ at 42°C for 24-48 h. Suspect colonies were Gram-stained. Gram-negative slender, seagull appearing microorganisms were subcultured onto blood agar and incubated at 42°C for 24 h in 8-10 % CO₂ and subsequently subjected to biochemical tests as suggested by Lior (31). Hippurate hydrolysis was used to distinguish between *C. jejuni* and *C. coli* (31).

To detect *Yersinia* spp., 25 ml of bulk milk or effluent were added to 225 ml of 0.067 M phosphate buffered saline (PBS), pH 7.6 while 1 g of faeces was enriched in 9 ml of 0.067 M PBS. Enrichment was done at 4°C for 3 weeks. Subcultures were made after 1 week and 3 weeks onto *Yersinia* agar containing *Yersinia* selective supplement (Oxoid, UK) and incubated at room temperature for 24-48 h. Typical colonies with "bull's eye" appearance demonstrated by a red or pink centre surrounded by a clear zone were subjected to biochemical tests (32). Serological identification of the isolates was kindly done at the Ontario Ministry of Health, Laboratory Services Branch, Ontario, Canada.

Cryptosporidium oocysts were detected in faeces and bulk milk processed within 2 h of collection by the formalin-ether concentration sedimentation technique (21). Ten millilitres of bulk milk samples were centrifuged to achieve sedimentation. The sediment was then added to formalin-ether as done for faecal samples. Smears were stained by the modified Ziehl-Neelsen method (21) and subjected to microscopic examination.

Determination of antibiograms of isolates

For the 26 isolates of *Campylobacter* spp., the following antimicrobial agents and concentrations were used: ampicillin (10 mcg), neomycin (30 mcg), streptomycin (10 mcg), kanamycin (30 mcg), chloramphenicol (30 mcg) and gentamycin (10 mcg). *Salmonella* isolates were tested for their sensitivity to: ampicillin (10 mcg), chloramphenicol (30 mcg), kanamycin (30 mcg), nalidixic acid (30 mcg), neomycin (30 mcg), streptomycin (10 mcg) and sulphamethoxazole/trimethoprim (30 mcg).

The susceptibility of *Listeria* spp. to the following antimicrobial agents and concentrations was determined using: penicillin (10 units), ampicillin (10 mcg), erythromycin (15 mcg), chloramphenicol (30 mcg), streptomycin (10 mcg), gentamycin (10 mcg) and sulphamethoxazole/trimethoprim (30 mcg). The antibiograms of *Yersinia* isolates were determined for streptomycin (10 mcg), gentamycin (10 units), chloramphenicol (30 mcg), sulphamethoxazole/trimethoprim (30 mcg), nalidixic acid (30 mcg) and kanamycin (30 mcg). The disc diffusion method (11) was used to determine the susceptibility of all the pathogens to the various antimicrobial agents.

Statistical analysis of data

The prevalences of enteropathogens in bulk milk, faeces and effluents and the antibiotic sensitivity of the isolates were compared amongst milking centres using the chi-square test for independence, with one degree of freedom.

■ RESULTS

The prevalence of selected enteropathogens in bulk milk samples is shown in table I. Of the 177 samples tested, 3 (1.7 %), 3 (1.7 %)

and 2 (1.1 %) were positive for *L. monocytogenes*, *Salmonella* spp. and *Y. enterocolitica*, respectively. All the samples were negative for *Campylobacter* spp. and *Cryptosporidium* spp. Both isolates of *Y. enterocolitica* belonged to serogroup 0:3 and biotype 4. All *Salmonella* isolates belonged to serotype *agona*.

All bulk samples collected from centres 5H, 6H and 3G were free of all the enteropathogens tested. Centres IC and 5C, however, accounted for 1 of the 3 *Listeria* isolates, 3 of 3 of the *Salmonella* isolates and 50 % (1 of 2) of the *Y. enterocolitica* isolates recovered.

Table II shows the prevalence of enteropathogens in the faeces of dairy cows and effluents from the milking parlour or shed. Amongst the 333 dairy cows sampled, 11 (3.3 %), 3 (0.9 %), 19 (5.7 %) and 7 (2.1 %) were shedders of L. monocytogenes, Salmonella spp., Campylobacter spp. and Cryptosporidium spp., respectively. There were statistically significant differences in the carriage of Campylobacter spp. (P < 0.001; χ^2), L. monocytogenes $(P < 0.05; \chi^2)$, compared to that for Y. enterocolitica. All faecal samples collected from cows in centres 3G and 6H were negative for the enteric pathogens cultured. Centres IC and 5C, however, accounted for 54.5 % (6 of 11) of the Listeria monocytogenes isolates, 68.4 % (13 of 19) of the Campylobacter isolates and 100 % (7 of 7) of the Cryptosporidium - positive samples. Effluents from the milking parlours were negative for L. monocytogenes and Yersinia spp., but, of the 168 samples tested, 1 (0.6 %) and 7 (4.2 %) were positive for Salmonella spp. and Campylobacter spp., respectively. Campylobacter spp. were isolated at a statistically significantly (P < 0.05; χ^2) higher rate than other pathogens. All 7 isolates of Campylobacter spp. originated from milking centres IC and 5C.

The sensitivity of Campylobacter spp. to 6 antimicrobial agents is shown in table III. Of a total of 26 strains of *Campylobacter* spp. made up of 16 (61.5 %) strains of C. jejuni and 10 (38.5 %) of C. coli, 19 (73.1 %) exhibited resistance to one or more antimicrobial agents. Resistance varied from 66.7 % (Centres IC, 2G, 2B and 3G) to 100 % (Centre 5H). Resistance was highest to ampicillin with 19 (73.1 %) strains and lowest to gentamycin with 1 (3.8 %) strain and the difference was statistically significant $(P < 0.001; \chi^2)$. A total of 5 (19.2 %), 4 (15.4 %), 3 (11.5 %) and 2 (7.7 %) strains were resistant to neomycin, streptomycin, kanamycin and chloramphenicol, respectively, but the differences were not statistically significant (P > 0.05; χ^2). Both strains of Y. enterocolitica were sensitive to streptomycin, gentamycin, chloramphenicol, sulphamethaxazole/trimethoprim (SXT), nalidixic acid and kanamycin. All of 7 isolates of Salmonella from bulk milk, cow's faeces and effluents were sensitive to the seven antimicrobial agents used, namely ampicillin, chloramphenicol, kanamycin, nalidixic acid, neomycin, streptomycin and sulphamethaxazole/trimethoprim.

For the 14 strains of L. monocytogenes tested, 13 (92.9 %), 13 (92.9 %), 12 (85.7 %), 4 (28.6 %) and 2 (14.3 %) exhibited resistance to ampicillin, streptomycin, sulphamethoxazole/trimethoprim (SXT), erythromycin and penicillin, respectively. All strains were sensitive to chloramphenicol and gentamycin. Overall, 7 resistance patterns were observed amongst L. monocytogenes strains, but the prevalent patterns were ampicillin-sulphamethoxazole/trimethoprim-streptomycin, ampicillinerythromycin-sulphamethoxazole/trimethoprim-streptomycin and ampicillin-streptomycin with 7 (50.0 %), 2 (14.3 %) and 1 (7.1 %) strains, respectively.

■ PATHOLOGIE INFECTIEUSE

Table II

Prevalence of selected pathogens in faeces of dairy cows and effluents of farms

			Yersinia	0.0)	(0.0)	0.0) 0	(0.0)	(0.0)	(0.0)	(0.0)	0.0) 0	0.0) 0	
tured	Effluents* of dairy farms	Number (%) of samples positive										0	
			ampyloba	2 (4.5)	5 (14.7)	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	7 (4.2)	
			Number (%) of sa	Salmonella*** Campylobacter	1 (2.3)	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	1 (0.6)
			Listeria	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	
			Num. of samples tested	44	34	30	16	15	14	2	10	168	
Sources of samples cultured	Faeces of dairy farms	Faeces of dairy farms Number (%) of faecal samples positive	Cryptosporidium	4 (3.4)	3 (5.4)	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	7 (2.1)	
Source			Yersinia	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	0.0) 0	
			Salmonella** Campylobacter	7 (5.9)	6 (10.7)	0.0) 0	3 (11.1)	0.0) 0	3 (7.3)	0.0) 0	0.0) 0	19 (5.7)	
			Salmonella**	0.0) 0	1 (1.8)	0.0) 0	2 (7.4)	0.0) 0	0.0) 0	0.0) 0	0.0) 0	3 (0.9)	
			Listeria	6 (5.0)	0.0) 0	1 (2.6)	0.0) 0	2 (10.5)	2 (4.9)	0.0) 0	0.0) 0	11 (3.3)	
			Num. of samples tested	119	26	39	27	19	41	11	21	333	
	Milking Centre				2C	2H	2G	2C	2B	Н9	3G	Total	

* Effluent samples were not processed for Cryptosporidium oocysts

^{**} Isolates from centre 5C was group D untypable, from centre 2G was S. typhimurium and S. javiana, respectively

^{***} Salmonella isolated from centre IC was S. agona

 Table III

 Resistance of Campylobacter strains to antibiotics tested

				Num. (%) of strains resistant to:							
Milking Centre*	Num. of Campylobacter** strains tested	Num. (%) of strains*** resistant	A***	N	S	К	С	CN			
IC	9	6 (66.7)	6 (66.7)	1 (11.1)	2 (22.2)	1 (11.1)	0 (0.0)	1 (11.1)			
5C	7	6 (85.7)	6 (85.7)	2 (28.6)	2 (28.6)	2 (28.6)	2 (28.6)	0 (0.0)			
5H	1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
2G	3	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
2B	3	2 (66.7)	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
3G	3	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
Total	26	19 (73.1)	19 (73.1)	5 (19.2)	4 (15.4)	3 (11.5)	2 (7.7)	1 (3.8)			

^{*} No Campylobacter isolate was recovered from milking centres 2C and 6H

■ DISCUSSION

It was significant that all the five enteropathogens of importance to the dairy industry studied were detected either in the bulk milk, faeces of lactating cows or the effluent of milking parlours. This is important because these agents are of zoonotic significance and, in addition, could cause economic losses to the dairy industry (24, 29).

Failure to detect a correlation between detection of enteropathogens in bulk milk, faeces of cows and effluents was not unexpected since only 20 % of lactating cows contributing milk to the bulk supply and of the effluents on each farm were sampled. It is also known that all four bacterial enteropathogens studied could cause subclinical or clinical mastitis (15, 24) and therefore be present in milking without being detected in the faeces of affected animals or the effluents.

Centres IC and 5C were observed to have comparatively high prevalences of enteropathogens from the three sources studied, suggestive of high carriage rates of these pathogens or poor sanitary practices during milking. The potential for contamination of milk during the milking process on dairy farms visited was high since 95 % of these farms (Adesiyun and others, unpublished data) practised hand-milking. The risk of faecal materials splashing into milk in milking buckets from defaecating cows was therefore high. Various practices during milking have contributed to poor microbial quality of milk on dairy farms (35).

The prevalence of *Campylobacter* species (5.7 %) in cattle's faeces is considerably lower than the rates of 12.7 % to 46.7 % reported by others (10, 23). Adesiyun *et al.* (5) had earlier reported a prevalence of 12.9 % and 16.5 % in diarrhoeic and non-diarrhoeic cattle, respectively. Although *Campylobacter* was detected in 5.7 % of cows' faeces and in 4.2 % of 168 effluent samples, none of the bulk samples was positive for the microorganism. Cerqueira *et al.* (17) also failed to isolate *Campylobacter* from raw milk, while a prevalence as high as 12.3 % was reported by Rohrbach *et al.* (37). The peroxidase system, not inactivated in the bulk milk in the present study, may have been responsible for the non-isolation of *Campylobacter* (13).

The predominance of *C. jejuni* over *C. coli* amongst *Campylobacter* spp. agrees with earlier reports of studies in Trinidad (6) and elsewhere (36). Similarly, the antibiograms of the isolates of *Campylobacter* were comparable to the findings of others (6, 36).

L. monocytogenes has been reported to cause milk-borne listeriosis following comsumption of raw or pasteurized milk and milk products (14, 20). The finding that 2 % of raw bulk milk in the present study contained L. monocytogenes is therefore of public health significance since over 30 % of dairy farmers and their families in Trinidad consume raw cows' milk (Adesiyun and others, unpublished data). The low rate of detection of L. monocytogenes in bulk milk (2 %) and faeces (3 %) of dairy cows agrees with published studies (25, 34).

Y. enterocolitica appeared to pose the least risk to consumption of milk from Trinidadian dairy farms with a failure to isolate the organism from faecal and effluent samples and only 1 % of bulk milk samples positive. Y. enterocolitica is known to be mostly harboured by pigs and the microorganism has been found on pork products (9) although other animals have been found to be carriers (5, 40). The isolation rate of Y. enterocolitica in bulk milk (1 %) is slightly lower than the 2.7 % reported by Davidson et al. (18).

Of epidemiological relevance was the finding that both *Y. enterocolitica* strains belonged to serotype 0:3 and biotype 4. All but one of the strains of *Y. enterocolitica* isolated from livestock (5) and slaughter pigs (7) in Trinidad were the same serotype 0:3 and biotype 4. *Y. enterocolitica* serotype 0:3 and biotype 4 may therefore be important as a cause of human yersiniosis in Trinidad, a prevalence presently unknown. Cryptosporidiosis, a disease not known to be associated with consumption of milk, has been associated with waterborne outbreaks (16, 33). The detection of *Cryptosporidium* oocysts in the faeces of cattle in milking parlours is therefore important from the viewpoint of environmental contamination. The faecal prevalence (2 %) of *Cryptosporidium* oocysts is however much lower than the 9.4 % and 7.6 % reported for diarrhoeic and non-diarrhoeic calves, respectively, in Trinidad (5). In addition to

^{**} Consisting of 16 (61.5 %) strains of C. jejuni and 10 (38.5 %) strains of C. coli

^{***} Resistant to one or more of the antimicrobial agents tested

^{****} A - Ampicillin; N - Neomycin; S - Streptomycin; K - Kanamycin; C - Chloramphenicol; CN - Gentamycin

Cryptosporidium being able to cause diarrhoea in dairy calves (12), parasitized dairy cows have been demonstrated to experience reduced milk yield, as much as 3.2 kg per day (19). The zoonotic significance of cryptosporidiosis was emphasized by reports that dairy farmers had significantly higher risk of contracting cryptosporidiosis compared to non-farmers (30).

Enteric pathogens on Trinidadian dairy farms

The 1.7 % prevalence of *Salmonella* in bulk milk compares favourably with prevalences of 2 % for milk filters (27) and 0.25 % for milk of dairy cows with subclinical mastitis (28). Rohrbach *et al.* (37) reported a much higher prevalence of 8.9 % for *Salmonella* in bulk milk. Adesiyun *et al.* (5) reported finding 6.0 % and 2.5 % of diarrhoeic and non-diarrhoeic calves, respectively, positive for *Salmonella*.

Acknowledgements

The technical assistance rendered by Nestlé Trinidad and Tobago Limited is appreciated. We thank the staff of the Veterinary Public Health Unit of the Ministry of Health, Port of Spain, for sample collection. The laboratory support provided by Mr. Gerard Ramirez and Mrs. Theresa Niles-Pierre is acknowledged. We are grateful to Ms. Camille Timothy for typing the manuscript.

REFERENCES

- 1. AARESTRUP F.M., WEGNENER H.C., ROSDAHL V.T., 1995. A comparative study of *Staphylococcus aureus* strains isolated from bovine subclinical mastitis during 1952-1956 and 1992. *Acta vet. Scad.*, **36**: 237-243,
- 2. ADESIYUN A.A., 1993. Prevalence of *Listeria* spp., *Campylobacter* spp., *Salmonella* spp., *Yersinia* spp. and toxigenic *Escherichia coli* on meat and sea foods in Trinidad. *Food Microbiol.*, **10**: 395-403.
- 3. ADESIYUN A.A., 1994. Bacteriological quality and associated public health risk of pre-processed bovine milk in Trinidad. *Int. J. Food Microbiol.*, **21**: 253-261.
- 4. ADESIYUN A.A., 1995. Bacteriologic quality of some Trinidadian ready-to-consume foods and drinks and possible health risks to consumers. *J. Food Prot.*, **58**: 651-655.
- 5. ADESIYUN A.A., KAMINJOLO J.S., 1994. Prevalence and epidemiology of selected enteric infections of livestock in Trinidad. *Prev. vet. Med.*, 19: 151-165.
- 6. ADESIYUN A.A., KAMINJOLO J.S., LOREGNARD R., KITSON-PIGGOTT W., 1992. *Campylobacter* infections in calves, piglets, lambs and kids in Trinidad. *Br. vet. J.*, **148**: 547-556.
- 7. ADESIYUN A.A., KRISHNAN C.A., 1995. Occurrence of *Yersinia enterocolitica* 0:3, *Listeria monocytogenes* spp. in slaughter pigs and carcasses in Trinidad. *Food Microbiol.*, **12**: 99-107.
- 8. ADESIYUN A.A., WEBB L., RAHAMAN S., 1995. Microbiological quality of raw cow's milk at collection centres in Trinidad. *J. Food Prot.*, **58**: 139-146.
- 9. ASPLUND K., TUOVINEN V., VEIJALAINEN P., HIRN J., 1990. The prevalence of *Yersinia enterocolitica* 0:3 in Finnish pigs and pork. *Acta. vet. Scand.*, **31**: 39-43.
- 10. BAFFONE W., PIANETTI A., BRUSCOLINI F., BRANDI G., ALBANO M., CANTONI G., SALVAGGIO I., 1988. Presence of *Campylobacter*, and *Yersinia* in some species of domestic animals of the area of Urbino, Italy. *Ig. Mod.*, **89**: 55-64.
- 11. BAUER A.W., KIRBY W.M., SHERRIS J.C., TURCK M., 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. clin. Path.*, **54**: 493-496.
- 12. BELLINZONI R.C., BLACKHALL J., TERZOLO H.R., MOREIRA A.R., ANZA N., MATTION N., MICHEO G.I., LA TORRE J.L., SCODELLER E.R., 1990. Microbiology of diarrhoea in young beef and dairy calves in Argentina. *Rev. Argent. Microb.*, **22**: 130-136.
- 13. BEUMER R.R., CRUYSEN J.J.M., BIRTANTIE I.R.K., 1988. The occurrence of *Campylobacter jejuni* in raw cows' milk. *J. appl. Bacteriol.*, **65**: 93-96.

- 14. BILLE J., GLAUSER M.P., 1988. Zur listeriose-situation in der Schweizbull. *Bundesmantes Gesundheitwe.*, **3**: 28-29
- 15. BOURRY A., POUTREL B., ROCOURT J., 1995. Bovine mastitis caused by *Listeria monocytogenes*: Characteristics of natural and experimental infection. *J. med. Microbiol.*, **43**: 125-132.
- 16. BRIDGEMAN S.A., ROBERTSON R.M., SYED Q., SPEED N., ANDREWS N., HUNTER P.R., 1995. Outbreak of cryptosporidiosis associated with a disinfected ground water supply. *Epid. Infect.*, **115**: 555-566.
- 17. CERQUEIRA M.O.P., SOUZA M.R., RODRIGUEZ R., FONSECA L.M., RUBINICH J., QUINTAES I.A.S., 1994. Microbiological characteristics of raw and pasteurized milk from Belo Horizonte, Brazil. *Arq. Bras. Med. vet. Zootec.*, **46**: 713-721.
- 18. DAVIDSON R.J., SPRUNG D.W., PARK C.E., RAYMAN M.K., 1989. Occurrence of *Listeria monocytogenes, Campylobacter* spp. and *Yersinia enterocolitica* in Manitoba, Canada raw milk. *Can. Inst. Food Sci. Tech. J.*, 22: 70-74.
- 19. ESTEBAN E., ANDERSON B.C., 1995. *Cryptosporidium muris*: prevention, persistence and detrimental effect on milk production in a dry lot dairy. *J. Dairy Sci.*, **78**: 1068-1072.
- 20. FLEMING D.W., COCHI S.L., MACDONALD K.L., BRONDUM J., HAYNES P.S., PLIKAYTIS B.Q., HOLMES M.B., AUDURIER A., BROOM C.V., REINGOLD A.L., 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Eng. J. Med.*, **312**: 404-407.
- 21. GARCIA L.S., BRUCKNER D.A., BREWER T.C., SHIMIZU R.Y., 1983. Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimens. *J. clin. Microbiol.*, **18**: 185-190.
- 22. GENIGEORGIS C.A., 1990. Present state of knowledge on staphylococcal intoxication. *Int. J. Food Microbiol.*, **9**: 327-360.
- 23. GIACOBONI G.I., ITOH K., HIRAYAMA K., TAKAHASHI E., MITSUOKA T., 1993. Comparison of faecal *Campylobacter* in calves and cattle of different ages and areas in Japan. *J. vet. Med. Sci.*, **55**: 555-559.
- 24. GROUNDSON J., CHIRINO TREJO J.M., 1993. A case of bovine mastitis caused by *Campylobacter jejuni. J. vet. Med.*, Series B, **40**: 326-327
- 25. IIDA T., KANZAKI M., MARUJAMA T., INOUE S., KANEUCHI C., 1991. *Listeria monocytogenes* in intestinal contents of healthy animals in Japan. *J. med. Sci.*, **53**: 873-875.
- 26. KARMALI M.A., 1989. Infection by verocytotoxin-producing Escherichia coli. Clin. Microbiol. Rev., 2: 15-38.
- 27. LANCE S.E., MILLER G.Y., HANCOCK D.D., BARTLETT P.C., HEIDER L.E., 1992. *Salmonella* infection in neonatal dairy calves. *JAVMA*, **201**: 864-868.
- 28. LANGONI H., PINTO M.P., DOMINGUES P.F., LISTONI F.J.P., 1991. Etiology and sensitivity test of bovine *Salmonella* enteritidis. *Arq. Braz. Med. vet. Zootec.*, **43**: 507-515.
- 29. LARKIN L.L., VASAVADA P.C., MARTH E.H., 1991. Incidence of *Campylobacter jejuni* in raw milk as related to its quality. *Milchwissenshaft*, **46**: 428-430.
- 30. LENQERICH E.J., ADDISS D.G., MARX J.J., UNGAR B.L., JURANEK D.D., 1993. Increased exposure to *Cryptosporidium* among dairy farmers in Wisconsin. *J. infect. Dis.*, **167**: 1252-1255.
- 31. LIOR H., 1984. New extended biotyping scheme for *Campylobactor jejuni, Campylobactor coli*, and *Campylobactor laridis*. *J. clin. Microbiol.*, **20**: 636-640.
- 32. MACFADDIN J.F., 1974. Biochemical tests for identification of medical bacteria. Baltimore, USA, Williams and Wilkins, p. 213-234.
- 33. MACKENZIE W.R., SCHELL W.L., BLAIR K.A., ADDISS D.G., PETER-SON D.E., HOXIE N.J., KAZMIERCZAK J.J., DAVIS J.P., 1995. Massive outbreak of *Cryptosporidium* infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. *Clin. infect. Dis.*, 21: 57-62
- 34. MASSA S., CESARONI D., PODA G., TROVATELLI L.D., 1990. The incidence of *Listeria* spp. in soft cheese, butter and raw milk in the province of Bologna. *J. appl. Bacteriol.*, **68**: 153-156.
- 35. OMAHONY P.A., AUSTIN F.H., 1991. A study of on-farm factors affecting bacteriological quality of bulk farm milk. *Irish J. agric. Res.*, **30**: 113-119.

Revue Élev. Méd. vét. Pays trop., 1996, 49 (4) : 303-309

- 36. REINA J., BORRELL N., SERRA A., 1992. Emergence of resistance to erythromycin and fluoroquinolones in thermotolerant *Campylobacter* strains isolated from faeces, 1987-91. *Eur. J. clin. Microbiol. infect. Dis.*, 11: 1163-1166.
- 37. ROHRBACH B.W., DRAUGHON F.A., DAVIDSON P.M., OLIVER S.P., 1992. Prevalence of *Listeria monocytogenes, Campylobacter, Yersinia* and *Salmonella* in bulk tank milk, risk factors and human exposure. *J. Food Prot.*, **55**: 93-97.
- 38. SUDHARMA D., NAIR G.K., PILLAIR R.M., SULOCHONA S., 1985. Bacterial organisms associated with mastitis of cattle and goats in Kerala and their antibiograms. *Kerala J. vet. Sci.*, **16**: 99-108.
- 39. TODD E.C.D., 1992. Foodborne diseases in Canada a 10 year summary from 1975 to 1984. *J. Food Prot.*, **55**: 123-132.
- 40. TURKSON P.K., LINDQUIST K.J., KAPPERUD G., 1988. Isolation of *Campylobacter* spp. and *Yersinia enterocolitica* from domestic animals and human patients in Kenya. *Acta Pathol. Mikrobiol. Infect. Scand.*, **96**: 141.146
- 41. WANG J.J., HAN L.L., MA J.X., ZANG X.M., MA L.K., 1985. Survey of carriers of *Campylobacter* in domestic animals. *Chin. J. vet. Sci. Technol.*, 1: 27-28.
- 42. WOOD R.C., MACDONALD K.L., OSTERHOLM M.T., 1992. *Campylobacter* enteritis outbreak associated with drinking raw milk during youth activities: a 10 year review of outbreaks in the United States. *JAMA*, **268**: 3228-3230.

Reçu le 29.8.96, accepté le 28.3.97

Résumé

Adesiyun A.A., Webb L.A., Romain H., Kaminjolo J.S. Prévalence de *Salmonella*, *Listeria monocytogenes*, *Campylobacter* spp., *Yersinia enterocolitica* et *Cryptosporidium* spp. dans du lait en vrac, des matières fécales de vaches et des effluents de fermes laitières à Trinidad

La prévalence de Listeria, Salmonella, Campylobacter, Yersinia et Cryptosporidium dans du lait en vrac, les matières fécales des vaches laitières et des effluents à lait des fermes laitières de l'île de la Trinité, a été evaluée. Des 177 échantillons de lait en vrac testés, 3 (1,7 p. 100), 3 (1,7 p. 100) et 2 (1,2 p. 100) étaient positifs respectivement pour Listeria monocytogenes, Salmonella spp. et Yersinia enterocolitica mais négatifs pour Campylobacter spp. et Cryptosporidium spp. Des 333 échantillons fécaux testés, L. monocytogenes, Campylobacter spp. et Cryptosporidium spp. étaient présents dans respectivement 11 (3,3 p. 100), 19 (5,7 p. 100) et 7 (2,1 p. 100) des cas. Ces taux étaient signifi-cativement (P < 0,05; χ^2) plus élevés, que le taux de détection (0 p. 100) pour Yersinia spp. Salmonella spp. a été detectée dans 3 (0,9 p. 100) échantillons fécaux. Des 168 échantillons prélevés des effluents à lait, Campylobacter spp. était présent dans 7 (4,2 p. 100) échantillons alors que Salmonella spp. l'était dans seulement 1 (0,6 p. 100) échantillon. Aucun échantillon n'était positif pour Yersinia spp. et Listeria spp. (différence significative, $P \le 0.05$; χ^2). Sur 26 isolats de *Campylobacter* spp., 19 (73,1 p. 100) étaient résistants à au moins un des six agents antimicrobiens testés. Les 14 isolats (100 p. 100) de L. monocytogenes étaient résistants à au moins un agent antimicrobien alors que les souches de Salmonella spp. et Y. enterocolitica étaient sensibles à tous les agents utilisés. En conclusion, les cinq espèces entero-pathogènes testées étaient présentes dans les fermes laitières de l'île de la Trinité. Les risques de maladie, dus à la présence de ces agents pathogènes dans le lait, existent pour le consommateur, renforçant ainsi la nécessité d'améliorer les pratiques sanitaires.

Mots-clés : Salmonella - Listeria monocytogenes - Campylobacter - Yersinia enterocolitica - Cryptosporidium - Vache laitière - Lait - Fèces - Eau usée - Exploitation laitière - Contamination - Trinité et Tobago.

Resumen

Adesiyun A.A., Webb L.A., Romain H., Kaminjolo J.S. Prevalencia de *Salmonella*, *Listeria monocytogenes*, *Campylobacter* spp., *Yersinia enterocolitica* y *Cryptosporidium*, en leche de tanque (no embalada), en heces de vacas lecheras y en efluentes de lecherías en Trinidad

Se investiga la prevalencia de Listeria, Salmonella, Campylobacter, Yersinia y Cryptosporidium spp. en leche de tanque (no embalada), en heces de vacas lecheras y en efluentes de lecherías en Trinidad. De las 177 muestras de tanque obtenidas, 3 (1,7 p. 100), 3 (1,7 p. 100) y 2 (1,2 p. 100) fueron positivas para L. monocytogenes, Salmonella spp. y Y. enterocolitica, respectivamente, pero todas fueron negativas para Campylobacter y Cryptosporidium spp. De las 333 muestras fecales, L. monocytogenes, Campylobacter spp. y Cryptosporidium spp. se detectaron en 11 (3,3 p. 100), 19 (5,7 p. 100) y 7 (2,1 p. 100), respectivamente, resultados significativamente (P < 0,05; χ^2) mas elevados que la tasa de deteccion (0 p. 100) para Yersinia spp. Salmonella spp. se detectó en 3 (0,9 p. 100) de las muestras fecales. De las 168 muestras obtenidas de efluentes y sometidas a cultivos bacterianos, Campylobacter spp. se aisló en 7 (4,2 p. 100), mientras que Salmonella se encontro unicamente en 1 (0,6 p. 100) muestra, ninguna muestra fue positiva para Yersinia o Listeria, La diferencia fue estadísticamente significativa (P < 0,05 ; χ^2). Un total de 19 (73,1 p. 100) de los 26 aislamientos de Campylobacter presentaron resistencia a uno o más de los seis agentes antimicrobianos examinados. Los quatorce (100 p. 100) aislamientos de L. monocytogenes fueron resistentes al menos a un agente, mientras que todas las cepas de Salmonella y Yersinia fueron susceptibles a todos los agentes antimicrobianos utilizados. Se concluye que los cinco enteropatógenos examinados estaban presentes en las lecherías en Trinidad y que el posibilidad de enfermedades relacionadas con la leche, debido a estos patógenos, existe para los consumidores, haciendo enfasis en la necesidad de buenas practicas sanitarias.

Palabras clave: Salmonella - Listeria monocytogenes - Campylobacter - Yersinia enterocolitica - Cryptosporidium - Vaca lechera - Leche - Heces - Agua residual - Granja lechera - Contaminación - Trinidad y Tobago.