Original Research Article

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Prevalence of *Yersinia enterocolitica* and *Cronobacter sakazakii* in Animals, Human and Dried Milk in New Valley Governorate, Egypt.

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ABSTRACT: There are limited reports that discuss the prevalence of *Yersinia enterocolitica* and *Cronobacter sakazakii* in Africa, especially in Egypt. The current study aimed to provide data about the prevalence of *Y. enterocolitica* and *C. sakazakii* in animals, humans, and dried milk in New Valley Governorate, Egypt. Samples were collected from fresh milk and feces from dairy cattle and sheep farms, in addition to human stool samples and also milk powder. The present study was the first report to isolate *Y. enterocolitica* from dried milk and *C. sakazakii* from sheep. By using bacteriological isolation, the prevalence rates of *Y. enterocolitica* in cow milk, cow feces, sheep milk, sheep feces, human stool, powdered milk and powdered infant formula were 12.5%, 14.85%, 5.71%, and 7.5%, 13.24%, 20% and 8.16%, respectively. On the other hand, the prevalence rates of *C. sakazakii* in cow milk, cow feces, sheep milk, sheep feces, of human stool, powdered milk and infant formula were 9.38%, 11.38%, 4.29%, 4.25%, 5.88%, 15%, and 6.8%, respectively. The presence of relatively high prevalence rates of *Y. enterocolitica* and *C. sakazakii* in animal and human samples reveals the high zoonotic importance of these bacteria.

KEYWORDS: Yersiniosis, Cattle, Sheep, Zoonotic pathogens.

1. Introduction

Yersinia spp., especially *Yersinia enterocolitica* (*Y. enterocolitica*), and Cronobacter spp., especially *Cronobacter sakazakii* (*C. sakazakii*), are important zoonotic pathogens that can cause many disorders in humans and animals [1]. *Y. enterocolitica* is a psychrotrophic, Gram-negative, facultative anaerobic zoonotic bacterium that belongs to the Enterobacteriaceae family [2, 3]. It causes yersiniosis which is an intestinal zoonotic infectious disease that affects humans and a wide variety of animals. The extended grazing periods of various animal populations cause severe contamination of pastures with feces, which increases the infection rate. Yersiniosis are foodborne illness; it is transmitted to humans through raw or undercooked meat, fresh or pasteurized milk, and dairy products.[4, 5]. *C. sakazakii* can cause many disorders in humans and animals [1] . The presence of *C. sakazakii* in animal feces poses a significant danger for environmental contamination and demonstrates the zoonotic potential of *C. sakazakii* infection [6]. Enterobacter sakazakii was the old name for *Cronobacter sakazakii*. It is an Enterobacteriaceae family member that is a motile, peritrichous, rod-shaped, Gram-negative bacterium. Besides that, this bacterium is frequently catalase-positive, oxidase-negative, and facultative anaerobic [7]. Food-producing animals and their food products are the major reservoirs of *C. sakazakii* and contribute to the infection cycle of humans as the main sources of Cronobacter species include meat, meat products, milk, milk powder, and powdered infant formula [7, 8]. Meningitis, septicemia, sepsis, and necrotizing enterocolitis (NEC) are all fatal neonatal illnesses that are caused by the opportunistic bacterium *C. sakazakii*. Moreover, Cronobacter infection has been linked to mortality rates in infants that range from 33 to 80%. In addition to infants, adults, and older children may be infected, and these infections can cause serious diseases such as septicemia, pneumonia, osteomyelitis, wound infections, and splenic abscesses [9, 10]. Despite the growing research interest in *Y. enterocolitica*, and *C. sakazakii* there is little data on the prevalence of these

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bacteria in Egypt, so this study aimed to provide data about the prevalence of isolated *Y. enterocolitica* and *C. sakazakii* in animals, humans, and dried milk in New Valley Governorate, Egypt.

2. Material and methods

2.1. Ethical declaration

This study was carried out according to the guidelines of the Institutional Animal Care and Use Committee of Assiut University. The collection of samples was approved with Institutional Approval Number (04-2023-200252).

2.2. Study area

This study was carried out in the New Valley Governorates. The new valley is the largest governorate in Egypt, consisting of roughly half of Egypt's area. It is in the southwestern part of the country, between the Nile, northern Sudan, and southeastern Libya. The study included the main center of the New Valley Governorate, El-Kharga, as shown in Fig. 1 .

2.3. Sampling

From the New Valley Governorates, 982 samples were collected which included fresh milk (230) and feces (282) from dairy cattle and sheep farms and human stool samples (136) were collected from different laboratories in El-Kharga. Moreover, milk powder (40) and infant formula (n=294) were collected from different supermarkets and houses.

Collection of samples

About ten grams of each fresh fecal sample were aseptically collected from cattle and sheep by using a sterile plastic spoon and vials according to [11]. Stool samples were aseptically collected from humans in a clean, sterile, clear plastic bag that may be opened over a toilet to collect it [12]. Milk samples were aseptically collected from cattle and sheep, from four quarters in dry, sterile vial condition after cleaning and disinfecting of milking equipment, and Proper hygienic preparation of udders according to [13]. Dried milk samples were aseptically collected from different supermarkets and infant formula samples were collected by mothers who feed their babies this formula. Dried milk and powdered infant formula samples were collected in aluminum foil bags according to [14]

Preparation of samples:

One gram of each sample was thoroughly mixed with nine ml of buffered peptone water (BPW) to make (1/10) dilution. In a sterile test tube, one ml of the dilution was mixed with nine ml of BPW.

Isolation of Y. enterocolitica:

Isolation of *Y. enterocolitica* was carried out according to the International Organization for Standardization (ISO) 10273:2017 [15]. The procedures were done as follows: The prepared samples were inoculated into a sterile test tube containing phosphate-buffered saline with the addition of 0.15% bile salts and 1% sorbitol and homogenized for 2 minutes then incubated at 25 °C for two to three days. Samples were subsequently combined with 0.5% potassium hydroxide (KOH). a loop-full of the previous mixture was streaked onto Yersinia selective agar base with Yersinia selective supplement and incubated for 24 h at 25°C for 48 h. Typical *Y. enterocolitica* appeared as a deep red center with a sharp border surrounded by a clear colorless zone with an entire edge (characteristic bull's eye appearance colonies). Colonies presumed to be *Y. enterocolitica* were transferred to trypticase soya agar (TSA) slopes and then incubated at 37 °C for 24 h, typical colonies on TSA slopes appeared as yellow-pigmented. The TSA slopes were preserved for further identification.

dentification of suspected colonies

The suspected pure colonies of *Y. enterocolitica* were identified morphologically and biochemically according to [16] Isolation of *C. sakazakii* according to [17] and [18]: One ml of the prepared samples was pre-enriched aerobically in nine ml of BPW at 37ºC for 24 h. From

Table 1: Prevalence of *Y. enterocolitica* in different animal species

Animal	Total No.	Positive No.	Positive %	Chi-square	Asymp. Sig.
Cattle	362	50	13.81	2.333	0.127
Sheep	150	10	6.67		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

each pre-enrichment tube, one ml was inoculated into nine ml was inoculated into a sterile test tube containing nine ml Enterobacteriacae enrichment broth (EEB) and another one ml from each pre-enrichment tube was inoculated into nine ml of tryptone soya broth (TSB), then each tube was incubated at 37 °C for 24 h. A loop full from each sample of incubated broths was streaked on MacConkey agar and chromogenic Cronobacter isolation agar, modified Druggan, Forsythe, and Iversen (mDFI), at 37°C for 24 h. The suspected colonies on MacConkey agar were the pink-mucoid (lactose fermenter colonies). Typical *C. sakazakii* colonies on mDFI appear as blue/ green colonies. Colonies that were presumed to be *C. sakazakii* were transferred to trypticase soya agar (TSA) slopes and then incubated at 37 °C for 24-48 h, typical colonies on TSA slopes appeared as yellow-pigmented colonies. The TSA slopes were preserved for further identification.

Identification of suspected colonies:

The suspected pure colonies of *C. sakazakii* were identified morphologically and biochemically according to [19].

2.4. Statistical analysis

The statistical analysis was carried out using Chi-square using SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to [20]. The significance level was set at < 0.05.

3. Results & Discussion:

Y. enterocolitica and *C. sakazakii* infections are the predominant foodborne zoonotic illness in the world. These

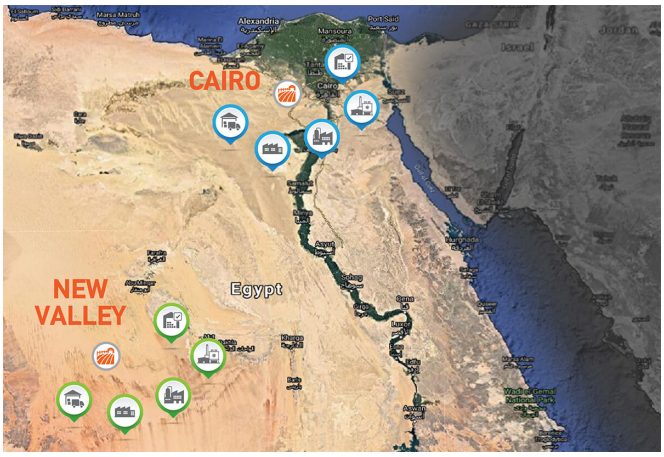

Figure 1: The locations of the study areas: New Valley Governorate, Egypt

Table 2: Prevalence of *Y. enterocolitica* in different types of collected animal, human, and dried milk samples.

Examined samples	Total No.	Positive No.	Positive %	Chi-square	Asymp. Sig.
Cattle milk	160	20	12.50	12.048	0.061
Cattle feces	202	30	14.85		
Sheep milk	70	4	5.71		
Sheep feces	80	6	7.50		
Human stool	136	18	13.24		
Milk powder	40	8	20.00		
Infant formula	294	24	8.16		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

infections primarily originate from food-producing animals and foods of animal origin like milk and milk products [21, 22]. Our results inFig. 1 revealed that the prevalence of *Y. enterocolitica* in cattle was 13.81%. The same prevalence was detected by [23], but different prevalence rates in cattle were reported in previous studies; 26% and 2.9 %, respectively [24]. On the other hand, the prevalence of *Y. enterocolitica* in sheep was 6.67%, a nearly similar result (5.1%) was reported in Iraq [25], but a higher prevalence in sheep (15.2%) was detected by [24]. The data illustrated in Table 2 showed that the prevalence of *Y. enterocolitica* was 12.5% in fresh cow milk samples and nearly the same percent (14.2%, and10.9%, respectively) was estimated by other researchers [26, 27]. In

Table 3: Prevalence of *C. sakazakii* in different animal species.

Animal	Total No.	Positive No.	Positive %	Chi-square	Asymp. Sig.
Cattle	362	38	10.50	2.250	0.134
Sheep	150	8	5.33		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

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Table 4: Prevalence of *C. sakazakii* in different types of collected animal, human, and dried milk samples

Examined samples	Total No.	Positive No.	Positive %	Chi-square	Asymp. Sig.
Cattle milk	160	15	9.38	10.069	0.122
Cattle feces	202	23	11.38		
Sheep milk	70	3	4.29		
Sheep feces	80	5	6.25		
Human stool	136	8	5.88		
Milk powder	40	6	15.00		
Infant formula	294	20	6.80		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

contrast, our result is higher than some previous studies; 7.6%, and 4%, respectively [28]. On the other hand, a higher prevalence rates of *Y. enterocolitica* were observed in another studies in India (64 %) [29] and in Egypt (36%), also [30] estimated a higher prevalence of *Y. enterocolitica* (34%) in examined cow milk samples. On the other hand, the prevalence of *Y. enterocolitica* in sheep milk was 5.7% in the New Valley Governorate, Egypt. A totally matched result was reported in Iran [31] and a nearly similar result (4.5%) was recorded in Italy [32]. Unsanitary condition on dairy farms, especially during milking, is the main cause of fecal contamination of fresh milk with *Y. enterocolitica* [33]. Our data exemplified that the prevalence of *Y. enterocolitica* in bovine feces in the New Valley Governorate, was 14.84%. Our result was higher than that reported in at Kaliobia Governorate (8.6%) [34], but it was lower than that previously reported in India (39.3%) [35] The prevalence of *Y. enterocolitica* in sheep feces, in was 7.5%. A nearly similar result was recorded in previous papers (8% and 5.16%, respectively) [4]. On the other hand, our result is mismatched with the results obtained in Kaliobia Governorate, Egypt, as *Y. enterocolitica* failed to be isolated from sheep feces [34]. Our results revealed that animals' feces is considered the source of infection for *Y. enterocolitica* because this pathogen is found in the digestive tracts and spreads in the environment through animals' feces[36] The prevalence of *Y. enterocolitica* in human stool was 13.24% and a nearly similar result (17%) was reported by [37]. On the contrary, a higher percentage of yersiniosis (50%) in human stool was reported in Mansoura Governorate, Egypt [38], also some previous studies recorded different prevalence rates; 55.4%, 2.4%, and 0.6%, respectively [39, 40, 41]. The prevalence of *Y. enterocolitica* in collected milk powder and powdered infant formula samples was 8%, and 24%, respectively, so this study revealed the important role of milk powder and powdered infant formula as a source of *Y. enterocolitica* infection in human. These types of products act as nutrient-rich media, it support the growth of bacteria [42]. Inadequate hygienic procedures in the production process of these products allow the contamination with Enterobacteriaceae [43]. children who consume significant amounts of powdered infant formula and powdered milk are more susceptible to diseases of the digestive system due to their incomplete immunity [44]. In Table 3, the prevalence rates of *C. sakassaki* in cattle and sheep were 13.8% and 6.67%, respectively. These results confirmed the zoonotic importance of cattle and sheep to the transmition of *C. sakassaki* infections. a Previous study repoted a lower prevalence (2.5%) in Egypt [45]. As shown in Table 4, the prevalence rates of *C. sakazakii* isolated from cattle feces and sheep feces were 11.38% and 6.25%, respectively. A nearly similar result of cattle feces (7.5%) was previusly reported in Egypt [45], but a higher result (37%) was estimated in japan [46]. Our results revealed the important role of animal feces in transmision of *C. sakazakii* from the infected animal to other animals through contamination of the farm environment [6]. In the current study, the prevalence rates of *C. sakassaki* in cow milk and sheep milk were 9.38% and 4.29%, respectively, a nearly similar result in cow milk (6.6%) was reported in Libya [47], but a lower prevalence (1.8%) was assumed in India [48]. Raw milk is a significant medium through which these bacteria are transferred in dairy products, and the animals and environmental sources of contamination with these pathogenic bacteria play an important role in the transmition of the infection [49] In New Valley Governorate, the prevalence of C. sakzakii in human stool was 5.88% and this result is

the same result of [19] and nearly similar to that reported in Iraq (6.5%) [50]. Previous study reported a higher result in the USA (55%)[51]. The data illustrated that the prevalence of isolated *C. sakazakii* from powdered infant formula was 6.8%. A totally matched result was assumed by [52] and nearly similar results were reported in previous studies; 4%, and 5.8%, respectively [27, 52]. In contrast, our result was lower than the reported result in Assuit, Egypt, (48%) [22]. The data revealed that the prevalence of isolated *C. sakazakii* from powdered milk was 20 % and this result is totally matched with that assumed in SaudArabi [53]. Unlike, other researchers did not isolate *C. sakazakii* from powdered milk in Egypt [54, 45]. Our results revealed that powdered milk and milk formulas are important vehicles for *C. sakazakii*, so it is the main cause of neonatal infections [55].

Conclusion:

This study provides information regarding the prevalence of *Y. enterocolitica* and *C. sakazakii* in animal, human, and dried milk. These bacteria have a negative effect on public health, the dairy industry, and consumer health, the presence of a relatively high prevalence of *Y. enterocolitica* and *C. sakazakii* in animal and human samples in the New Valley, Egypt reveals the high zoonotic importance of these bacteria. Animal feces, fresh milk and powdered milk act as an important source of *Y. enterocolitica* and *C. sakazakii* and contribute to food-borne infection.

Recommendations

The application of strict proper management and hygienic measures in animal farms is recommended to prevent the fecal-oral route of animal infection. It's essential for people to preserve sanitary practices and personal hygiene to protect themselves from infection with food-borne pathogens by washing of hands after contact with animals or raw milk and disinfection of any surfaces or utensils that come in contact with raw milk or any animal products. Boiling of milk before consumption or its use in dairy products is recommended.

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