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Prevalence and risk factors of Campylobacteriosis in Animals and Humans in New Valley Governorate, Egypt.

Mohamed Gomaa Sayed [1], Nehal Kamal Alamedldin [1], Mohamed Ezzat Kholief [1] Mohamed Said Diab [1]

[1]Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, New Valley University, Kharga Oasis, Egypt

*Corresponding author: mohamedgomaa@vet.nvu.edu.eg

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ABSTRACT:

*Campylobacter*iosis is a significant zoonotic disease with substantial public health and economic impacts. This study aimed to investigate the prevalence of *Campylobacter* spp. in animals and humans in the New Valley Governorate, Egypt, and identify potential risk factors. A total of 432 samples were collected, including animal samples (rectal swabs and milk) and human samples (stool and hand swabs). Isolation and identification of *Campylobacter* spp. were performed using conventional cultural and biochemical methods. Out of all examined samples, the overall prevalence of *Campylobacter* spp. was 32.9% in animals and 74.2% in humans. Sheep exhibited the highest prevalence (38.7%), followed by goats (27.4%), and cattle (24.3%). The most important risk factors for *Campylobacter*iosis are locality, age, sex, and health status of animals and humans. The high prevalence of *Campylobacter* spp. in both animal and human samples reveals that animal feces act as a serious source of *Campylobacter*iosis and alarm the circulation of *Campylobacter* spp. between animals and humans as an important zoonotic pathogen.

KEYWORDS: *Campylobacter* spp.,Cattle, Sheep, Goats, Risk factors.

1. Introduction

Among the human pathogens, about 61% are zoonotic, and recent diseases discovered in humans were of animal origin, often associated with animal-origin foods [1]. Among these, *Campylobacter*iosis is one of the most significant food-borne bacterial zoonosis worldwide that caused by *Campylobacter* species [2]. While the genus encompasses numerous species, *C.jejuni* and *C. coli* are the primary human pathogens [3]. *Campylobacter* species exhibit a wide host range, infecting various animals, including livestock, and wildlife. Food-producing animals, including cattle, and sheep, are key reservoirs for *Campylobacter* species. Transmission typically occurs through the fecal-oral route, with contaminated environments playing a crucial role [4, 3]. The main animal sources of *Campylobacter* infection are feces, urine, milk, aborted fetuses, and uterine discharges of infected animals. While there are many environmental sources such as farm mud, sewage sludge, surface water, farm water, contaminated animal feed, and contaminated silage. In animals, *Campylobacter* infections may lead to gastroenteritis manifestations such as enteritis, diarrhea, fever, anorexia, and dehydration, particularly calves and lambs, may experience severe manifestation, and death if untreated. Reproductive effects include abortion in the last trimester, placentitis, and infertility, particularly in ruminants. Neurological symptoms are rare but possible in systemic infections [5, 6]. For humans, the consumption of raw or undercooked contaminated food is the most critical source of *Campylobacter* infection, such as raw or unpasteurized milk, untreated water, undercooked poultry and eggs, seafood, red meat, and meat products [7, 3]. In addition, contact with infected animals, or exposure to environments contaminated with animal feces are important sources of infection [8, 9]. Human *Campylobacter*iosis has usually appeared as acute gastroenteritis with fever, abdominal cramps, and diarrhea, often watery, but may become bloody in severe cases. Other symptoms may include nausea, vomiting, headache, and myalgia. The illness is the most often self-limiting, with symptoms

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declining after a few days up to two weeks, although complications like bacteremia, meningitis, reactive arthritis, and Guillain-Barré syndrome may occur in infants, young children, and patients with the compromised immune system, elderly, pregnant women, and newborns as they at a greater risk of severe *Campylobacter* infections [10, 11, 12]. The current study aims to investigate the prevalence of *Campylobacter* in animals and humans in New Valley Governorates, Egypt, identify the most important risk factors associated with *Campylobacter* infection, and suggest the most important prevention and control measures.

2. Material and method

2.1. Ethical declaration:

This study was conducted in accordance with the ethical guidelines of the "Institutional Review Board" of the Faculty of Veterinary Medicine, New Valley University (Approval Number: NVREC 0213-20249). Informed consent was obtained from all farm owners involved in the study after explaining the study objectives and procedures.

2.2. Study area and design:

The study was conducted in the New Valley Governorate, Egypt, from November 2023 to December 2024. The New Valley Governorate is a vast oasis region located in southwestern Egypt, known for its fertile oases surrounded by desert (Figure. 1). The study included El. Dakhla, Balat, and El. Kharga Centers in the New Valley Governorate.

2.3. Sampling:

Milk and rectal swabs were collected from diarrheic and non-diarrheic animals of different ages and sex. On the other hand, stool samples were collected from clinical labs, hospitals, and farm workers. A total of 432 samples were collected, including: cattle rectal swab (n=62), sheep rectal swab (n=75), goat rectal swab (n=70), cattle milk (n=41), sheep milk (n=36), goat milk (n=36), human stool samples (n=66) and hand swab (n=46). All samples were transported to the microbiology laboratory of the Faculty of Veterinary Medicine, New Valley University with the minimum delay as possible.

2.4. Bacteriological examination of Campylobacter Spp.:

Isolation of *Campylobacter* was done following the instruction of the International Organization of Standards (ISO10272-1) method [13]. One milliliter from the collected samples was mixed with 9 mL Bolton selective enrichment broth (HiMedia) supplemented with selective antibiotics (cefoperazone, vancomycin, trimethoprim, Amphotericin B) and 5% lysed horse blood then incubated at 37°C for 6 hours under microaerobic conditions in Co2 incubator (5% of O2, 10% of CO2 and 85% of N2) and then, transferred at 42°C for 48 hours of incubation for resuscitation. After incubation, a loopful of enriched broth was streaked onto modified charcoal cefoperazone desoxycholate agar (MCCDA) (HiMedia) plates with a selective supplement (Cefoperazone, Amphotericin B) and incubated at 42°C under microaerobic conditions for 48 hours. The characteristic colonies were round, creamy-grey, moist in texture, and slightly raised colonies on MCCDA plates.

2.5. Identification of Campylobacter spp.:

Suspected colonies were examined microscopically for characteristic morphology according to [14]. In addition, biochemical tests were performed for definitive identification[15].

3. Results

The results presented in Table 1 showed that the overall prevalence of *Campylobacter* spp. was 32.9%, and 74.2% in animals, and humans, respectively. The prevalence of *Campylobacter* spp. was higher in sheep 38.73% than in goats and cattle (27.4%, and 24.3%) respectively, as shown in Table 2. The results illustrated in Table 3 showed that the prevalence of *Campylobacter*iosis in cattle in relation to locality was higher in Balat 29.2% and

lower in El-Kharga 12.5%, in sheep the occurrence was higher in El-Dakhla 48.7% and lower result in El-Kharga 24.1%, and in goat the higher occurrence obtained from El-Dakhla 34.6% and lower result in Balat 21.8%. As shown in Table 4 the prevalence of *Campylobacter*iosis was higher in human stool samples obtained from El-Kharga 78.6% than in Balat 77.5% and El-Kharga 58.3%, and the occurrence was higher in hand swabs obtained from Balat than in other locality. Considering sex as a risk factor, the results shown in Table 5 show that infection rates in female animals are higher than males, except for cattle, they are higher in males than females. By contrast, the prevalence of *Campylobacter*iosis in humans was 50% higher in females than in males at 43.05% as shown in Table 6. Considering the age effect the data presented in Table 7 revealed that, the prevalence of *Campylobacter*iosis in cattle was higher in the age group of 1<6 months, while the microorganism was not isolated in the age group 2 to < 4 years. Additionally, the prevalence rate in sheep and goats was higher in the age group 1 to > 6 months at 48.4% and <4 at 44.4% respectively. The data in Table 8 shows that the prevalence of *Campylobacter*iosis in humans in relation to age was higher in age group 1Mounth < 6 Years 75% and lower in age group 6<16 Years 33.33%.

Table 1: Overall prevalence of *Campylobacter* spp. based on Rectal, Stool, and Environmental Samples.

Sample	Total No.	Positive		Chi-square	P-value
		No.	%		
Animal rectal swab	207	68	32.9	15.710	0.000***
Human stool	66	49	74.4		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
: High significant (P<0.01) *: Very high significant (P<0.001)

The results in Table 9 show that the prevalence of *Campylobacter*iosis in Diarrhetic animals is higher than in Non-Diarrhetic animals. The data illustrated in Table 10 revealed that the Occurrence of *Campylobacter*iosis in Diarrhetic patients was 84.71% higher than in Non-Diarrhetic patients 59.26%. The results presented in

Table 2: Prevalence of *Campylobacter*iosis in relation to animal species.

Animal	Total No.	Positive		Chi-square	P-value
		No.	%		
Cattle	103	25	24.3	4.200	0.122
Sheep	111	43	38.7		
Goat	106	29	27.4		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
: High significant (P<0.01) *: Very high significant (P<0.001)

Table 3: Prevalence of *Campylobacter*iosis in animals in relation to locality

Animal	Location	Total No.	Positive		Chi-square	P-value
			No.	%		
Cattle	Balat	65	19	29.2	34.931	0.000***
	El-Dakhla	22	4	18.2		
	El-Kharga	16	2	12.5		
Sheep	Balat	45	18	40.00		
	El-Dakhla	37	18	48.7		
	El-Kharga	29	7	24.1		
Goat	Balat	55	12	21.8		
	El-Dakhla	26	9	34.6		
	El-Kharga	25	8	32.00		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
: High significant (P<0.01) *: Very high significant (P<0.001)

Table 4: Prevalence of *Campylobacter*iosis in humans in relation to locality.

Sample type	Location	Total No.	Positive		Chi-square	P-value
			No.	%		
Stool	Balat	40	31	77.5	59.565	0.000*
	El-Dakhla	12	7	58.3		
	El-Kharga	14	11	78.6		
Hand swab	Balat	25	2	8		
	El-Dakhla	10	0	0		
	El-Kharga	11	0	0		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
: High significant (P<0.01) *: Very high significant (P<0.001)

Table 5: Prevalence of *Campylobacter*iosis in animals in relation to sex.

Sex	Animal	Total No.	Positive		Chi-square	P-value
			No.	%		
Male	Cattle	12	6	50.00	16.761	0.005**
	Sheep	25	9	36.00		
	Goat	22	6	27.3		
Female	Cattle	91	19	20.9		
	Sheep	86	34	39.5		
	Goat	84	23	27.4		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
: High significant (P<0.01) *: Very high significant (P<0.001)

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Table 6: Prevalence of *Campylobacter*iosis in humans in relation to sex.

Sex	Total No.	Positive		Chi-square	P-value
		No.	%		
Male	72	31	43.1	0.527	0.468
Female	40	20	50.00		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
∗∗: High significant (P<0.01) ∗∗∗: Very high significant (P<0.001)

Table 7: Prevalence of *Campylobacter*iosis in animals in relation to age

Animal	Age	Total No.	Positive		Chi-square	P-value
			No.	%		
Cattle	0-<6 M	20	14	70.00	72.781	0.000***
	6 M-<1 Y	11	5	45.5		
	1-<2 Y	7	1	14.3		
	2-<4 Y	4	0	0.00		
	>4 Y	61	5	8.2		
Sheep	0-<6 M	31	15	48.4	8.755	0.068
	6 M-<1 Y	19	9	47.4		
	1-<2 Y	22	7	31.8		
	2-<4 Y	16	5	31.3		
	>4 Y	23	7	30.4		
Goat	0-<6 M	25	8	32.00	18.159	0.001**
	6 M-<1 Y	22	5	22.7		
	1-<2 Y	21	3	14.3		
	2-<4 Y	20	5	25.00		
	>4 Y	18	8	44.4		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
∗∗: High significant (P<0.01) ∗∗∗: Very high significant (P<0.001)

Table 8: Prevalence of *Campylobacter*iosis in humans in relation to age

Age	Total No.	Positive		Chi-square	P-value
		No.	%		
1M-<6Y	12	9	75.00	19.159	0.000***
6-<16 Y	18	6	33.3		
16-60 Y	70	30	42.9		
>60 Y	12	6	50.00		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
∗∗: High significant (P<0.01) ∗∗∗: Very high significant (P<0.001)

Table 9: Prevalence of *Campylobacter*iosis in animals in relation to health status.

Animal	Health Status	Total No.	Positive		Chi-square	P-value
			No.	%		
Cattle	Diarrhetic	39	17	43.6	11.951	0.001**
	Non-Diarrhetic	23	4	17.4		
Sheep	Diarrhetic	43	19	44.2	5.232	0.022*
	Non-Diarrhetic	32	8	25.00		
Goat	Diarrhetic	41	14	34.2	3.073	0.080
	Non-Diarrhetic	29	6	20.7		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
∗∗: High significant (P<0.01) ∗∗∗: Very high significant (P<0.001)

Figure 1: Map of New Valley Governorate showing the location of the study area.

Table 10: Prevalence of *Campylobacter*iosis in humans in relation to health status.

Health Status	Total No.	Positive		Chi-square	P-value
		No.	%		
Diarrhetic	39	33	84.6	4.694	0.030*
Non-Diarrhetic	27	16	59.3		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
∗∗: High significant (P<0.01) ∗∗∗: Very high significant (P<0.001)

Table 11 show that the prevalence of *Campylobacter*iosis was higher in individuals who contact with animals than in non-contact

4. Discussion:

*Campylobacter*iosis is one of the leading causes of bacterial foodborne illnesses worldwide, with fatality rates typically lower than 1%, but capable of causing severe morbidity, especially in immune-compromised individuals, young children, and the elderly [7]. Our results shown that the overall prevalence of *Campylobacter* spp. in animal samples was 32.9 %. This finding is comparable to the prevalence of 30.9% reported in livestock in Kajiado County, Kenya [16]. However, it is higher than the prevalence reported in other studies; 20.06% in Assiut, Egypt

Table 11: Prevalence of *Campylobacter*iosis in humans in relation to animal contact.

Items	Total No.	Positive		Chi-square	P-value
		No.	%		
Contact	67	31	46.3	0.044	0.833
Non-contact	45	20	44.4		

∗: Non significant (P>0.05) ∗: Significant (P<0.05)
∗∗: High significant (P<0.01) ∗∗∗: Very high significant (P<0.001)

[17], 14.6%, 15.7% and 12.9% in Ethiopia [18],[19] and [20], respectively, 17.69% in Catalonia, Spain [21], and 28.8% in South Africa [22]. The obtained result revealed that the overall prevalence of *Campylobacter* spp. in humans (stool samples) was 74.2% which is nearly similar to findings reported in Ethiopia (72.7%)[23] and Egypt (76.9%) [24]. However, it is higher than a previous studies reported in Cambodia (12%) [25, 26], Bangladesh (31.5%), Egypt (16.7% and 66.6%) [27] and [28], respectively, Ethiopia (9%) [18], and Iran (8.4) % [29]. In contrast, it is lower than the prevalence reported in other Egyptian studies (90.91%) [30], 85.7% (Ammar et al., 2021). The *Campylobacter* infection rates were higher in humans than in animals with a very high significant difference (P value <0.01). Many factors can affect the prevalence of *Campylobacter* spp. such as animal species, sample type, geographical location, age, and gender. Our findings revealed varying prevalence rates among different animal species. Sheep exhibited the highest prevalence (38.7%), followed by goats (27.4%), and cattle (24.3%), but statistically, there wasn't a significant difference (P value = 0.122). This finding is consistent with a previous study in Ghana that recorded higher prevalence in sheep and goat (18.5%, and 18.6%, respectively) compared to cattle (13.2 %) [31], and with a previous study in Ethiopia that recorded higher prevalence in sheep (38%) than cattle (12.6%) [32]. However, it disagrees with (Ocejo et al[33]) who reported a higher prevalence in cattle (81.2%) than sheep (45.2%), and with (G. Chala et al.,[34]) who recorded that the most susceptible animal for *Campylobacter*iosis is cattle (18.5%) followed by sheep (13.3%) then goat (7.1 %). Our results showed that the prevalence of *Campylobacter*iosis varied across different regions within the New Valley Governorate. The prevalence in sheep and goat samples collected from El. Dakhla center were higher than that in Balat and El. Kharge center, but the prevalence of *Campylobacter*iosis in cattle samples collected from Balat center was higher than that in El. Dakhla and El. Kharga Center. There is a statistically high significant difference between the results (P value <0.01). In contrast, a higher prevalence of *Campylobacter*iosis in humans was detected in El. Kharga center (78.6%) than in Balat center (77.5%) and El. Dakhla center (58.3%), there is a statistically high significant difference between the results (P value <0.01). These results illustrated that the prevalence of *Campylobacter*iosis is affected by geographical distribution and relative population size in every region, also the climatic conditions, sampling season. In addition, contact with the infected farm animals and pet animals, higher consumption of raw and undercooked meat and poultry, unpasteurized milk, ready-to-eat food, and contaminated water [35, 36, 9, 37]. According to our results, female sheep and female goats displayed a higher prevalence (39.5, and 27.4%, respectively) than males (36%, and 27.3% %, respectively), while the opposite pattern was observed in cattle, males was higher than females (50%, and 20.9%, respectively) with a high statistically significant different between the results (P value <0.01). Hormonal, physiological factors and immunity may contribute to variations in susceptibility to *Campylobacter* infection [38, 39]. In our study, a higher prevalence was observed in females (50%) than males (43.1%), this difference was not statistically significant. This finding is consistent with a previous study that reported a higher prevalence in females than males in Zuru Kebbi State, Nigeria [40], but it contradicts other studies that found a higher prevalence in males than females in South Africa [41], and Beijing, China [37]. Sex-related differences in susceptibility to

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Campylobacter infection may be influenced by various factors, including sex hormone influences, immune responses, and physiological differences between males and females [38], and may be due to behavioral differences such as handling of raw food and contaminated article [42]. Our results suggested potential age-related variations in susceptibility to *Campylobacter* infection. In cattle and sheep, younger animals (0-<6 months) exhibited higher prevalence, conversely, older goats (>4 years) showed higher prevalence, this difference was not statistically significant. This finding is consistent with previous studies that reported the highest prevalence in young animals [43], and newborns [44]. This finding is potentially due to immature immune systems [45, 46] in relation to age, the highest prevalence of *Campylobacter*iosis observed in the age group (1 month < 6 years) (75%) and the age group (>60 years) (50 %) with a very high significant different between the results (P value <0.001). This result agrees with a previous study that reported a nearly similar prevalence in children in the age group (0-12 months) (78.8%) [47], and also agrees with a study in sub-Saharan Africa found that the highest prevalence was in children below 5 years [48]. On the other hand, our result disagrees with a study in Zuru Kebbi State, Nigeria reported that the prevalence of *Campylobacter*iosis was higher in age group (>30 years) (73.5%) than the youngest age group (0-9 years) (56.8%) [40]. According to the results, the prevalence of *Campylobacter*iosis in diarrheic animals was higher than in non-diarrheic animals with a statistically significant difference in cattle and sheep samples and not in goat samples (P value = 0.001**, 0.022*, and 0.080, respectively). This result agrees with [44] who reported that the prevalence of *Campylobacter* was 100% in diarrheic cattle and sheep, [17] who reported that the prevalence was higher in the diarrheic calves (23.4%) than from the apparently healthy calves (10.83%), and [49] who reported that the isolation rate of *Campylobacter* was higher in diarrheic sheep (72.2%) than non-diarrheic sheep (27.8%) in the Vhembe district of South Africa. The data illustrated that a higher prevalence of *Campylobacter* in diarrheic humans (84.61%) than non-diarrheic humans (59.26%) with a statistically significant difference between the two results (P value = 0.030*). Isolation of *Campylobacter* from non-diarrheic individuals highlights the importance of asymptomatic carrier in the transmission of infection [50, 51]. This result agrees with Rouby et al., [44] who isolated *Campylobacter* with 71.4% from diarrheic human samples, and 17.6% from non-diarrheic samples, and [52] who isolated *Campylobacter* with 48% from diarrheic samples, and 32.1% from the non-diarrheic sample, but this result disagrees with [53] who don't isolate *Campylobacter* from any patient stool. Our results showed a slightly higher prevalence of *Campylobacter*iosis in humans in with animal contact (46.3) than non-contact humans (44.4%), but there wasn't a statistically significant difference between the two results. Although not statistically significant (P value = 0.833), a higher prevalence was observed in individuals with animal contact, emphasizing the importance of zoonotic transmission routes and the contact with animal feces represents a common source of *Campylobacter* infection in humans [35, 33, 9].

Conclusion

The study findings highlight the need for improved hygiene practices and biosecurity measures in animal husbandry to reduce the risk of *Campylobacter* transmission. Furthermore, public health education campaigns should be implemented to raise awareness about the importance of proper food handling and personal hygiene in preventing *Campylobacter*iosis. These results underscore the importance of a One Health approach in addressing zoonotic diseases like *Campylobacter*iosis, emphasizing the interconnectedness of animal, human, and environmental health.

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Conflict of interest

The authors don't have any competing interests.

Authors' contribution

All authors cooperate with each other in work design, experimental plan, sample collection, carrying out the practical part, and lettering of the manuscript. MSD, MEA, MGS, NKA and conceived and designed the experiments. MSD, MEA, and MGS measured the parameters. NKA and MGS statistically analyzed the data. MSD, NKA, and MGS wrote the manuscript. All authors accepted and permitted this manuscript.

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