Epidemiological study of thermophlic *Campylobacter* isolated from diarrheic and non diarrheic cows in Baghdad governorate

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Summary

The study achieved isolating Campylobacter spp from diarrheic and non-diarrheic cows and studying the percentage of diarrhea in 10 areas in Baghdad governorate (Al-Shulah, Abou Ghrayb, Kadimyiah, Sadr City, Suwayrah, Mahmoodyiah, Latifyiah, Al-Radwaniyah, Howr Rijab and Yousfyia). One thousand fecal samples were cultured on different selective culture media specific for Campylobacter spp, and different biochemical tests were used (Oxidase reaction, Oxoid biochemical identification system campy, Tripple Sugar Iron (TSI), hippurate hydrolysis and the use of Vietik (NH). Eighty isolates of Campylobacter spp were recorded (8%). Campylobacter spp isolates included C. jejuni 30(3%), C. coli 30(3%) and C.lari 20(2%). High percentage of diarrhea was recorded in Howr Rijab and Abou Ghrayb 70(82.35%), 81(81.81%) respectively, while lower percentage of diarrhea was in Mahmoodyiah and Yousfyia in percentage 43(39.09%) and 50(46.72%), High percentage of *campylobacter* isolates was recorded in Al-Shulah 15(18.75%), while lower percentage of isolates was recorded in Howr Rijab and Yousfyia 5(6.25%) and 4(5%) respectively. Age group one day to one month gave the highest isolation rate 50% (40) while lowest isolation rate in age group (6 months to 1year) 5% (4) under (P<0.0001) level. The highest isolation rates were recorded in Summer 2016 (19.16%) and Summer 2017 (24.32%) while the lowest isolation rates was recorded in Winter (0.8%). Animals infected with C. jejuni and C coli, showed obvious clinical signs including depression, loss of appetite and inability to feed newborn calves, they suffered from severe diarrhea and dehydration, and the presence of blood and thick mucus, high fever were observed in some animals, while in animals infected with C. lari the clinical signs were mostly within normal limits. It can be concluded that two species of campylobacter (C. coli and C. Lari) isolated from diarriheic cows are new species added to the two species of campylobacter (C. fetus and C. Jejuni) previously isolated from cows in Iraq and increasing the total *campylobacter* species confirmed in Iraq to four spp.

Keywords: Thermophlic Campylobacter, Diarrhea, Cows.

Introduction

Neonatal calve diarrhea is a significant cause for calf mortality and morbidity during the first few weeks of life and it stand out among the most difficult clinical disorders that face animals production and leading to economic losses at cattle industry in most countries (1). A variety of pathogens including viruses, bacteria, protozoa and intestinal parasites can cause diarrhea in calves and other animals, Campylobacter is one of the most widely recognized causative agent of bacterial foodborne gastroenteritis in human and healthy domestic and wild animals because the intestinal tracts of warm-blooded are animals a natural reservoir for Campylobacter spp (2). Ruminants play an

important role in the epidemiology of this zoonosis and the disease occours sporadically in developed countries and more generally in undeveloped countries The (3). family Camplyobacteraceace includes more than 19 species and 9 subspecies (4) most of species are thermophlic, such as C. lari, C. coil and C. jejuni (5). These organisms live and colonize in the mucosal surfaces of intestinal tracts, oral cavities, or urogenital tracts of a variety animal including chickens, turkeys, dogs, cat, cattle, sheep and goats (6). Cattle including dairy calves represent strong reservoirs of C. jejuni and represent a risk for contamination of environment (7). Campylobacter is recognized as an important zoonotic pathogen of worldwide economic significance.

Campylobacteriosis in cattle can cause sporadic abortions, temporary or permanent sterility, irregular heats due to early embryonic death, and disruption of the breeding regime. This can lead to heavy economic losses which occur not only from mortality but also from treatment costs and time spent on care as well as subsequent chronic ill thrift and impaired growth performance (8). Campylobacteriosis causes significant reproductive wastage in infected beef and dairy herds and represents a large economic loss for producers, particularly in the first year of infection (9). The aim of this study was to achieve isolating Campylobacter spp from diarrheic and nondiarrheic cows and to study the percentage of diarrhea in 10 areas in Baghdad governorate.

Materials and Methods

A total of 1000 fecal samples from diarrheic and non-diarrheic cattle and calves were collected from Veterinary Clinic and slaughter houses from (May 2016 to July 2017) from different areas in Baghdad governorate (Al-Shulah. Ghravb. Abou Kadimyiah, Suwayrah, Sadr City, Mahmoodyiah, Latifyiah, Al-Radwaniyah, Howr Rijab and Yousfyia) by taking 67 fecal sample every month; sterile cotton swab were used for taking fecal samples and transported by using ransport media (Amies or Preston broth) via cool box as fast as possible to the laboratory. In order to isolate *Campylobacter* from samples that may contain different varieties of bacteria, selective media were used to inhibit the growth of the more rapidly growing components of the enteric bacterial flora because *Campylobacter* species multiply much more slowly than other enteric bacteria (10). To isolate *Campylobacter* spp, one gm of fecal samples was collected and enriched within 24 hrs in 1ml of Preston broth (nutrient broth no.2 CM0067B Campylobacter selective supplement SR0117E and lyzed horse blood SR0048, Oxoid England), Incubation of Preston broth for 48 hrs. at 41°C took place under microaerophilic conditions by using (Campygen, CN0025 or CN0035, Oxoid England) as primary isolation then incubate at 37°C on same media and then made serial dilution in Peptone Water (CM0009B, Oxoid England). Following this, 0.1 ml of each serial

dilution was streaked onto mCCDA plate (modified Charcoal Deoxycholate Agar, Campylobacter blood-free selective medium, CM0739 and CCDA selective supplement, SR0155, Oxoid England) and was incubated for 24 to 72 hrs. at 41°C in a microaerophilic atmosphere conditions (11). One presumptive Campylobacter colony from each selective agar plate was subcultured into Columbia blood agar (Oxoid) and incubated for 24 hrs under the same conditions and tested by standard microbiological and biochemical procedures (12), differentiated at species level by gram stain, oxidase and catalase activities, hippurate hydrolysis, TSI, hydrogen sulfide production and susceptibility to nalidixic acid by using a commercially available species differentiation kit (Vitek nh compact 2 bioMérieux, Marcy-l'Etoile, France and oxoid biochemical identification system campy (O.B.I.S oxoid England). The VITEK 2 Neisseria-Haemophilus identification card (NH) is intended for use with the VITEK 2 systems for the automated identification of most clinically significant fastidious organism.

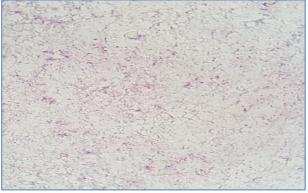
Results and Discussion

The presumptive *Campylobacter* colony from the selective mCCDA and Preston agar plate appeared as shown in (Fig. 1). Gram negative, rods, S-shaped and motile (Fig. 2). C. coli and C. jejuni and C. lari are all thermotolernt spp grow at 41°C catalase and oxidase postive and TSI negative, C. coli and C. jejuni are sensitive to naldixic acid and resistance to cephalothin, while C. lari resistance to cephalothin and naldixic acid, C. coli and C. jejuni and C. lari differ biochemically in their ability to hydrolyze sodium hippurate (HIP). C. coli cannot hydrolyze hippurate and there are some C. jejuni subspecies that are hippurate negative (13). The 80 suspected isolates were positive for o.b.i.s test of Campylobacter spp (it has been designed for the differentiation of Campylobacter, Helicobacter and Arcobacter species from other gram negative organism (Fig. 3), according to the Vietek compact results the isolate was oxidase and catalase positive, Esculin and nitrate reduction negative, sodium hippurate positive for C.

jejuni and negative for *C. coli* and *C. Lari* (Table, 1).



Figure, 1: Growth of *Campylobacter* spp on selective Preston and MCCD mCCDA plat.



Figure, 2: Morphological properties of Campylobacter spp cultured on mCCDA examined by light microscope (X100) after 24 hours.



Figure, 3: Oxoid biochemical identification system campy (3, 4.5 No) colorless of colonies indicate the organism is Campylobacter spp.

	Catalase	Oxidase	TSI	Hippurate hydrolysis	Growth at 41°C		eptibility to cephalothin	
C.jejuni C. coli	+	+	_	+	+	S	R	
C. coli	+	+	_	_	+	S	R	
C. lari	+	+	_	_	+	R	R	
W= weak reaction; S=Susceptible; R=Resistant; TSI=Triple								

W= weak reaction; S=Susceptible; K=Kesistant; ISI=Iriple Sugar Iron.

Eighty thermophilic *Campylobacter* spp were isolated in a ratio (8%) the predominant spp 30 (3%) found to be *C. jejuni*, and 30 (3%) were found *C. coli* and 20(2%) were found to be *C. lari* (Table, 2), similar results were found in a Brazilan study which showed that *Campylobacter* species specially *C. jejuni* and *C. coli*. were that most implicated in outbreaks of gastrointestinal disease (14). Our result also agreed with (15) who showed that *C. jejuni* is a common commensal in the gastrointestinal tracts of wild and farm animals and is ubiquitous in the natural environment. Our results also disagree with (16), although other worker found the prevalence of *campylobacter* colonization in cattle showed high variation extent from 0%–80%.

 Table, 2: Percentages of Campylobacter in Baghdad

 governorate.

No of fecal samples	Biotype	No of Isolate %	Chi Square Value	Р
1000	C. jejuni C. coli	30(3%) 30(3%)	2.33	0.33
	<i>C. lari</i> Total	20(2%) 80		

High significant percentages of diarrheic cows under (P < 0.0001) level were recoreded in Howr Rijab 70(82.35%) and Abou Ghrayb, 81 (81.81 %), while the lower percentage of diarrheic cows were recoreded in Mahmoodyiah 43(39.09%) and Yousfyia 50(46.72%) (Table, 3), this might be attributed to many different reasons as the environment, husbandry condition, rearing method, feeding regimen, crowding, treatment used, all these factors can account and contribute for significant difference in isolation rates and species isolate from area to area. These results are expected because of diarrhea represent a symptom of most infectious diseases, non-significant differences among diarrheic cows which were positive to campylobacter isolates between areas of study were noticed because diarrhea due to primary Campylobacter infection is not thought to be a common cause of scouring in calves however, Campylobacter may be found in fecal sample examination in combination with other pathogens or may be a secondary pathogen resulting from opportunistic infection following previous gut damage, on other hand diarrheic cows showed high percentage of Campylobacter isolates was recorded in Al-Shulah 15(20%), followed by Mahmoodyiah 7(16.27%) Kadimyiah 9(15%) Abou Ghurayb 12(14.81%), AlRadwaniyah 6 and Sadr City, (13.63%)8 (13.33%)respectively, Suwayrah 8(10%), Latifyiah and Yousfyia 6(8%), 4(8%) and Howr Rijab 5(7.14%). Al-Shulah represented the highest number of *Campylobacter* from total number of isolates (80) in ratio 15 (18.75%), followed by Abou Ghrayb 12(15%), Kadimyiah 9 (11.25%), Sadr City and Suwayrah 8(10%), Mahmoodyiah 7(8.75%), Latfyia and Al-Radwaniyah 6(7.5%), Howr Rijab 5(6.25%), and Yousfyia 4(5%) (Table, 4). This can be attributed to two general reason, first most of these animals graze near the waste dumps and slaughter houses all around the year, feeding and drinking water contaminated with fecal material of infected and aborted animals from abortion and calving, this contributes to the distribution and shedding of Campylobacter to other healthy animals, these areas were with close results mostly to increase their nearby geographically. This is supported by (17) whom showed that communicability of promptly *Campylobacter* species occurs between animals when organisms are present in feces, vaginal discharges, and the products of abortions and can be spread by direct contact, on fomites and by arthropods acting as mechanical vectors. Contaminated food and water is often the source of infections. Also our result were supported by (18) who transmission between concluded that individuals within the herd may be sufficient to maintain a steady C. jejuni population independent of environmental influx, the lowest isolation rate was recorded in southern of Baghdad Howr Rijab 5(6.25%), and Yousfyia 4(5%) compared with isolation rate in north of Baghdad and its west area. This can be attributed to the fact that these animals graze or rear on pastures and feed alfa alfa as well as interest of animals by its owner through provision of necessary treatment in case of diarrhoea through veterinary clinic and services in this regions this lead to reduce or decrease isolation rate of theromophilic Campylobacter similar result found by (19) whom showed that lower prevalence of Campylobacter is observed on the farm compared with continuous surveillance at slaughter houses.

Area	Total no of fecal	Diarrheic	positive	non diarrheic	positive
Al-Shulah	106	75 (70.75%)	15(20%)	31	0
Kadimyiah	90	60 (66.67%)	9 (15%)	40	0
Sadr City	109	60 (55.04%)	8(13.33%)	39	0
Howr Rijab	85	70 (82.35%)	5 (7.14%)	25	0
Mahmoodyiah	110	43 (39.09%)	7(16.27%)	67	0
Yousfyia	107	50 (46.72%)	4(8%)	26	0
Latifyiah	103	75 (72.81%)	6(8%)	28	0
Abou Ghrayb	99	81 (81.81%)	12(14.81%)	39	0
Al-Radwaniyah	77	44 (57.14%)	6(13.63%)	33	0
Suwayrah	114	(70.17%)	8(10%)	34	0
Total	1000	638	80	362	
Chi Square Value		82.43	10.02		

Table, 3: Percentage of	positive diarrheic cows and	percentage of	positive diarrheic to Campy	lobacter isolates.

Table,4:DistributionofCampylobacter spp. according to locations in Baghdad governorate.

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Area	% (No of isolate)	C. jejuni	C. coli	C. lari							
Al- Shulah	15(18.75%)	4	4	7							
Kadimyiah	9(11.25%)	4	3	2							
Sadr city	8(10%)	5	2	1							
Howr Rijab	5(6.25%)	0	4	1							
Mahmoodyiah	7(8.75%)	1	5	1							
Yousfyia	4(5%)	3	0	1							
Latifyiah	6(7.5%)	4	2	0							
Abou Ghrayb	12(15%)	3	5	4							
Al-Radwaniyah	6(7.5%)	1	2	3							
Suwayrah	8(10%)	5	3	0							
Total	80	30	30	20							

High significant isolation rate was recorded in young animals (1 day - 1 month) 40(50%)followed by age group (1 - 3 months)23(28.7%) and age group (3 - 6 months)13(16.25%) while non significant difference observed among aged group (6 months_1 year) 4(5%). The reason for the significant increase or high incidence of Campylobacter in calves in first age group may be due to the population of *Campylobacter* in the intestine change with the age of the animals as normal gut microflora developed. Similar results have been obtained by a number of workers: In an Austrian study (20) isolated C. jejuni and coli from calves as young as 3 days and up to 67 days, also our results agree with (21) who explained that *Campylobacter* spp are colonized in cattle, sheep and swine as higher in young animals than in older animals, and showed that in older animals, the organism are occasionally detected in feces, and this may be contributed to by low levels and its possible elimination or intertomittent excretion (Table, 5).

Table, 5: Isolation rates of Campylobacter spp.according to ages of animals.

Distribution of isolates by age	No of isolates (%)	C. jejuni	C. coli	C. lari
1day - months	40(50 %)	18	12	10
1 _3 months	23(28.75%)	11	8	4
3_6 months	13(16.25%)	0	8	5
6 months_1 year	4(5%)	1	2	1
Total	80	30	30	20
Chi Square Value	35.7			10.58
Р	<0.0001			0.10

Significant difference at P <0.0001 Chi Square Value 35.7.

Females showed high non-significant differences isolation rate of *C. jejuni* (27.5%) and *C. coli* (21.25%) and *C. lar*i (11.25%) respectively, while the male showed high significant differences (P=0.002) but the relationship between type of isolates and sex was not significant (P=0.26) (Table, 6).

Table, 6: Isolation rates of Campylobacter sppaccording to sex of animals.

Bacteria	S	Chi	Р	
	Male No (%)	Female No (%)	Square Value	
C. jejuni	18(22.5%)	22(27.5%)	2.62	0.26
C. coli	12(15%)	17(21.25%)		
C. lari	2(2.5%)	9(11.25%		
Total	32	48		
Chi Square	12.11	5.37		
Value				
Р	0.002	0.06		

High significant difference at (P=0.002) Chi Square Value 12-11. In male No significant difference at (P=0.26) Chi Square.

In this study, the relationship between months and percentage of infection of *Campylobacter* during the period of study in 10 area of Baghdad governorate was studied over a period (15 months May 2016 to July 2017), thus incorporating of all season in Baghdad governorate. Results showed the significant differences P<0.0001 among seasons, the highest percentage of infection was detected on Summer 2017 (24.32%) and Summer 2016 (19.16) while the lowest percentage of infection was detected on Winter (0.8%) (Table, 7). Our result agree with (22) who found that there is the annuals peak was in late June, early July and is more evident in rural\semi rural than urban areas. Our results also agree with (23) whom found that June and July have the highest rates of *Campylobacter* isolation at (14.68%), (12.35%) respectively.

Table, 7: Isolation of Campylobacter spp. duringdifferent seasons.

month	Number of diarrheic	No of isolates (%)
Summer 2016	167	32(19.16%)
Autumn	97	12(12.37%)
Winter 2017	122	1(0.8%)
Spring 2017	178	17(7.86%)
Summer 2017	74	18(24.32%)
Total	638	80
Chi square value		28.33
P		< 0.0001

signs and severity differed Clinical according to the causative agent, but they were all involved in various types of diarrhea (mild, moderate and severe), in animals infected with Campylobacter jejuni, animals showed clear clinical signs including lethargy, loss of appetite and inability to feed newborn calves. Thev suffered from severe diarrhea. dehydration. presence of blood and thick mucus, and there was an increased in temperature of infected animals with severe diarrhea, reached the range at (41°C) with an increase in pulse and respiration rate, which a range reached at (135) per minute in pulse rate and (50) per minute in respiration rate (10), the rest of the animals also suffered from depression, loss of appetite, but temperature, pulse and respiratory activity were in normal limits, the animals were suffering from watery diarrhea but normal in colour, also mucus or blood was not observed in the faeces of those animals and the degree of deyndration was of medium type (22). While the animals infected with C. coli suffered from depression, lethargy, wasting and loss of appetite, and high fever was observed (40.5°C) also, pulse and respiratory rate were (132 and 50 per minute) respectively (17). The animals suffered from severe bloody diarrhea and dehydration, and the presence of Thick bile-streaked mucus, the rest of the animals (13) which infected with Campylobacter coli were also suffering from depression, but loss of appetite and fever, pulse were within normal limits. Also there was not observe any mucus or blood in the faeces of calves that were infected with this type of bacteria and the degree of diarrhea and devdration was in middle type. In animals infected with Campylobacter lari the clinical signs, mostly were within normal limits including temperature (39.7), pulse rate (115) per minute, respiratory rate (30) but there was watery diarrhea, mild dehydration, with threads of mucus, but no blood in faeces of these animals, also there is anorxia, depression and lethargy. This can be attributed to the presence of cases of carrying the organism without the emergence or showing of clinical signs. In animals infected with C. jejuni and C. coli the animals suffered from severe diarrhea and dehydration, and the presence of blood, thick mucus, and Thick bile-streaked mucus and high fever were observed in some animals; this could be attributed to that this type of Campylobacter possessed virulence genes associated with invasiveness an adherence (24). Our result disagree with (25) who showed that C. jejuni could be found in the faeces of diarrheic and healthy calves, but both C. jejuni and C coli can cause a mild selflimiting enteritis and bacteremia when inoculated orally into new born calves; but the results agree with him in that many different animal species maintain Campylobacter spp, without showing clinical signs (Table, 8).

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Table.	X:	Clinical	signs	of cows	infected	with	Campylobacte	r Snn.
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Campylobacter Spp.	No.	No.	Temp. Rate	Mean ± SE	Plus	Mean ±SE	Resp. Rate	Mean ±SE	Mucus	Blood		Dgree of Diarrioea			Dgree of yhdration	
											Sever	Middle	Mild	Sever	Middle	Mild
C. jejuni	30	10	41	39.97	135	120.60	55	37.00	++T	++bl	++			++		
		12	39.5	±0.13a	115	±1.91ab	30	±2.39a	-	-W	-	+			+	
		8	39.4		111		25		-	-W	-	+			+	
C. coli	30	17	40.5	40.12	132	123.80	50	40.83		++ B	++bl	++			++	
		8	39.6	±0.07a	110	±1.80a	25	±2.03a	+	-	-		+			+
		5	39.7		118		35		+	-	-		+			+
C. lari	20	12	39.7	39.60	115	117.50	30	28.50	+	-W			+			+
		6	39.5	±0.04b	120	±0.76b	25	±0.52b	+	-W			+			+
		2	39		125		30		+	-W			+			+
LSD				0.2982		5.002		5.8993								

Means with different letter in the same column significantly different (P<0.05). ++T= Thick mucus, ++ bl= sever bloody diarrhea, -W=watery diarrhea ++B=bile streaked mucus + = mild and middle diarrhea and dehydration - = not presence.

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دراسة وبانية للكمبايلوبكتر المحبة للحرارة المعزولة من الأبقار المصابة بالإسهال وغير المصابة في محافظة بغداد

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الخلاصة

صُممت الدراسة لعزل بكتريا الكمبايلوبكتر ومعرفة نسبة الإصابة بها في الأبقار المصابة وغير المصابة بالإسهال ودراسة نسبة الإسهال في 10 مناطق من محافظة بغداد وهي (الشعلة، ابو غريب، الكاظمية، مدينة الصدر، الصويرة، المحمودية، اللطيفية، الرضوانية، هور رجب واليوسفية). زرعت 1000 عينة براز على مختلف الأوساط الزرعية والأختبارات الكميوحيوية الاختيارية الخاصة ببكتريا الكمبايلوبكتر (O.B.I.S الاوكسيديز، TSI, hippurate hydrolysis (Vietik NH . وأظهرت النتائج الحصول على 80 عزلة بكتيرية مثلت (8%) شملت العزلات ثلاثة انواع مختلفة من بكتريا الكمبايلوبكتر وهي C.Jejuni (3%) و C.coli و 30(3%) و C.lari (3%) (20(2%) . سُجلت أعلى نسبة للإسهال في هور رجب وأبو غريب بنسبة (82.35%) 70 (81.81%) على التوالي، بينما انخفضت نسبة الإسهال في المحمودية واليوسفية بنسبة (39.09%) 43 و(46.72%) وقد مثلت الشعلة أعلاها بالنسبة لعدد عز لات الكمبايلوبكتر بنسبة (١8.75%) 15 بينما سجلت منطقة هور رجب واليوسفية أقل نسبة عزل وكانت (5/6,25%), (6.25%) على التوالي أما فيما يخص تأثير العمر فقد أظهرت النتائج ان الفئة العمرية للعجول من (1 يوم -شهر) أكثر نسبة عزل وبلغت (%50/40 بينما أقل نسبة عزل سجلت في الفئة العمرية (6 شهور-سنة واحدة) بنسبة (%5) وبفارق معنوي واضح P<0.0001 أما تأثير الموسم فقد كانت أعلى نسبة للإصابة في صيف 2016 و2017 حيث بلغت النسبة (24.32%) في 2017 و (19.16%) في 2016 بينما كانت أقل نسبة عزل في فصل الشتاء حيث بلغت النسبة (0.8%) والحيوانات المصابة ب C.jejuni وC.jejuni أظهرت علامات سريرية واضحة تمثلت بالخمول وفقدان الشهية وعدم القدرة على تغذية العجول حديثة الولادة فيما يخص الحيوانات البالغة وقلة الرضاعة للعجول الصغيرة وكذلك عانت من الإسهال الدموي والجفاف الشديدين ووجود مخاط سميك وكذلك إرتفاع درجة الحرارة، أما الحيوانات المصابة ببكتريا C.lari فقد أعطت أعراضا تمثلت بالإسهال المائي والجفاف المعتدل وخيوط من المخاط ولا وجود للدم في بر از الحيوانات المصابة بالإسهال فضدلاً عن فقدان الشهية والخمول وكانت الحرارة والنبض والتنفس ضمن المعدل الطبيعي يمكن أن نستنتج ان النوعين المعز ولين من الأبقار المصابة بالإسهال (C.Coli و C.lari) هي أنواع جديدة تضاف إلى نوّعي الكمبايلوب اكتر (C.fetus و C.fejuni) المعزولة سابقا من الأبقار في العراق وهذا أدى إلى زيادة مجموع أنواع الكمبايلوباكتر في العراق إلى أربعة أنواع. الكلمات المفتاحية: الكمبايلوبكتر المحبة للحرارة، إسهال، أبقار.