Electrophoretic Protein Patterns of campylobacter Species in Different Farm Animals

El-Ged, A.M.S.[1]; A. M. Khalid, A.M.[1]; Mona M. Sobhy[2] and Eman S. Ramadan[2]

1- Dept. of Bact., Imm. & Myc., Fac.Vet. Med., Benha Univ.;

2- Dept. of Reprod. Dis., Animal Reprod. Res. Inst., ARC

Abstract

A total number of 9 *Campylobacter* isolates were collected from different farm animals (cattle, buffaloes, sheep and camels). All strains were identified biochemically. Outer membrane protein (OMP) of each isolate was extracted and then subjected to Sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for determination of molecular weight (MW) of each protein component. The biochemical identification of the nine isolates revealed that, three isolates were belonged to *C.fetus* subsp. *venerealis*, two isolates were *C.fetus*. subsp. *fetus*, two isolates were *C.jejuni* and two isolates were *C.coli*. The electrophoretic patterns of *Campylobacter* OMPs showed (6-11 bands) with different MWs ranging from 219 to 14 kDa. Seven strains had a common band with molecular weight of 97.633 KDa, six strains had 84.703 kDa, five strains expressed 157.325, 155.91 & 75.632 kDa, four strains expressed kDa with 111.55, 107.38, 54.24, 47.418, 41.375& 27.348, three strains expressed kDa with 175.57, 111.19, 109.43, 105.115, 102.575, 63.795, 34.579 & 22.1165, two strains expressed KDa with 219, 213.805, 165.795, 71.671 & 17.882 and only one strain expressed kDa with 205, 162.92, & 15.432. From the obtained results of the present study, it can be concluded that, each *Campylobacter* species has a distinct protein pattern which can be used to distinguish between different strains. Protein bands with MW of 97.633 and 84.703 kDa showed to have immunogenic activity. SDS-PAGE can be considered as a rapid, reliable, sensitive and diagnostic tool for characterization of *Campylobacter* isolates.

Introduction

Campylobacteriosis is considered as an important zoonotic disease causing economic losses in farm animals due to abortions, still births, weak or unhealthy feti and enteritis. *C. fetus, C. jejuni* and *C. coli* are the causative agents of this disease (11,15).

Campylobacter fetus is a microaerophilic bacterium that is able to colonize a variety of mucosal sites. There are two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, which are responsible for

causing substantial veterinary problems associated with ruminant infertility (9,26). Thermophilic Campylobacters, particularly *C. jejuni* and *C. coli* are the major cause of infectious enteritis and diarrheal illness in both human and animals (14, 17).

During the last decade, the outer membrane protein (OMP) and its composition has been a subject of keen interest as they are known to act as receptors, adhesions, sensors, transporters and signal-transductors of living cells (20). OMPs appear to protect the bacterium from phagocytosis and serum killing and comprise a family of highly antigenic proteins with variable molecular masses (27,10,4).

The classical methods, including biotyping, serotyping and phage-typing have been widely used for differentiation between *Campylobacter* species (30, 29). However, even these typing methods are not available for this differentiation. Sodium dodecyle-sulphate polyacrylamide gel electrophoresis(SDS-PAGE) offering a stable fingerprint of each strain, has been applied successfully for identification of several groups of *Campylobacters* and is considered to be an applicable method for differentiation of these organisms (12,16,28).

The present study aimed to study the epidemiological status of *Campylobacters* in different Egyptian farm animals through using of recent technique for identification and molecular characterization of the prepared *Campylobacter* OMPs using SDS-PAGE.

Material and Methods

Bacterial strains:-

A total number of 9 *Campylobacter* isolates collected from different farm animals were examined, 3 of cattle origin (No. 1, 5 & 6) from (aborted feti, preputial washes & vaginal swabs respectively) , 3 of sheep origin (No.4,7&9) from (aborted feti, bile secretions &fecal swabs respectively), 2 of camel origin (No.3&8) from (vaginal swabs & fecal swabs respectively) and 1 of buffaloe origin (No.2) from preputial washes. (Table, 1).

Table (1):- Sources of *Campylobacter* isolates used in the current study

Animal species	Type of samples	No. of Lab. strain
Cattle	Aborted feti	1
	Preputial washes	5
	Vaginal swabs	6
Sheep	Aborted feti	4
	Bile secretions	7
	Fecal swabs	9
Camels	Vaginal swabs	3
	Fecal swabs	8
Buffaloes	preputial swabs	2

All strains were identified as *Campylobacter* species according to(13) following catalase reaction, oxidase test, motility, H_2S production, growth at 25 °C, 37 °C &42 °C, growth in presence of 3.5% NaCl, growth in 1% glycine, sodium hippurate hydrolysis and susceptibility to nalidixic acid and cephalothin.

The tested isolates were cultured for growth by their inoculation into tubes containing Mueller Hinton broth and were incubated in a microaerophilic atmosphere (5%O_2, 10%Co_2, &85% N_2) at 37 °C

All strains were maintained frozen at -70 °C in brucella broth containing 15% glycerol till used (24).

Determination of electrophoretic protein profiles of *Campylobacters*:-

1-Extraction of outer membrane proteins(OMPs):-

Campylobacter Strains were grown on tryptose soya agar with sheep blood and incubated overnight at 37°C in a microaerophilic atmosphere. Each bacterial strain was harvested in 10 mm. HEPES buffer (pH=7.4).The cells were disrupted using a sonicator power 60 for 15 minutes which caused mechanical shearing of the cell wall. The intact cells and large debris were removed by cooling centrifugation at 8000 xg (4 °C for 20 min.) and washed twice in PBS. The washed cells were suspended in HEPES buffer. The total protein preparations were harvested from the supernatant by ultracentrifuge at 100.000 xg (4°C for 60 min). The clear gel-like pellets were suspended in 10mm HEPES buffer to which equal amount of 2% sodium sarcocele was added. The mixture was left over night at 4°C, then the insoluble fractions were harvested by centrifugation at 100.000 xg (4°C for 60 min.), then washed twice with distilled water by ultracentrifugation at 100.000 xg (4°C for 60 min.).The pellets were dialyzed against distilled water for about 48 hrs. Finally the protein concentrations were adjusted to contain 10 mg./dl. (3)

2-Sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE):-

Protein components were identified by electrophoretic mobility in 8 % poly-acrylamide gel as following:-

The vertical electrophoretic unit (miniprotein II Bio-Rad) was assembled as recommended by the manufacturer. The running gel was poured into glass plate with 1.5 mm thickness spacer using a pipette. The gel was covered with 2-3 ml. of isobutanol alcohol to keep the upper edge of the running (separating) gel flat and prevent the inhibitory effect of air oxygen on gel polymerization. The gel was left for 45 minutes till complete polymerization. The solution was decanted from the gel surface, dried using filter paper, then the stacking gel was poured above the separating gel, a comb with lodges was immersed into the gel and left to polymerize. After one hour, the comb was gently removed and the plate was fixed to the upper electrophoresis chambers, then the running buffer was poured into the upper and lower chambers. Twenty micrograms of the purified antigen per 20 microlitre of loading buffer were boiled at 95 °C for 4 min. and loaded in each well. A standard molecular weight protein marker was loaded to estimate the molecular weight of the separated antigens. The samples were

electrophoresed at 90 mv. for 1.5-2 hrs. in SDS-PAGE. After dye front of the sample was migrated to the end of the gel, the electric source was turned off and the gel was transferred to a plastic container containing staining buffer overnight for protein staining. Then the gel was transferred to another plastic container containing destaining buffer. The buffer was changed every 10 min. till no traces of the dye was left in the destaining buffer. The gel then washed several times with distilled water and stored immersed in the destaining buffer. (18)

Results

All tested strains had typical biochemical characteristics of *Campylobacter* species as shown in (Table, 2).

Table (2): Characteristics of *Campylobacter* organisms isolated from different farm animals

Characteristic		*C.f.* subsp. *venerealis*	*C.f.* subsp. *fetus*	*C.jejuni*	*C.coli*
Deep stab growth		-	-	-	-
Temp. Tolerance	At 25°	+	+	-	-
	At 37°	+	+	+	+
	At 42°	-	-	+	+
Oxidase test		+	+	+	+
Catalase test		+	+	+	+
Nitrate reduction		-	-	-	-
H_2S production		-	-	+	+
Hippurate hydrolysis		-	-	+	-
Growth on3.5% NaCl		-	-	[illegible]	+
Growth on1%glycine		-	+	+	+
Nalidixic acid		R	R	S	S
Cephalothin		S	S	R	R

S: Sensitive. R: Resistant.

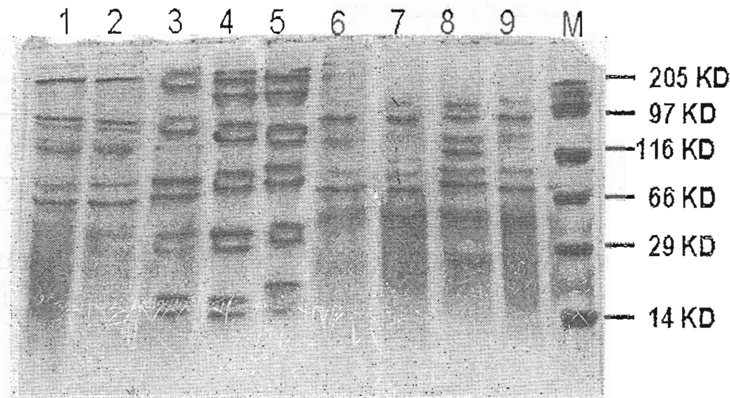
The nine isolates of *Campylobacter* were identified as: 3 out of them (No. 1,2and 3) were belonged to *C.fetus* subsp. *venerealis*, 2 isolates (No.4and5) were *C.fetus*. subsp. *fetus*, 2 isolates (No.6 and 9) were *C.jejuni* and 2 isolates (No.7 and 8) were *C.coli* (Table, 3).

Table (3):-*Campylobacter* strains isolated from different farm animals which used for characterization of OMPs:

Animal Species	*C. fetus* spp. *venerealis*	*C. fetus* spp. *fetus*	*C.jejuni*	*C.coli*	Total
1- Cattle	1(N1)	1(N4)	1(N6)	-	3
2-Buffaloes	1(N2)	-	-	-	1
3- Sheep	-	1(N5)	1(N9)	1(N7)	3
4- Camels	1(N3)	-	-	1(N8)	2
Total	3	2	2	2	9

N : Strain number.

Photo (1) and Table (4) showed the SDS-PAGE (silver stained) patterns of the outer membrane protein of *Campylobacter* strains (9 strains) with different molecular weights (MW) ranging from 219 to 14 kDa : each *Campylobacter* species of the nine strains, had 5-11 protein bands of molecular weights (219-14kDa). Seven strains had a common band with molecular weight of 97.633 kDa, six strains had 84.703 kDa, five strains expressed 157.325, 155.91 & 75.632 kDa, four strains expressed kDa with 111.55, 107.38, 54.24, 47.418, 41.375& 27.348, three strains expressed kDa with 175.57, 111.19, 109.43, 105.115, 102.575, 63.795, 34.579 & 22.1165, two strains expressed kDa with 219, 213.805, 165.795, 71.671 & 17.882 and only one strain expressed kDa with 205, 162.92, & 15.432 .



Photo(1) : Lanes (1-9): SDS-PAGE profiles of OMPs of 9 Campylobacter isolates. Lane (M): protein marker (205-14 kDa).

Discussion

Campylobacteriosis is known to have a world-wide distribution and to be associated with infertility and abortion of different farm animals. Once the disease is established in a herd, the syndrome of fetal death, repeat breeding and irregular estrus occur, resulting in great economic losses. The disease is generally venereal in cattle, buffaloes and camels caused by *C.fetus* particularly subspecies *venerealis*. The natural habitat of this organism in female animals is within vagina, cervix and uterus, while in males (healthy carriers) is confined to the mucosa of glans penis and prepuce (8,26,21). It is worth to mention that, due to limited biochemical activity of *Campylobacters*, the definitive identification relied on few phenotypic characteristics. Analysis using sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was considered as a powerful tool to differentiate genetically related microorganisms and permitted the identification of multiple outer membrane proteins (OMPs) of different molecular weights with an advantage of minimizing the visual errors and facilitating the simultaneous interpretation of large numbers of strains (23,1).

The present investigation determined the locations and alternations of these protein components of the nine *Campylobacter* isolates using SDS-PAGE. As shown in table(4) and photo(1), *Campylobacter* strains had 6-11 discrete protein bands with molecular weights (MWs) ranging from 219 to 14kDa. All isolates of *C.fetus* shared common bands with MW of 157.325 & 155.91kDa. These results suggested that MW of expressed OMP might affect the virulence of such species to cause abortion (19, 31, 25).In addition, *C. fetus* subsp. *venerealis* isolated from cattle and buffaloes showed sharing protein bands with MW of 175.57, 109.43, 107.38 & 97.633 kDa. Moreover, *C. fetus* subsp. *venerealis* of camels expressed protein bands with MW of 111.19, 105.115 34.597, 17.882 & 15.439 kDa. However, isolates of *C.fetus* subsp. *fetus* shared common protein patterns with MW of 213.805 & 34.597 kDa. These results showed that, there were some differences in expressed protein patterns within the same species of *Campylobacter*, due to difference in the affected animal species. This

finding reported earlier (5, 6). On the other hand, isolates of *C.jejuni* and *C.coli* expressed sharing protein patterns with MW of 97.633, 84.703, 47.418, 41.375 & 27.348 kDa. Moreover, isolates of *C.jejuni* showed common bands with MW of 75.632, 63.795 & 54.24 kDa, while isolates of *C.coli* gave sharing protein patterns with MW of 71.671 & 22.165 kDa. These results coincided to the results stated by (22,7) who added that these OMPs represented more than 50% of protein presented, plus several minor bands. From all of above results, The molecular weight of OMPs of *C.fetus* differed from *C.jejuni* and *C.coli* and the SDS-PAGE provided discrimination between them (2, 6).

From the obtained results, it could be concluded that: each *Campylobacter* species has a distinct protein pattern which can be used to distinguish between different strains. SDS-PAGE can be considered as a rapid, reliable, sensitive and diagnostic tool for characterization of *Campylobacter* isolates.

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Table (4): Comparison between number and molecular weight of reactive bands (OMP) produced by SDS-PAGE of Campylobacters isolated from farm animals:.

Species	C.F. spp. venerealis			C.F. spp. fetus		*C.jejuni*	*C.coli*	*C.coli*	*C.jejuni*	Marker	No. reactive bands
Animal	Cattle	Buff	Camel	Sheep	Cattle	Cattle	sheep	Camel	Sheep		
sample	ab	ps	v s	a f	ps	v s	b s	f s	f s		
Lane M.W. (KDa)	1	2	3	4	5	6	7	8	9	10	
219	+	+									2
213.805				+	+						2
205										+	1
175.57	+	+	+								3
165.795		+		+							2
162.92		+									1
157.325	+	+	+	+	+						5
155.91	+	+	+	+	+						5
116										+	1
111.55	+				+			+	+		4
111.19			+			+	+				3
109.43	+	+			+						3
107.38	+	+		+		+					4
105.115			+	+			+				3

ab: aborted feti ; ps: preputeal swabs ; vs: vaginal swabs; bs: bile secretion; fs: fecal swabs

Table (4): Continue

102.575	+				+			+			3
97										+	1
97.633	+	+			+	+	+	+	+		7
84.703				+	+	+	+	+	+		6
75.632		+	+		+	+			+		5
71.671							+	+			2
66										+	1
63.795						+		+	+		3
54.24	+					+	+		+		4
47.418						+	+	+	+		4
41.375						+	+	+	+		4
34.597			+	+	+						3
29										+	1
27.348						+	+	+	+		4
22.165						+	+	+			3
17.882			+					+			2
15.439			+								1
14										+	1
Total	10	10	9	8	10	11	10	11	9	6	94

التحليل الكهربائي للطبقات البروتينية لأصناف الكامبيلوباكتر في حيوانات المزرعة المختلفة

علي محمد سليمان الجد؛ عادل محمود عبد المجيد خالد
مني محمد صبحي؛ إيمان شوقت رمضان
1-قسم البكتريولوجي والمناعة والفطريات-كلية الطب البيطرى-جامعة بنها ؛
٢- قسم الأمراض التناسلية -معهد بحوث التناسليات الحيوانية-الهرم-الجيزة)

خلال هذا البحث تم فحص عدد ٩ عترات من الكامبيلوباكتر المعزولة من حيوانات المزرعة المختلفة، ثلاثة عترات من الماشية (جنين مجهض، غسول جراب ذكري، مسحة مهبلية)؛ ثلاثة من الخراف (جنين مجهض، مسحة مهبلية)؛ اثنين من الجمال (مسحة مهبلية، مسحة برازية) و عترةً واحدة من غسول الجراب الذكري للجاموس. وقد تم تصنيف هذه العترات التسعة كيميائيا إلي؛ ثلاثة كامبيلوباكتر جنيني تناسلي ضاري؛ اثنين كامبيلوباكتر جنيني معوي ضاري؛ اثنين كامبيلوباكتر جيجوناي؛ اثنين كامبيلوباكتر كولاي.
وقد تم تحديد مواقع وتناوب المكونات البروتينية للعترات التسعة عن طريق استخراج الغشاء البروتيني الخارجي ثم التحليل الكهربائي باستخدام (إس دي إس- باج). وقد
كشفت النتائج أن عترات الكامبيلوباكتر التسعة تحتوي علي عدد ٦ – ١١ طبقة بروتينية تتراوح أوزانهم الجزيئية بين ٢١٩ إلي ١٤ ك.د. ٧ عترات منهم أعطت طبقة بروتينيه مشتركة بوزن جزيئي ٩٧,٦٣٣ ك.د.؛ ٦ عترات أعطت ٨٤,٧٠٣ ك.د.؛ ٥ عترات أعطت ١٥٧,٣٢٥، ١٥٥,٩١ ٧٥,٦٣٢، ك.د.؛ ٤ عترات أعطت ١١١,٥٥، ١٠٧,٣٨، ٥٤,٢٤، ٤٧,٤١٨، ٤١,٣٧٥& ٢٧,٣٤٨ ك.د.؛ ٣ عترات أعطت ١٧٥,٥٧، ١١١,١٩، ١٠٩,٤٣، ١٠٥,١١٥، ١٠٢,٥٧٥، ٦٣,٧٩٥، ٣٤,٥٧٩، ٢٢,١١٦٥، ك.د.؛ ٢ عترة أعطت ٢١٩، ٢١٣,٨٠٥، ١٦٥,٧٩٥، ٧١,٦٧١، ١٧,٨٨٢، ك.د.؛ و عترة واحدة أعطت ٢٠٥، ١٦٢,٩٢، ١٥,٤٣٢ ك.د.
مِنْ نَتائِجِ هذه الدراسة نستخلص أنْ كلّ نوع كامبيلوباكتر لهُ نمط بروتيني مُتميّز والذي يمكن بواسطته التمييز بين الأنواع المختلفة للعتراتِ . يمكن أن تكون الطبقات البروتينية (ذات الوزن الجزيئي ٩٧,٦٣٣ & ٨٤,٧٠٣ ك.د.) لميكروبات الكامبيلوباكتر ذات تأثير محفز للمناعة. يعتبر التحليل البروتيني الكيميائي بواسطة إس دي إس- باج وسيلة تشخيصية حسّاسة وموثقة وسريعة لتمييز ميكروبات الكامبيلوباكتر المعزول.