Detection of campylobacter infection in ruminants in Sharkia Province

Saleh, M.A., Kadry, M. B, EL-Shafei A. A. and EL Said, M. M.*

Bacteriology Dept., Animal Health Research Institute, Zagazig Provincial Lab.
*Faculty of Vet. Medicine Zagazig University, Dept. Of Zoonosis

Abstract

A total number of 800 samples were collected from males (preputeal wash and semen) and females (vaginal mucous membrane swabs, aborted fetus, milk and serum samples) of bovine, ovine and caprine species located in different localities of Sharkia province. All these samples were subjected to detailed culturing methods for isolation and identification of campylobacter species. Tube agglutination test and enzyme linked immunosorbent assay (ELISA) were applied on all previous samples for detection of campylobacter antibodies. In the present study sero-prevalence of *Camp.* species found in bovine sera showed that 10/50 (20 %),10/50 (20 %), 12/50 (24 %) , 8/50 (16 %) , 11/50 (22 %) and 10/50 (20 %) were serologically positive for the presence of campylobacter species antibodies for preputeal wash, semen, vaginal mucous membrane swabs, aborted fetus, milk and serum samples, respectively. Whereas sero-prevalence of *Camp.* antibodies in ovine species showed that 16/50 (32 %), 12/50 (24 %), 13/50 (26 %), 15/50 (30 %), 5/50 (10 %) and 12/50 (24 %) were serologically positive for the presence of campylobacter species antibodies for preputeal wash, vaginal mucous membrane swabs, aborted fetus, milk and serum samples respectively. Sero-prevalence of *Camp.* antibodies in Caprine species showed that 14/50 (28 %), 7/50 (14 %), 7/50 (14 %), and 15/50 (30 %) were serologically positive for the presence of campylobacter species antibodies for vaginal mucous membrane swabs, aborted fetus, milk and serum samples, respectively.

The serologically positive cases were tested bacteriologically and biochemically differentiated into *Camp. fetus* subspecies *venerialis, Camp. fetus* subspecies *fetus, Camp. Jejuni*, *Camp. fetus* subspecies *bubules* and *Campylobacter sputorum.*

Key words: *membrane swabs, aborted fetus, milk , Campylobacter sputorum.*

Introduction

Campylobacteriosis is known as a worldwide distribution and constituents one of the most significant problem, which affects animal breeding and has been reported as one of the major sources of serious economic losses **(Prescott, 1985)**. Bovine Campylobacteriosis is generally venereal and mainly caused by *Camp. fetus* and in particular subspecies *venerealis*. The natural habitat of this organism is the genital tract of bovine. In bulls, it is confirmed to be present in the mucous membrane of glands penis and prepuce of who are acting as healthy carrier. In heifers and cows, the sites of infections are present in vagina, cervix and uterus (**Morris and Patton, 1985** and **Eaglesome and Garcia, 1992).**

Campylobacter causes abortion of sheep and goats and the disease is considered as an acute infectious disease characterized by abortion during the last half of gestation period (**Dennis, 1984)**. It caused by *Camp. fetus* subspecies *fetus*. It originates in the intestine and is acquired through ingestion, the ram has no role in transmission of the disease (**Stanely and Jones, 2003)**. Also, it can cause sporadic abortion in bovine without infertility **(Maclaren and Agumbah, 1998)**. Other species such as *Camp*. jejuni might cause few abortions in cattle and sheep **(Donkergoed *et al*., 1989)**.

The present study was aimed to spot light on the prevalence of *Camp.* species in ruminants in Sharkia province and re-evaluate the efficacies of conventional selective methods for isolation and identification of *Camp.* species to decrease the infection.

Material and Methods

1- Samples :

A total of number of 800 samples from males (preputeal wash and fresh semen of bull and ram) and females (vaginal mucous membrane swab, aborted fetus and milk samples of cow, sheep and goats) also serum samples were collected from both males and females of bovine ovine and caprine to evaluate the spreading of *Camp.* species.

Table (1). Types and numbers of samples collected from ruminants (bovine, ovine and caprine) from different localities in Sharkia Province for isolation and identification of *Camp.* spe-

Samples \ Location		Abo Kabeer	Abo Hammad	Fakous	Kafr Sakr	Zagazig	Total No. of examined samples
Preputeal wash	Bull	6	10	12	12	10	50
	Ram	12	13	8	7	10	50
Semen	Bull	6	10	14	12	8	50
	Ram	11	13	10	9	7	50
Serum	Cow	13	12	8	10	7	50
	Ewe	10	16	9	9	6	50
	Goat	12	10	6	14	8	50
Vaginal swabs	Cow	12	13	8	7	10	50
	Ewe	11	14	8	7	10	50
	Goat	10	9	8	13	10	50
Aborted Fetus	Cow	8	10	9	8	15	50
	Ewe	12	14	9	8	7	50
	Goat	15	7	10	8	10	50
Milk sample	Cow	10	9	12	8	11	50
	Ewe	6	8	10	16	10	50
	Goat	9	10	12	10	9	50

- Media used for preputeal washings (Bactotryptose broth, Difco)
- Media used for transportation of samples (Bactothiol broth, Difco) ,
- Media used for isolation and identification of *Camp.* species :

(i) Semisolid thiol medium, **(Edwin *et al.*, 2006)**.
(ii) Semisolid thioglycolate medium,
(iii) blood agar platerogen, **Anderson *et al.*, (1983) .**
(iv) Modified Charcoal cefoperazone deoxycholate agar (mCCDA) based blood-free medium (Oxoid Ltd., Basingstoke, United Kingdom) and Cefazone (32 mg/Liter) (Sigma, St. Louis, Mo.) **(Aspinall *et al.*, 1993).**
(v) brucella blood agar (Oxoid) .

- Media used for maintenance of *Camp.* isolates : (i) Semisolid thiol medium. (ii) Semisolid thioglycolate medium.
- Stains : (i) Gram ′s stain **(Nachamkin, 1995).** (ii) Modified Ziehl Neelsen stain (Manual of clinical Microbiology, 4th ed.).
- Tube agglutination test and ELISA technique were applied on serum samples and vaginal mucus collected from investigated animals **(Eide, 1964** and **Walsh and White, 1968)** to detect antibodies against *Camp.* species.

2- Methods:

Suspected samples to be infected with campylobacter species were inoculated into thiol media and incubated at 37˚C in a hydrogen- enriched microaerobic atmosphere and examined after 2 days. To assess the recovery of isolates after a prolonged incubation period .

Camp. species isolation was continued using the conventional methods described by **Smibert, (1964)** and identified biochemically according to **Holt *et al.*, (1994)** and **Garrity (2005)**. The vaginal mucous membrane samples from each female bovine, ewe and she-goat were examined serologically and immunologically by using tube agglutination test **(Eide, 1964** and **Walsh and White, 1968)** and ELISA test **(Ibrahim, 1992)** for the detection of antibodies against Campylobacter species.

Two reference strains of *Camp. fetus subsp. Fetus* and *Camp. fetus subsp. Venerealis* and their relevant anti-sera were obtained from National Veterinary and Food Research Institute, Helsinki, Finland and used for preparation of antigen negative and positive sera used in ELISA test as control.

Results

Table (2). Prevalence of *Camp.* species isolated from ruminants (bovine, ovine and caprine) from different localities in Sharkia Province (number of samples = 50).

Samples \ Location		Abo Kabeer	Abo Hammad	Fakous	Kafr Sakr	Zagazig	Total No. of (+)ve samples
Preputeal Wash	Bull	2	4	0	3	1	10
	Ram	3	3	6	2	2	16
Semen	Bull	2	3	1	2	2	10
	Ram	4	2	2	2	2	12
Vaginal Swab	Cow	2	3	1	2	4	12
	Ewe	1	2	5	2	3	13
	Goat	3	2	3	4	2	14
Aborted Fetus	Cow	0	2	2	1	3	8
	Ewe	2	1	5	3	4	15
	Goat	0	2	0	3	2	7
Milk sample	Cow	4	2	3	1	1	11
	Ewe	2	1	0	0	2	5
	Goat	3	0	2	2	0	7
Total		28*	27*	30*	27*	28*	140

Data were analyzed using the SPSS statistical analysis system package using Chi-Square test **(SPSS, 2001).**
* Significant $P < 0.05$

Table (3). Prevalence of *Camp.* Species isolated from bovine species collected from different localities in Sharkia Province.

Sex	Type of Specimen	No. of isolates	Campylobacter species				
			c.f.s.s. venerialis	*c.f.s.s. fetus*	*c. jejuni*	*c.f.s.s. bubulus*	*C.sputorum*
Male (Bull)	Preputeal wash	6	2	1	1	1	1
	Semen	8	2	3	2	0	1
Female	Vaginal swab	10	3	3	1	1	2
	Aborted fetus	6	1	2	2	0	1
	Milk samples	8	2	3	2	1	0

Table (4). Prevalence of *Camp.* Species isolated from sheep (Ovine) collected from different localities in Sharkia Province**.**

Sex	Type of Specimen	No. of isolates	Campylobacter species				
			c.f.s.s. venerialis	*c.f.s.s. fetus*	*c. jejuni*	*c.f.s.s. bubulus*	*C.sputorum*
Male (Ram)	Prepateal wash	12	5	2	3	0	2
	Semen	8	3	0	3	1	1
Female	Vaginal Swab	10	3	2	3	1	1
	Aborted fetus	9	4	2	2	0	1
	Milk Samples	4	1	1	1	0	1

Table (5). Prevalence of *Camp.* Species isolated from goat species collected from different localities in Sharkia Province (number of samples = 25).

Type of Specimen	No. of isolates	Campylobacter species				
		c.f.s.s. venerialis	*c.f.s.s. fetus*	*c. jejuni*	*c.f.s.s. bubulus*	*C.sputorum*
Vaginal mucous membrane swab	11	4	3	2	0	2
Aborted fetus	5	2	2	0	0	1
Milk samples	4	1	1	0	2	0

Table (6). Prevalence of *Camp.* Species antibodies in serum of Ruminants (bovine, Ovine and Caprine species) collected from different localities in Sharkia Province (number of samples = 50) by using of tube agglutination test and ELISA technique.

Species	No. of (+)ve samples by Tube agglutination test	No. of (+)ve samples by ELISA technique
Bovine serum	9	10
Ovine serum	10	12
Caprine serum	11	15

Discussion

Results of isolation and identification of *Camp.* species from ruminants collected from different localities in Sharkia province are shown in **Table (2)** .

The sero-prevalence of *Camp.* antibodies in collected serum of bovine species collected from different localities in Sharkia province revealed that out of 10/50 (20 %), ovine serum revealed out of 12/50(24%) and Caprine serum were 15/50 (30 %).

Vaginal mucous membrane swabs revealed isolation percentages reached 12/ 50 (24 %) from bovine, 13/ 50 (26 %) ovine samples and 14/ 50 (28 %) caprine samples this agrees with **(Garcia *et al*., 1983)** who isolated *Camp. jejuni* from vaginal mucous membranes of diseased bovine female genitalia in an incidence of 1.06 % and from mastitic bovine milk in an incidence of 2.22 % and also agrees with **Sobhy (1994)** and **El-Rashidy *et al.* (1996).**

Aborted fetal tissues revealed that 8 / 50 (16 %) from bovine, 15/ 50 (30 %) ovine samples and 7/ 50 (14 %) caprine samples. This finding attracts the attention to the significant role of *Camp.* species especially *Camp. fetus* subspecies *venerealis* as an important cause of abortion. This point of view has been supported intensively by **Garcia *et al,* (1983)** and **Kholeaf *et al.* (1985a).**

Milk samples revealed that 11 /50 (22 %) from bovine, 15/ 50 (30 %) ovine samples and 7/ 50 (14 %) caprine samples which agrees with **(Garcia *et al*., 1983)** who isolated *Camp.* jejuni from mastitic bovine milk in an incidence of 2.22 %.

Table (3) showed that from that from 10 seropositive bovine preputeal wash samples 6 isolates were classified as follows *isoletes 1 C.sputorum, 2 isolates c.f.s.s. venerialis, 1 c.f.s.s. fetus, 1 c. jejuni* and *1 c.f.s.s. bubulus,* but from bovine semen 10 seropositive samples 8 isolates were recoverd and classified as follows *isoletes 1 C.sputorum, 2 isolates c.f.s.s. venerialis, 3 c.f.s.s. fetus, 2 c. jejuni and 0 c.f.s.s. bubulus* this agrees with **Khalid (1986); Joan (1987), Elegbe *et al.*, (1987), Varga *et al.* (1990)** and **Lovett *et al.* (1993)** who isolated campylobacter species from preputeal wash and fresh collected semen.

From 15 seropositive bovine vaginal mucous membrane swabs 10 samples isolates were re-

coverd and classified as follows *isoletes 2 C.sputorum, 3 isolates c.f.s.s. venerialis, 3 c.f.s.s. fetus, 1 c. jejuni and 1 c.f.s.s. bubulus* this agrees with **Sobhy (1994)** and **El-Rashidy *et al.* (1996)** who isolated Campylobacter species from the vaginal mucus of diseased bovine female genitalia and it may be the causative agent of mastitic bovine milk.

From 8 seropositive bovine aborted fetus samples 6 isolates were classified as follows *isoletes 1 C.sputorum, 1 isolates c.f.s.s. venerialis, 2 c.f.s.s. fetus, 2 c. jejuni and 0 c.f.s.s. bubulus whereas* From 11 seropositive bovine milk samples 8 isolates were recoverd and classified as follows *isoletes 0 C.sputorum, 2 isolates c.f.s.s. venerialis, 3 c.f.s.s. fetus, 2 c. jejuni and 1 c.f.s.s. bubulus*

Table (4) showed that from 16 seropositive ovine preputeal wash samples 12 isolates were isolated and classified as follows *isoletes 0 C.sputorum, 5 isolates c.f.s.s. venerialis, 3 c.f.s.s. fetus, 2 c. jejuni and 2c.f.s.s. bubulus.* From 12 seropositive ovine semen samples 8 isolates were detected and classified as follows *isoletes 1 C.sputorum, 3 isolates c.f.s.s. venerialis, 3 c.f.s.s. fetus, 0 c. jejuni and 1 c.f.s.s. bubulus,* this agrees with **Khalid (1986), Sobhy *et al.*, (1998)** and **Lovett *et al.* (1993)** who isolated campylobacter species from preputeal wash and freshly collected semen.

From 13 seropositive ovine vaginal mucous samples 10 isolates were found and classified as follows *isoletes 1 C.sputorum, 3 isolates c.f.s.s. venerialis, 3 c.f.s.s. fetus, 2 c. jejuni and 1 c.f.s.s. bubulus* this agrees with **Hisham (1992)** who isolated *C.fetus* subspecies fetus from sheep and goats genitalia.

From 15 seropositive ovine aborted fetus samples 9 isolates were classified as follows *isoletes 0 C.sputorum, 4 isolates c.f.s.s. venerialis, 2 c.f.s.s. fetus, 2 c. jejuni and 1 c.f.s.s. bubulus whereas* from 5 seropositive ovine milk samples 4 isolates were classified as follows *isoletes 0 C.sputorum, 1 isolates c.f.s.s. venerialis, 1 c.f.s.s. fetus, 1 c. jejuni and 1 c.f.s.s. bubulus*.

Table (5) showed that 14 seropositive from goat vaginal mucous membrane swabs samples 11 isolates were found and classified as follows isolates 2 *C.sputorum,* 4 isolates *c.f.s.s. venerialis,* 3 *c.f.s.s. fetus,* 2 *c. jejuni* and 0 *c.f.s.s. bubulus*. From 7 seropositive goats aborted fetus samples 5 isolates were classified as follows *isolates* 1 *C.sputorum,* 2 *isolates c.f.s.s. venerialis,* 2 *c.f.s.s. fetus,* 0 *c. jejuni* and 0 *c.f.s.s. bubulus whereas* From goat milk samples 7 seropositive samples 4 isolates were classified as follows isolates 0 *C.sputorum,* 1 isolates *c.f.s.s. venerialis,* 1 *c.f.s.s. fetus,* 0 *c. jejuni* and 2 *c.f.s.s. bubulus*

Table (6) showed that ELISA technique is more sensitive and accurate than the tube agglutination test which agrees with **Kendrick (1968)** who approved that vaginal tube agglutination test was less sensitive than ELISA test for detection of venereal Campylobacteriosis and a titer of 1:40 was usually regarded as indication of infection. **El-Jakee (1989), Hum *et al.* (1994)** and **Sobhy *et al.* (1996)** stated that the ELISA test is satisfactory and more sensitive than agglutination test and early demonstrates the antibody of *Camp.* species than tube agglutination test.

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الكشف عن عدوي الكامبيلوبكتر في المجترات بمحافظة الشرقية

د.محمد علي صالح*, د.محمد قدري بكري إبراهيم*, د. أشرف عبدالرحمن الشافعي*, د.محمد السيد محمد محمد**

*قسم البكتريولوجى - معهد بحوث صحة الحيوان – معمل الزقازيق

**قسم الأمراض المشتركة كلية الطب البيطري – جامعة الزقازيق

الملخص العربي

الهدف من هذه الدراسة هو التحقق من وجود عترات بكتريا ميكروب الكامبيلوبكتر في العينات المأخوذة من مجترات سليمة ظاهريا ومريضة وخصوصا العدوي المصاحبة للإجهاض لتقييم الطرق المختارة للعزل و التصنيف لعترات الميكروب المختلفة في أماكن متفرقة من محافظة الشرقية.

فقد تم إجراء البحث علي عدد 800 عينة موزعة كالتالي :0 5 عينة من كل من مسحات بكتريولوجية معقمة لكيس الصفن للذكور - عينة سائل منوي حديث التجميع – عينة مصل – مسحة مهبلية معقمة – عينة من أجزاء من الأجنة المجهضة –عينة ألبان لكل من الماشية و الأغنام و الماعز من مراكز مختلفة في محافظة الشرقية بجمهورية مصر العربية.

وقد تم أخذ العينات و زرعها علي أوساط غذائية خاصة بالميكروب وتم عمل بعض الإختبارات السيرولوجية المختلفة مثل إختبار الأنبوبة المتلبد و إختبار المادة المرتبطة بالإنزيم .

ومن مجموع العينات المجمعة من الماشية وجد أن العينات الإيجابية هي 10 (20 %) , 10 (20%) , 12 (24 %) ، 8 (16 %) ، 11(22 %) و 10 (20 %) لكل من مجموع العينات المأحوذة من مسحات كيس الصفن للأعضاء التناسلية للذكور ' عينات السائل المنوي حديث التجميع , عينات المصل ، عينات المسحات المهبلية , العينات المأخوذة من أجزاء من الأجنة المجهضة , العينات المأخوذة من ألبان الماشية علي الترتيب .

بينما في الأغنام وجد أن العينات الإيجابية هي 16 (32 %) , 12 (24 %) , 13 (26 %) ، 15 (30 %) و5 (10 %) و 12 (24 %) لكل من مجموع العينات المأخوذة من مسحات كيس الصفن للأعضاء التناسلية للذكور ' عينات السائل المنوي حديث التجميع , عينات المصل ، عينات المسحات المهبلية , العينات المأخوذة من أجزاء من الأجنة المجهضة , العينات المأخوذة من ألبان الأغنام علي الترتيب .