

Animal Health Research Institute  
Assiut Regional Laboratory

**PREVALENCE OF CAMPYLOBACTERIOSIS  
IN DAIRY FRIESIAN CATTLE WITH GENITAL  
TROUBLES IN SOME FARMS  
OF ASSIUT GOVERNORATE**  
(With 3 tables)

By

**A.M. SAYED; M.M. ABD EL-HAFEEZ;  
SHEHATA, S.H\* and H.A. ABD EL-KADER**

\* Dept. of Theriogenology, Fac. Vet. Med., Assiut University.  
(Received at 24/3/2001)

مدى تواجد ميكروب الكامبيلوباكتر في الأبقار الفريزيان المصابة باضطرابات  
تناسلية في بعض مزارع محافظة أسيوط

أحمد مصطفى سيد ، محمد عبد الحفيظ ،  
شحاتة حسن ، حسين علي عبد القادر

لجريت هذه الدراسة بهدف معرفة معدل انتشار ميكروب الكامبيلوباكتر بين الطلائق السائمة ظاهريا والأبقار الحلوب التي تعاني من اضطرابات تناسلية في بعض مزارع الأبقار الفريزيان بمحافظة أسيوط. وقد تم فحص ٤٢٦ بقرة و ١٦ طلوقة في اثنتين من كبرى المزارع بالمحافظة. وقد أوضحت نتائج فحص الجهاز التناسلي للأبقار والمدعومة بقراءة السجلات الخاصة بالمزارع أن ٢٦ بقرة تعاني من اضطرابات تناسلية مختلفة وقد تم تسجيل أهم الأعراض. وتم أخذ عينات للفحص البكتريولوجي من الأبقار المصابة وكل الطلائق لاستبيان وجود هذا الميكروب. وقد تبين أن نسبة إصابة الطلائق ( ٣١.٢٥%) أعلى نسبيا منها في الأبقار (١٩.٢%). وقد أثبتت الدراسة أن ميكروب الكامبيلوباكتر الجيني فيرياليز هو المسبب الرئيسي لهذا المرض بتلك المزارع حيث تم عزله وتصنيفه من ٨٠% ، ٦٠% من الحالات المصابة في كل من الطلائق والأبقار على التوالي. هذا وقد تم عمل اختبار حساسية عترات الكامبيلوباكتر المعزولة للمضادات الحيوية المختلفة وقد أسفرت النتائج عن أن ميكروبي كامبيلوباكتر الجيني فيرياليز والكامبيلوباكتر الجيني شديدي الحساسية للبيفالوسين ومقاومين لحمض النالديكسيك. كما وجد أن الاسترثام والامستربتوميسين والنتلاميسين والاميكين والجاراميسين هم أشد المضادات الحيوية تأثيرا على العترات المعزولة. هذا وقد تم مناقشة الشروط الصحية اللازمة لمنع انتشار هذا المرض.

### SUMMARY

A survey of bovine campylobacteriosis in apparently clinically healthy bulls and cows suffered from genital troubles was carried out in two farms in Assiut Governorate. A total number of 452 dairy Friesian cattle (436 lactating cows and 16 breeding bulls) were gynecologically examined. Out of the examined cows 26 showed signs of genital troubles during rectal palpations. All breeding bulls and only these cows with genital problems were sampled for isolation and identification of different campylobacter species. The rate of campylobacter infection in breeding bulls 31.25% (5/16) was higher than that in cows with reproductive problems 19.2% (5/26). The result of the study identifies *Campylobacter fetus subsp. venerealis* as the main cause of campylobacteriosis in these farms. It was identified from 80% (4/5) and 60% (3/5) of campylobacter isolated strains in bulls and cows respectively. Results of antibiogram indicated that all *C. fetus subsp. venerealis* and *fetus* were susceptible to Cephalothin and resistant to Nalidaxic acid. Spectram, Streptomycin, Netilmycin, Amikin and Garamycin had high inhibitory effect on isolated campylobacter strains. The hygienic measures for controlling the disease were discussed.

**Key words:** *Campylobacteriosis, genital troubles, dairy friesian cattle, Assiut, Egypt.*

### INTRODUCTION

Efficient production of milk and beef in cattle industry is very much dependent on maintenance of a high level of fertility in both male and female cattle. For each calf that is not born or does not survive, there arises an economic loss two years later. Campylobacteriosis in cattle is a widespread disease associated with bovine infertility and abortion (Bawa *et al.* 1991 and Eaglesome and Garcia, 1992). Shum, (1987) and Shum *et al.* (1994) classified *Campylobacter fetus* into *campylobacter fetus subsp. venerealis* and *Campylobacter fetus subsp. fetus* on the basis of biochemical characteristics correlated with pathogenicity. *Campylobacter fetus subsp. venerealis* is transmitted venereally and produces a syndrome of infertility in female cattle associated with inflammation of the genital tract, abortion and repeat breeders (Schurig *et al.* 1973). The other subspecies, *Campylobacter fetus subsp. fetus* is known to cause sporadic abortion in cattle but is not usually associated

with early embryonic loss. This subspecies has also been isolated from the intestinal and reproductive tracts of healthy cattle (Clark, 1971).

Therefore, the aim of this study of two folds: (a) to evaluate the relative prevalence of *Campylobacter fetus*, the main campylobacter species associated with bovine reproductive problems and to focus attention about the level of infection on apparently healthy bulls at Assiut Governorate, (b) Antibioqram for isolated campylobacter species to reach available and specific treatment.

## **MATERIALS and METHODS**

### **Animals:**

A total of 452 dairy Friesian Cattle consisting of 436 lactating cows and 16 breeding bulls belonging to Farm A (210) and Farm B (242) in Assiut Governorate were gynecologically examined. Twenty six cows (12 from A and 14 from B) showed signs of endometritis by rectal examination, this diagnosis was supported by failure of conception for more than three successive inseminations. Age of the examined cattle was ranged from 3 - 8 years. All breeding bulls and only cows with signs of genital troubles in the studied farms were sampled for campylobacter isolation.

### **Samples:**

After cleaning the perineum and vulva of the examined cows using 70% ethanol, a sterile swab was introduced into the vagina up to the cervix. Contamination was avoided by opening the vaginal cleft and placing the swab cranial to the external urethral opening. The swab was then turned and slightly pulled back and forth several times to ensure saturation (Shum, 1987). Uterine samples were collected by aspiration using a hand-operated vaccum pump and sterile 16 gauge uterine catheters (Scott, 1994).

In the bull, preputal washings were collected as described by Lander (1990 b). All samples were transported as rapidly as possible within two hours in ice back container to the laboratory where isolation and identification of the organisms were performed.

### **Isolation and identification of Campylobacter species:**

The samples were enriched under asptic conditions in Campylobacter enrichment broth base (Biolife) supplemented with campylobacter growth and antimicrobial supplement. The enriched broth was incubated at 37°C for 48 h under microaerophilic conditions (5%

O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>) in an anaerobic jar without a catalyst, but with a gas generating kit (Oxoid, code BR 038B) (Garcia *et al.*, 1983). A loopful of enriched broth of each sample was streaked on campylobacter selective agar (Defco) with Butzler antibiotic supplement for selective isolation of campylobacter fetus which contain Bacitracin, Cycloheximide, Colistin sulphate, Cephazolin sodium and Novobiocin (Oxoid code SR 85). The inoculated plates were incubated at 37° C for 72 hours under the same microaerophilic conditions.

Suspected typical campylobacter colonies were identified morphologically, culturally and biochemically according to Garcia *et al.* (1983) using gram stain, motility, catalase test, H<sub>2</sub>S on TSI, H<sub>2</sub>S on lead acetate strips, growth in 1% glycine, 3.5% sodium chloride, and 1% bile salt, growth at 25°C and 43°C, susceptibility to Nalidixic acid, Cephalothin and hippurate hydrolysis test.

Antibiogram sensitivity testing was carried out using the disc and agar diffusion method according to Quinn *et al.* (1994). Twelve different antibiotic discs, supplied by Oxoid were used. These antibiotics were Cephalothine (30 µg), Nalidixic acid (30 µg), Spectramin (10 µg), Streptomycin (10 µg), Garamycin (30 µg), Unasin (20 µg), Cefobid (75 µg), Amikin (30 µg), Duracif (30 µg), Claforan (30 µg), Spiramycin (100 µg) and Netilmycin (30 µg). The results were interpreted according to Konemen *et al.* (1992).

## RESULTS

The main clinical signs of the infected lactating cows were endometritis, cervicitis and vaginitis. The mucous varied from slightly cloudy to extremely purulent. Other signs include long oestrus cycle interval (over 40 days), metritis and early abortion within 40-50 days. The examined breeding bulls were completely apparently clinically healthy.

The bacteriological examination of the collected samples showed the prevalence of *C. fetus* infection. The incidence was 31.25% (5/16) in the breeding bulls and 19.2% (5/26) of the cows with genital troubles. *Campylobacter fetus subsp. venereales* were the main cause of campylobacteriosis in this study. Types, incidence and percentage of

different campylobacter species isolates and antibiogram assay were illustrated in tables 1-3.

## DISCUSSION

*Campylobacter fetus* continues to be a major microbial agent causing reproductive problems in breeding cattle. It is considered to be the third most common cause of infertility and abortion (Villar and Spina, 1982, and Kimberling, 1988). The disease is characterized by temporary infertility of female cattle as a result of subacute diffuse mucopurulent endometritis, salpingitis, cervicitis and vaginitis (Shum, 1987). Similar clinical signs were observed in this study.

Clark *et al.* (1974); Winter and Gaveney (1978), and Scott, (1994) recorded that the only unequivocal means of demonstrating *C.fetus* infection in individual animals is to be isolated and identified. The isolation from clinical samples is not easy chiefly because of the microaerophilic nature of campylobacters and the rapid overgrowth of more vigorous multiplying contaminating organisms (Lander, 1990 a). To overcome these problems, microaerobic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>) and campylobacter selective agar media (CSA) with Butzler antibiotic supplement for selective isolation of *Campylobacter fetus* were used. After receipt of the samples, pre-culture selective enrichment on campylobacter selective broth takes place for 2 day before subculturing onto CSA. Shum (1987) recorded that CSA is the most reliable media for the isolation of *C.fetus*, especially if samples processed immediately or not later than 6 h after collection. Similar techniques for isolation of *C.fetus* were used by Garcia *et al.* (1983), Lander (1990 a) and Eaglesome and Garcia (1992).

Tables 1 and 2 give details of the prevalence of *C.fetus* isolated from bulls and cows. 31.25% (5/16) of the examined bulls and 19.2% (5/26) of cows with reproductive problems were infected. Nearly similar findings had been reported by Campero *et al.* (1987), Villar and Spina, (1982) and Akhtar *et al.* (1990 b). Lower percentages were stated by Stoianov (1982), Jerrett *et al.* (1984) and Bawa *et al.* (1991), whereas higher percentages were recorded by Akhtar *et al.* (1990 a). These variations could be attributed to the contamination level and methods of isolation (Shum, 1987). The present study indicated a higher prevalence of the organism was found in bulls than cows (Table 1 and 2). This may

be due to that the infection in the bull is accompanied by neither histological changes nor modification in the characteristics of the semen (Clark, 1971). Garcia *et al.* (1983) explained the higher incidence of carrier bulls especially among those of over five years of age due to increase in the size and number of crypts in the epithelium of penis which provide favourable conditions for the persistence of organism.

In this work *C.fetus subsp. venerealis* was more common than *C.fetus subsp. fetus*. It was identified from 80% (4/5) and 60% (3/5) of campylobacter isolated strains in both bulls and cows respectively (Tables 1 and 2). These agree well with findings of Garcia *et al.* (1983) and Eaglesome and Garcia (1992). Hoerlein and Carroll (1970) recorded that *C.fetus subsp. fetus* is of little epidemiological importance in bovine genital campylobacteriosis. In bulls, *C.fetus subsp. venerealis* appears to be confined to the preputal cavity, in particular to the mucosa of glans penis and transmission occur only during coitus or by artificial insemination (Garcia *et al.*, 1983). In heifers and cows, the sites of infection are within the lumen of uterus, cervix, and can be established in the vagina up to two years (Corbeil *et al.*, 1981). Ware (1980) suggested that *C.fetus subsp. venerealis* may cause infertility in cows by restricting the supply of oxygen available to the preimplantation embryo. In contrast, *C. fetus subsp. fetus* strain has a tropism principally for fetal placenta tissues and the transmission of infection occur through fecal-oral route. (Schurig *et al.*, 1973 and Garcia *et al.*, 1983).

*Campylobacter jejuni* was isolated from 3.8% (1/26) of examined cows (Table 2). Garcia *et al.* (1983) reported that *C.jejuni* caused enteritis and abortions among milk herds. This strain was distinguished biochemically from the *C.fetus subsp. venerealis* and *fetus* by its ability to grow at 43°C but not at 25°C and it appears to be the only campylobacter species that hydrolyzes hippurate (Hebert *et al.*, 1982).

Results in Table 2 revealed that 31.2% (5/16) of the examined preputal washings were contaminated by *Campylobacter fecalis*. This is a non pathogenic strain present in the intestinal tract and had been isolated from semen and vagina of cattle. It is the only campylobacter strain that produce H<sub>2</sub>S on both TSI and lead acetate (Garcia *et al.*, 1983 and Quinn *et al.*, 1994).

Results of antibiogram indicates that all *C.fetus subsp. venerealis* and *fetus* were susceptible to Cephalothin and resistant to Nalidaxic acid. Spectram, Streptomycin, Netilmicin, Amikin and Garamycin had high

inhibitory effect on isolated *Campylobacter* strains (Table 3). Nearly similar results were recorded by Das *et al.* (1996). Streptomycin is used extensively for controlling *C.fetus* infection. Preputial infusion of an aqueous solution of dihydrostreptomycin concomitant with subcutaneous injection of antibiotic has been recommended for treating bulls (Garcia *et al.*, 1983).

From the achieved results in the present work, we can conclude that a number of effective measures should be taken for controlling bovine campylobacteriosis include separation of infected from non-infected herds, culling older bulls and replacement with younger bulls, treatment of infected cows with antibiotics and artificial insemination.

## REFERENCES

- Akhtar, S.; Riemann, H.P.; Thurmond, M.C.; Favrer, T.B. and Franti, C.E. (1990a): Multivariate logistic analysis of repeat cross-sectional surveys of *Campylobacter fetus* in dairy cattle. Preventive Veterinary Medicine 10, 15-24.
- Akhtar, S.; Riemann, H.P.; Thurmond, M.C.; Favrer, T.B. and Franti, C.E. (1990b): The use of loglinear model to evaluate factors associated with sero-prevalence of *Campylobacter fetus* in dairy cattle. Theriogenology 34, 989-1001.
- Bawa, E.K.; Adekeye, J.O.; Oyedipe, E.O. and Umoh, J.U. (1991): Prevalence of bovine Campylobacteriosis in indigenous cattle of three states in Nigeria. Top. Anim. Hlth. Prod. 23, 157-160.
- Campero, C.M.; Ballabene, N.C.; Cipolla, N.C. and Zamora, A.S. (1987): Dual infections of bull with campylobacteriosis and trichomoniasis: Treatment with dimetridazole chlorohydrate. Aust. Vet. J. 64 (10): 320-321.
- Clark, B.L. (1971): Review of bovine vibriosis. Aust. Vet. J. 47, 103-107.
- Clark, B.L.; Mon Sborough, M.J. and Duffy, J.H. (1974): Isolation of *Campylobacter fetus* subsp. *venerealis* and *Campylobacter fetus* subsp. *intermedius* from the preputial secretions of bulls. Aust. Vet. J. 50, 324.

- Corbeil, I.B.; Schwig, G.G.; Duncan, J.R.; Wilkie, B.N. and Winter, A.J. (1981):* Immunity in the female bovine reproductive tract based on the response to *Campylobacter fetus*. *Adv. exp. Med. Bio.* 137, 729-743.
- Das, S.G.; Nair, G.B.; Mallick, S.G.; Biswas, G.; Sdkar, A. and Bhattach, A.D. (1996):* Study on in-vitro antimicrobial sensitivity of *Campylobacter* species of animal and human origin. *Ind. J. of Animal Health* 35 (2) 193-196.
- Eaglesome, M.D. and Garcia, M.M. (1992):* Microbial agents associated with bovine genital tract infections and semen. Part I. *Brucella abortus*, *Lepiospira*, *Campylobacter fetus* and *Trichomonas foetus*. *Vet. Bull.* 62 (8): 757-768.
- Garcia, M.M.; Eaglesome, M.D. and Rigby, C. (1983):* Campylobacters important in veterinary medicine. *Vet. Bull.* 53, 793-818.
- Hebert, G.A.; Hollis, D.G.; Weaver, R.E.; Lambert, M.A.; Blaser, M.J. and Moss, C.W. (1982):* 30 years of Campylobacters: biochemical characteristics and a biotyping proposal for *campylobacter jejuni*. *J. Clin. microbiol.* 15, 1065-1073.
- Hoerlein, A.B. and Carroll, E.J. (1970):* Duration of immunity to bovine genital vibriosis. *J. Am. Vet. Med. Ass.* 156, 775-778.
- Jerrett, J.V.; Steven, M.; John, W.; Jeffrey, W.; Jack, M., and Jan, P.M. (1984):* Diagnostic studies of the fetus, placenta and maternal blood from 265 bovine abortions. *Cornell Vet.* 74, 8-20.
- Konemen, E.W.; Allen, S.; Janda, W.M.; Schrecrenberger, P.C. and Winn, W.C. (1992):* Diagnostic Microbiology. 4th Ed. Lippincott Co. USA.
- Kimberling, C.V. (1988):* Diseases causing abortion. Kimberling, C.V. Ed. Jensen and Swifts diseases of sheep 3<sup>rd</sup> Philadelphia: Lea Febiher, 57.
- Lander, K.P. (1990 a):* The development of a transport and enrichment medium for *Campylobacter fetus*. *British Vet. J.* 146, 327-333.
- Lander, K.P. (1990 b):* The application of transport and enrichment medium to the diagnosis of *Campylobacter fetus* in bulls. *British Vet. J.* 146, 334-340.



- Quinn, P.J.; Carter, M.S.; Markyl, B. and Carter, G.R. (1994): Clinical Vet. Microbiology, Mosby- Year Book Europe Limited.
- Schurig, G.D.; Hall, C.E.; Burda, M.S.; Corbeil, I.B.; Dunncan, J.R. and Winter, A.J. (1973): Persistent genital tract infection with *Vibrio fetus intestinalis* associated with serotypic alteration of infected strain. Am. J. Vet. Res. 34 (11) 1339-1403.
- Scott, P.R. (1994): Control of venereal campylobacteriosis in beef herd. Vet. Record, 135, 162-163.
- Shum (1987): Bovine abortion due to *Campylobacter fetus*. Aust. Vet. J. 64 (10) 319-320.
- Shum; Quinn, C.; and Kennedy, D. (1994): Diagnosis of venereal campylobacteriosis by ELISA. Aust. Vet. J. 71 (5) 140-143.
- Stoianova, T.O. (1982): Localization of *Campylobacter fetus* in genital organs of cows and its sensitivity to antibiotics and chemotherapeutic agents. Vet. Med. Nauki, 19 (4): 69-74.
- Villar, J.A. and Spina, E.M. (1982): Bovine Campylobacteriosis. A survey of its prevalence in Argentina between 1966-1981. Gaceta Veterinaria, 44, 647-658.
- Ware, D.A. (1980): Patgogenccity of *Campylobacter fetus subsp. venerealis* in causing infertility in cattle. Br. Vet. J. 136, 301-303.
- Winter, A.J. and Gaveney, N.T. (1978): Evaluation of transport medium for *Campylobacter fetus* J. Am. Vet. Med. Ass. 173, 472-473.

**Table 1:** Prevalence of *Campylobacter fetus* in breeding bulls and cows with genital troubles in two farms at Assiut Governorate

Farms	Cows					Bulls		
	No. of examined cows	Cows with genital troubles (infected cows)		No. of <i>C.fetus</i> isolates from infected cows		No. of examined bulls	No. of <i>C.fetus</i> isolates.	
		No.	%	No.	%		No.	%
A	201	12	5.97	2	16.6	7	2	28.5
B	235	14	5.95	3	21.5	9	3	33.3
Total	436	26	5.96	5	19.2	16	5	31.25

**Table 2:** Different subspecies of campylobacter isolated from breeding cattle.

Isolates	Cows (n = 26)		Bulls (n = 16)	
	No. of isolates	%	No. of isolates	%
<b>A- Pathogenic subspecies:</b>				
<i>C.fetus subsp. venerealis</i>	3	11.53	4	25
<i>C.fetus subsp. fetus</i>	1	3.84	1	6.25
<i>C.jejuni</i>	1	3.84	-	-
Total	5	19.21	5	31.25
<b>B- Non pathogenic subspecies:</b>				
<i>C.fecalis</i>	-		5	31.25

Table 3 : Antibiogram of campylobacter isolates

Isolated organism	No. of isolates	Cephalexithine 30 µg	Nalidixic acid 30µg	Spectin-ama 10 µg	Streptomycin 10 µg	Garamycin 30µg	Unasin 20µg	Cefobid 7½ µg	Amikin 30 µg	Duraef 30 µg	Claforan 30 µg	Spiramycin 100 µg	Netilmycin 30 µg
<i>C.fetus venerealis</i>	7	7 +++	-	6+++	5+++	4+++	7-	6-	3+++	6-	7-	5-	5+++
				1-	1+	3-		1++	2++	1+		2++	2+++
<i>C.fetus subsp. fetus</i>	2	2++	-	1++	1+	1+	2-	1++	2++	2-	2-	2-	1++
				1+	1-	1-		1-					1+
<i>C.fetus</i>	1	-	+++	+	+	-	-	-	+	-	-	-	++