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CAMPYLOBACTERIOSIS IN REPEAT BREEDER BUFFALO-COWS: ITS PREVALENCE, TREATMENT AND SUBSEQUENT FERTILITY IN ASSIUT GOVERNORATE

(With 3 Tables and One Figure)

By

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مدى تواجد الكمبيلوباكترز فى الجاموس ذى الشياع المتكرر بمحافظة أسيوط ، العلاج والخصوبة التالية

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أجريت هذه الدراسة لتحديد وجود ميكروب الكمبيلوباكتر فى الجهاز التناسلى لإناث الجاموس المصاب بالشياع المتكرر مع تحديد نسبة الخصوبة بعد العلاج لهذه الحيوانات. تم فحص ١٥٠ جاموسة فحصاً تناسلياً ومن هذه الحيوانات وجد ٩٦ جاموسة مصابة بالشياع المتكرر مع سلامة الجهاز التناسلى ظاهرياً. وأخذت مسحة مهبلية من هذه الحيوانات لعزل وتصنيف ميكروب الكمبيلوباكتر. وتم التعرف على هذا الميكروب باستخدام الـ PCR وكذلك باستخدام تفاعلات الكيمياء الحيوية. وكذلك تم علاج الحالات باستخدام المضادات الحيوية (فيت بايوتك). وأظهرت النتائج أن معدل انتشار أو تفشى الكمبيلوباكتر فى الجاموس ذى الشياع المتكرر كان ١٠,٤٢%. وكانت معدلات الكمبيلوباكتر المختلفة ٦,٢٥% للكمبيلوباكتر الجنينى فيتريالز ، ٤,١٧% للكمبيلوباكتر الجنينى الجنينى. ولم يتم عزل العترات الأخرى للكمبيلوباكتر كولاى أو جوجينى. كما أوضحت النتائج أن نسبة الشفاء بعد العلاج وصلت إلى ٩٣,٧٥% وأيضا معدل الشياع المخصب بعد العلاج وصل إلى ٨٧,٧٨% مع نسبة عشر ٨٦,٠٨%.

SUMMARY

This study was carried out to evaluate the prevalence of *Campylobacter spp.* present in the genitalia of repeat breeder buffalo-cows, as well as the subsequent fertility after treatment. A total of 150 buffalo-cows were

examined gynaecologically and out of these animals, 96 were found to suffer from repeat breeding with apparently healthy genitalia. Sterile swabs were collected from the portio-vaginalis and enriched under aseptic conditions in campylobacter enrichment broth base supplemented with campylobacter growth supplement. The typical campylobacter colonies were identified by using PCR and biochemical reactions. The prevalence of campylobacteriosis in this study was 10.42% (10/96). *Campylobacter fetus* subsp. *venerealis* is a main cause of Campylobacteriosis in repeat breeder buffalo-cows (6.25%) and other subsp. (*C. fetus* subsp. *fetus*) was 4.17%. The other *Campylobacter spcies* (*C. jejuni* and *C. Coli*) were not isolated. After treatment, the recovery rate was 93.75% and 6.25% did not responde to treatment. Moreover, the rate of fertile heat was 87.78% with 86.08% pregnancy rate.

Key words: *Campylobacteriosis, Buffaloes, Treatment, Fertility.*

INTRODUCTION

Ruminants are considered a source of high quality protein and the economic viability of a dairy animal is dependent upon normal reproduction. Milk and beef production in cattle industry is very much dependent on maintenance of a high level of fertility in both male and female cattle (Foote, 1996). The reproductive performance of dairy animals (especially cattle and buffaloes) is adversely affected by various factors, but pathological changes in the genital tract caused by microorganisms appear to be the most important factors (Krishnan, *et al.*, 1994). In Egypt, animal's infertility leads to reduction or dropping in the personal and national economical income.

Campylobacteriosis is a wide spread disease associated with infertility (Eaglesome and Garcia, 1992). This infertility is due to early death of the embryo which inturn is the result of uterine infection. Repeat breeding activity is seen with campylobacteriosis and irregular estrus cycles are common. It is a chronic venereally transmitted disease caused by a bacterium named *Campylobacter (C.) fetus* subspecies *venerealis* (Schurig, *et al.*, 1973 and Scott, 1994). This microorganism is transmitted by infected bulls during mating. The practice of artificial insemination also can spread the disease if *C. fetus* is present in the semen (Hartwig, 2000). Moreover, it can be self-limiting and some of the cattle recover within a year. However, carrier cows are common, and *C. fetus* can be spread to non-infected bulls during mating (Rice and Rogers, 2002).

The infection is usually cleared and pregnancy can occur (Hartwig, 2000). He added that, in the male, especially aged bulls, the infection can be carried over until the next breeding season. A typical history is that significant infertility that occurs the first year, then less so in succeeding years because of immunity formation, but infertility is still a problem in heifers (Rice and Rogers, 2002). *Campylobacter spp.* isolated from animals particularly *C. jejuni* and *C. coli* are recognized as one of the etiologic agents of gastrointestinal illness (Linton, 1996 and Bourke, *et al.*, 1998) and acute diarrheal disease in humans worldwide (Skirrow, 1994 and Nachamkin, *et al.*, 1998).

The present study was carried out to evaluate the prevalence of *Campylobacter spp.* commonly present in the genitalia of repeat breeding buffalo-cows. Moreover, the field application of treatment was also evaluated as well as the subsequent fertility.

MATERIALS and METHODS

A total of one hundred and fifty repeat breeder buffalo-cows (2-3 parity) were recorded from various Assiut localities for this study. These animals had a regular estrus cycle but failed to conceive following three either natural or artificial inseminations (frozen semen). Out of these animals, 96 animals were sampled for campylobacter isolation. The selection of these animals was based on the criteria that they had clinically normal genitalia as revealed by rectal examination but failed to conceive. The materials collected from those animals were cultured for isolation and classification of *Campylobacter species*.

Collection of samples:

The perineum and vulva lips were thoroughly cleaned with mild antiseptic solution (0.01% potassium permanganate) then wiped with clean sterile cotton prior to sample collection. The protected sterilized cotton swab was carefully passed into vagina till portio-vaginalis under complete aseptic conditions. The sterile swab was then pushed out of its protective sheath and moved gently around portio-vaginalis and external os of the cervix. After retraction into the tube, the swab was gently removed. All swabs were brought on ice to the laboratory and kept at 4 °C until processed.

Isolation of *Campylobacter species*:

Each swab was suspended in sterile physiological saline (10 ml). The saline suspension of each swab (take about 5 ml) was enriched under aseptic conditions in campylobacter enrichment broth base

(Biolife) supplemented with campylobacter growth supplement and Skirrow antimicrobic supplement. The enriched broth was incubated at 37°C for 48 h under microaerophilic conditions (5% O_2, 10% Co_2 and 85% N_2) in a Co_2 incubator (Wegmuller, *et al.*, 1993). A loopfol of enriched broth of each sample was streaked on campylobacter agar kit Skirrow (Defico Lab. Detroit Michigan, USA) to which 10% sterile defibrinated sheep blood and 10 ml Bacto campylobacter antimicrobic supplement "S" were added (Skirrow, 1990). The inoculated plates were incubated at 37°C for 48 -72 h under microaerophilic conditions. The colonies were enriched under aseptic conditions in campylobacter enrichment broth base and incubated at 37°C for 24 h under microaerophilic conditions.

Identification of *Campylobacter* species:

A) Through PCR

Pretreatment of samples for amplification:

Nucleic acids were extracted from the campylobacter enrichment broth base culture as described by Boom *et al.* (1990). Briefly 1 ml of an overnight culture of campylobacter was centrifuged (30 s at 12.000 Xg) and the bacterial pellet was suspended in 50 µl of TE buffer, then added to 1 ml of buffer [120 g of guanidinium thiocyanate, 100 ml of 0.1 M Tris (pH 6.4), 22 ml of 0.2 M EDTA (pH 8.0), 2.6 g of Trition X-100] and 20 µl of Celite (10 g in 50 ml of H_2O and 500 µl of 32% [wt/vol] HCL). The samples were vortexed and incubated at room temperature for 10 min. After centrifugation at 12.000 Xg (Ependorf centrifuge, fixed angle), the pellets were washed twice with guanidinium thiocyanate buffer that mentioned before, twice with 70% ethanol, and once with acetone. The pellets were dried and suspended in 100 µl of 10 mM Tris (pH 8.0). After incubation at 56°C for 10 min, the samples were centrifuged at 12.000 Xg. The supernatant was used for the PCR.

PCR Technique:

Two synthetic oligonucleotides were CF03 and CF04 (Amersham pharmacia biotech, Austria) were selected and used to generate 340 to 400 bp DNA fragment from the *C.jejuni* and *coli* in the PCR. Sequences of the oligonucleotides are as follows:
CF03,5`-GCTCAAAGTGGTTCTTATGCNATGG-3` (sense), and CF04, 5`-GCTGCGGAGTTCATTCTAAGACC-3`(antisense). PCR reaction was carried out using ready-TO-GO PCR Beads (Amersham pharmacia biotech, product number 27-9555-01) with 25 µl reaction volumes containing 0.25 mM of each primer, 2 µl of template DNA (50

ng). Samples were covered with mineral oil (Sigma) and subjected to PCR on a biometra thermal cycler (Germany) with the following temperature program:
Denaturation at 94°C for 4 min.; 40 to 50 cycles at 95°C for 5 s, 53°C for 30 s, and 72°C for 40 s; and a final extension at 72°C for 5 min. A total of 20 µl of PCR products was analyzed by agarose gel electrophoresis and made visible by ethidium bromide staining and UV transillumination (Nuijten, *et al.*, 1990).

B) Through biochemical reaction

Suspected typical C. colonies were identified morphologically, culturally and biochemical reaction. Biochemical identification carried out according to Garcia *et al.* (1983) using gram stain, motility, catalase test, H_2S on lead acetate strips, growth in 4% glycine, 3.5% sodium chloride at 37 °C for 24 h.

Treatment application:

Selected buffalo-cows were subjected to field application of Vetbiotic vial (Nile company for pharmaceuticals, Egypt) as an intrauterine infusion (100 ml solution which contain about 3 millions I.U penicillin and 4.0 g. streptomycin) for treatment as a weekly dosage for two weeks.

Follow up of the treated animals:

The treated animals were observed for heat. The fertile heat was calculated as the animals, which did not return to heat after 21 days following natural insemination using fertile bulls. Pregnancy diagnosis was carried out on 60-75 day post-insemination by rectal palpation.

RESULTS

The bacteriological examination of the collected samples showed the prevalence of *Campylobacter fetus* infection. *Campylobacter jejuni* or *coli* were not detected. The results present in Table (1) revealed that, out of 96 samples from apparently healthy repeat breeder buffalo-cows, 10 samples were positive for *Campylobacter spp*. The incidence was 10.42% (10/96) of the animals with failed to conceive following three either natural or artificial insemination. All the samples positive to campylobacter enrichment broth were prepared for PCR testing. All the samples were not positive for *Campylobacter jejuni* and *coli* (Fig.1). Table (2) revealed the different *Campylobacter species*,which tested through biochemical reaction. *C. fetus* subsp. *Venerealis* was the main isolates (6.25%) from the animals in this study and *C. fetus* subsp. *fetus* was 4.17%.

The obtained results after application of the treatments and subsequent fertility are shown in Tables (1 and 3). Out of 96 treated animals, 90 (93.75%) responded for treatment but only 6 (6.25%) animals did not respond (Table 1). However, the follow up for the positive cases (for campylobacteriosis) are presented in Table (3). Only one case did not respond after treatment and nine cases responded after treatment. All treated animals showed the first fertile heat as 87.78% as well as 86.08% total pregnancy rate (Table 1). Moreover, the positive animals for compylobacterosis, show the first fertile heat as 88.89% and 87.5% pregnancy rate.

DISCUSSION

A prevalence of campylobacteriosis of 10.42% was found in buffalo-cows in this study. Similar findings have been reported by Das *et al.*, (1995) who demonstrated that *Campylobacter spp.* from animals with reproductive disorders, especially repeat breeder, was isolated in 10.4%. Lower findings have been reported by Akpokodje (1984), Finlay *et al.* (1985) and Bawa *et al.* (1991) who reported 2.9% and Sobhy *et al.* (1996) recorded 7.5%. Higher percentages of campylobacteriosis were stated by Villar and Spina (1982), Campero *et al.* (1987), Akhtar *et al.* (1990) and Sayed *et al.* (2001). These variations could be attributed to the contamination level and methods of isolation (Shum, 1987). Moreover, detection of *Campylobacter* infections can be influenced by the effectiveness of the selective media and time of sampling (Bawa, *et al.*, 1991). The isolation from clinical samples is not easy chiefly due to the microaerophilic nature of *Campylobacters* and the rapid overgrowth of more vigorous multiplying contaminating organisms (Lander, 1990 and Eaglesome and Garcia, 1992). To overcome these problems, microacrobic conditions, 5% O_2, 10% Co_2 and 85% N_2 and campylobacter selective agar media with antibiotic supplement for selective *Campylobacter spp.* were used (Shum, 1987).

Several methods for genetic analysis of bacteria have been described, but the method applied most often is the polymerase chain reaction (PCR). *Campylobacter spp.* are known to be difficult to isolate and require very elaborate culture techniques to show satisfactory growth. PCR may help in overcoming this problem (Atanassova and Ring, 2001). The obtained results after tested the *Campylobacter spp.* genome PCR suggests that the samples have not *Campylobacter jejuni* and *coli*. This assumption was confirmed by the fact that a diagnostic laboratory screening for *Campylobacter spp.* by a conventional culture

method was unable to isolate *C. jejuni* and *C. coli* (Wegmuller, *et al.*, 1993). We concluded that the PCR systems described are excelently suited for detection and confirmation of *C. jejuni* and *C. coli*. The described sample preparation protocol is generally applicable and relies on the isolation of the total bacterial population present in samples.

In this study, *C. fetus* subsp. *venerealis* was more (6.25%) common than *C. fetus* subsp. *fetus* (4.17%) in buffalo-cows which suffered from repeat breeding syndrome. Concerning the incidence of *Campylobacter* subsp., the obtained results did not agree with that reported by Sobhy *et al.* (1996) and Sayed *et al.* (2001) who recorded that, *C. fetus* subsp. *venerealis* was isolated as 11.53%, 6% and subsp. *fetus* as 3.84%, 1.5% respectively. The obtained result indicates that the *C. fetus* subsp. *venerealis* may cause infertility in animals by restricting the supply of oxygen available to the preimplantion embrys (Ware, 1980). Moreover, *C. fetus* subsp. *fetus* plays an important role in reproductive disorder in animals and probably the organism remains as commensal in animals and become pathogenic under favourable condition (Das, *et al.*, 1995).

Concerning the treatment in the positive cases, about 10% did not responde to the used drugs. The result coincid with that reported by Dekeyser (1986) who mentioned that *C. fetus* subsp. *venerealis* and subsp. *fetus* were sensitive to penicillin and streptomycin. Bacterial resistance to antimicrobial agents, which is increasing worldwide, is frequently caused by the acquisition of new genes rather than by mutation (Recchia and Hall, 1995 and Hall and Collis, 1995). An efficient means of acquiring new genes is by mobile genetic elements such as resistance(R)-plasmids and transposons. *Campylobacter spp.* have a natural ability for transformation (Wang and Taylor, 1990) and in shared animal reservoirs, interspecies transfer of DNA, including antimicrobial resistance encoding genes and other unrelated genes, may occur by strategies analogous to site-specific recombination (Jackson, *et al.*, 1998 and Nielsen *et al.*, 1998).

In conclusion, the results of the study identify *Campylobacter fetus* subsp. *venerealis* as the agent of infertility in buffalo-cows and suggest that it may be a significant problem. Fortunately, the fertile heat and pregnancy rates after treatment were higher by using penicillin and streptomycin. It is recommended that routine periodical examination of the genitalia of buffalo-cows should be made in the organized animal to ascertain the presence of microbial agent, which may help for the effective treatment and control of female reproductive disorder. Furthermore, this protocol decreased or stops the financial loss

associated with campylobacteriosis, which resulted from a high culling rate in the herd's animals due to failure to conceive and the seasonal shift in the calving pattern resulting from delayed conception.

REFERENCES

Akhtar, S.; Riemann, H. P.; Thurmond, M.C.; Favrer, T. B. and Franti, C.E. (1990): The use of loglinear model to evaluate factors associated with sero-prevalence of *Campylobacter fetus* in dairy cattle. Theriogenology, 34:989-1001.

Akpokodje, J.U. (1984): Incidence of bovine campylobacteriosis. 4th Annual Conference, Nigeria Society for the Study of Reproduction, NAPRI, ABU, Zaria, Nigeria.

Atanassova, A. and Ring, C.H. (2001): Application of molecular biological methods (PCR) in food hygiene. 1st Cong. of Food Hygiene and Human Health 6-8 Feb., Faculty Vet. Med., Assiut Univ., Egypt.

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. Trop. Anim. Hlth. Prod., 23:157-160.

Boom, R.; Sol, C.J.; Salimans, M.M.; Jansen, C.L.; Werthein-van Dillen, P.M. and van der Noordaa, J. (1990): Rapid and simple method for purification of Nucleic acids. J. Clin. Microbiol., 28(3): 495-503.

Bourke, B.; Chan, V.L. and Sherman, P. (1998): Campylobacter upsaliensis: waiting in the wings. Microbiol. Rev., 11:440-449.

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. Aus. Vet. J., 64(10):320-321.

Das, S.C.; Mullick, S.G.; Roy, P.K.; Biswas, G. and Roy, P. (1995): Isolation and characterization of some bacteria associated with reproductive disorders of dairy cows with special reference to campylobacter species. Ind. J. Anim. Hlth., 34(1): 25-28.

Dekeyser, D.V. (1986): Bovine genital campylobacteriosis. In: Current Therapy in Theriogenology. D.A. Morrow (ed.), Saunders Com., Philadelphia, pp. 263-266.

Eaglesome, M.D. and Garcia, M.M. (1992): Microbial agents associated with bovine genital tract infections and semen. Part. I Brucella abortus, Leptospira, *Campylobacter fetus* and Trichomonas fetus. Vet. Bull., 62(8):757-768.

Finlay, R.C.; Ruckerbauer, G.M. and Stovell, L.P. (1985): Campylobacter infection in man and animals. Canadian J. Comparative Med., 49:231-232.

Foote, R.H. (1996): Review: Dairy cattle reproductive physiology research and management-progress and Future Prospects. J. Dairy Sci., 79:980-990.

Garcia, M.M.; Eaglesome, M.D. and Rigby, C. (1983): Campylobacter important in veterinary medicine. Vet. Bull., 62(8):757-768.

Hall, R.M. and Collis, C.M. (1995): Mobile gene cassettes and integrons : capture and spread of genes by site-specific recombination. Molecular Microbiol., 15:593-600.

Hartwig, N.R. (2000): Infectious diseases that affect cattle fertility. The National Dairy Database.

Jackson, C.J.; Fox, A.J.; Jones, D.M.; Wareing, D.R. and Hutchinson, D.N. (1998): Associations between heat-stable (O) and heat-labile (HL) serogroup antigens of *campylobacter jejuni*: evidence for interstrain relationships within three O/HL serovars. J. Clin. Microbiol., 36:2223-2228.

Krishnan, R.; Tanwani, S.K. and Moghe, M.N. (1994): Antibiotic sensitivity pattern of bacteria isolated from repeat breeding and normal cows. Indian Vet. J., 71:315-137.

Lander, K.P. (1990): The development of a transport and enrichment medium for *Campylobacter fetus*. Br. Vet. J., 146:327-333.

Linton, D. (1996): Old and new *Campylobacters*: a review. PHLS Microbiol. Digest., 13:10-15.

Nachamkin, I.; Allos, B.M. and Ho, T. (1998): Campylobacter species and Guillain-Barre syndrome. Clin. Microbiol. Rev., 11: 555-567.

Nielsen, E.M.; Engberg, J. and Madsen, M. (1998): Validity of Sma I-defined genotypes of *Campylobacter jejuni* examined by Sal I, Kpn I, and Bam HI polymorhisms: evidence of identical clones infecting humans, poultry and cattle. Epidemiol. Infect., 120:231-237.

Nuijten, P.J.; van Asten, F.A.; Gaastra, W. and van der Zeijst, B.A. (1990): Structural and functional analysis of two *Campylobacter jejuni* flagellin genes. J. Biol. Chem., 265:17798-17804.

Recchia, G.D. and Hall, R.M. (1995): Gene cassettes: a new class of mobile element. Microbiol., 141:3015-3027.

Rice, D.N. and Rogers, D. (2002): Common infectious diseases that cause abortions in cattle. The National Dairy Database.

Sayed, A.M.; Abd El-Hafeez, M.M.; Shehata, S.H. and Abd El-Kader, H.A. (2001): Prevalence of campylobacteriosis in dairy friesian cattle with genital troubles in some farms of Assiut governorate. Assiut Vet. Med. J., 45(89): 278-288.

Schurig, G.D.; Hall, C.F.; Burda, M.S.; Corbeil, L.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. Rec., 135:162-163.

Shum, F.S. (1987): Bovine abortion due to *Campylobacter fetus*. Aust. Vet. J., 64(10): 319-320.

Skirrow, M.B. (1990): Campylobacter. The Lancet, 336:921-923.

Skirrow, M.B. (1994): Diseases due to *Campylobacter*, Helicobacter and related bacteria. J. Comp. Pathol., 111:113-149.

Sobhy, M.M.; Kholeaf, Z. M.; Ibrahim, I.G. and Farag, Y.A. (1996): Campylobacteriosis in cervicovaginal mucus of female cattle and buffaloes. 8th Annual Congr. Egypt Soc. Anim. Reprod. Fertil. Cairo, pp. 305-311.

Villar, J.A. and Spina E.M. (1982): Bovine campylobacteriosis. A survey of its prevalence in Argentina between 1966-1981. Gaceto Veterinaria, 44:647-658.

Wang, Y. and Taylor, D. E. (1990): Natural transformation in *Campylobacter species*. J. Bacteriol., 172:949-955.

Ware, D.A. (1980): Pathogenecity of *Campylobacter fetus* subsp. *venerealis* in causing infertility in cattle. Br. Vet. J., 136:301-303.

Wegmuller, B., Luthy, J. and Candrian, U. (1993): Direct polymerase chain reaction detection of *campylobacter jejuni* and *campylobacter coli* in raw milk and dairy products. Environ. Microbiol.,59:2161-2165.

Table 1: Distribution of the results.

Item	No.	%
-Total examined animals*	150	
-No. of animals used for treatment**	96	
-No. of positive samples***	10/96	10.42
-Treatment response	90/96	93.75
-Non-response for treatment	6/96	6.25
-Fertile heat	79/90	87.78
-Pregnancy rate	68/79	86.08

* Number of examined animals in this study.

** Numbers of animals with clinically normal genitalia (repeat breeder).

*** Number of animals had a positive campylobacteriosis.

Table 2: Incidence of *Campylobacter species* and subspecies (ss) from positive animals

Isolates	(n=96)	%
- *C. fetus*	10	10.42
C. fetus ss *venerealis*	6	6.25
C. fetus ss *fetus*	4	4.17
- *C. jejuni*	-	-
- *C. coli*	-	-

Table 3: Follow up of treatment in positive animals

Item	(n=10)	%
- Treatment response	9	90
- Non- response	1	10
- Fertile heat	8*	88.89
- Pregnancy rate	7**	87.5

* From the treated response animal (n=9).

** From the fertile heat's animals (n=8).

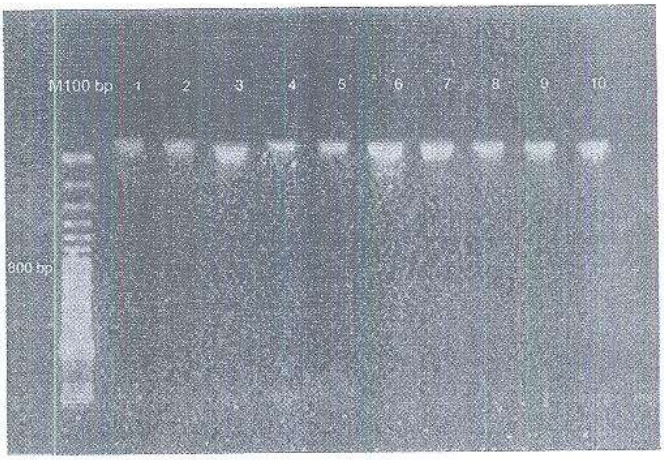

Fig. (1): Sensitivity of CF03 – CF04 PCR assay.

The figure shows the detection limit of CF03 – CF04 PCR products from 10^5 cells of *Campylobacter species* as obtained by ethidium bromide-stained agarose gel.PCR. Lanes:-

M, 100-bp DNA ladder (Amersham pharmacia Biotech).

1-10 are the samples which showed no bands (negative *C. jejuni* and *C. coli*).