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RAPID LABORATORY DIAGNOSIS OF CAMPYLOBACTER INFECTION USING IMMUNOFLUORESCENT ANTIBODY TECHNIQUE (IFAT)

BY

JAKEEN, EL-JAKEE,* R. SOLIMAN,* A.KHALID** and M. ISMAIL***

* Dept. Microbiology, Fac. Vet. Med. Giza Univ.
** Dept. Microbiology, Fac. Vet. Med. Moshtohor. Banha Univ.
*** Dept. Vet. Medicine, Fac. Vet. Med. Cairo Univ.

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INTRODUCTION

Campylobacter species are frequent aetiological cause of bacterial diarrhoea all over the world (Blaser et al. 1979 and Walder, 1982). Since 1977, campylobacter jejuni has been increasingly recognized as a cause of sporadic and epidemic diarrhoea, not only in animals but also among humans (Jones et al., 1981). Also different campylobacter species have been increminated as a cause of mastitis, infertility and abortion among various animal species (Safford, 1969).

The cultural recovery of campylobacter species from clinical specimens has been a constant problem, firstly, because of the relatively low numbers of organisms in the clinical specimens and the usually present contaminants and secondary because of the possibility of cold injury on campylobacter cells, especially when cultured on antibiotic media (Humphrey and Gruickshank, 1985).

For these reasons, it was the aim of the present investigation to compare and to evaluate the efficacies of immunofluorescent antibody technique (IFAT) and the conventional culturing procedures in definitive diagnosis of campylobacteriosis.

MATERIAL AND METHODS

Materials

Specimens:

A total of 200 different samples were collected from various animal species as shown in Tab. (1). The collected specimens were examined for the presence of campylobacter microorganisms using both the conventional culturing procedures and the IFAT.

Table (1): Different types of specimens collected from various animal species and examined for campylobacter species

Source of specimens	Type of specimens			
	Faeces	Mastitic milk	Preputial wash	Aborted feti
Diarrhoeic calves	100	-	-	-
Buffaloes	-	50	-	-
Infertile Bull	-	-	20	-
Sheep	-	-	-	17
Diarrhoeic chicken	13	-	-	-

Laboratory animals: Eight white New Zealand, male rabbits (2 kg/each) were used for preparation of anti-campylobacter antisera.

Campylobacter strains: The following campylobacter species were used; C.jejuni, C.fetus ss. fetus, C.fetus ss venerealis and C.bubulus. These strains were isolated and typed in Dept. of Microbiology, Fac. of Vet. Med. Cairo Univ.

Fluorescein isothiocyanate (Sigma Micro.) was used to label the globulin fraction of the anticampylobacter antisera.

Bacterial culture media:

Camp BAP medium: Brucella agar base containing 5 % sheep RBCs and supplemented with the following antibiotics/liter; vancomycin, 10 mg; Trimethoprim, 5 mg; polymyxin B, 25000 iu; amphotericin B_2, 2 mg and cephalothin, 15 mg (Kaplan et al. 1985).

Thioglycollate agar:

Methods:

Bacteriological examination:

All samples were inoculated immediately after collection onto camp-BAP and incubated at 37°C for 48 hrs under microaerophilic conditions. Colonies showing characteristic morphology of campylobacter microorganisms were picked up and re-inoculated onto tubes of semisolid thioglycollate agar media and at the same time streaked over brucella agar plates enriched with 10 % defibrinated sheep blood. Six plates were used for inoculation by each of the suspected colonies.The plates were incubated under micro-aerophilic conditions for 48 hrs at 25, 37 and 42°C (2 plates at each temperature). The isolates were identified according to Krieg and Holt (1984).

Anti-Campylobacter rabbit antisera:

Anti sera against C.fetus ss. venerealis, C.fetus ss. fetus, C.jejuni and C.bubulus were separately prepared in rabbits according to Chang et al. (1984). Titres and specificity of antisera were determined.

Preparation of fluorescein isothiocynate (FITC) conjugated anti campylobacter antisera was made according to Hijmans et al. (1969).

Examination of the collected specimens using direct IFAT:

From each specimen several smears were made on clean glass-slides. The slides were air dried and fixed with 95 % ethanol at room temperature. The prepared smears were separately stained with FITC-conjugates prepared against C.fetus ss. venerealis, C.fetus ss. fetus, C.jejuni and C.bubulus. A conjugate dilution of 1/100 was used for staining. After 30 min. incubation at 37°C in humid chamber, the slides were washed with 3 changes of PBS (pH 7.2, 10 minutes), then mounted with buffered glycerol and examined with fluorescent microscope. Negative and positive controls were included where smears of E.coli were made and similarly stained with the different conjugates as negative controls. On the other hand, the known identified campylobacter smears were stained with the corresponding conjugates as positive controls. Also, the cross reactivity of the prepared conjugates was tested.

The intensity of fluorescence was recorded as 0 (negative) through 4+.

RESULTS AND DISCUSSION

From data presented in Tab. (2) it is clear that IFAT was more sensitive than the culturing procedure for detection of campylobacter microorganisms in clinical specimens.

Using IFAT, out of 200 specimens 36 (18 %) were positive for campylobacter species, compared with only 8 (4 %) successful isolations using the conventional culturing procedure.

The only campylobacter species isolated from faecal samples of diarrhoeic calves was C.jejuni, where 3

isolates were recovered from 100 faecal samples.When the same samples were examined by IFAT using anticampylobacter conjugates, 20 samples reacted positively with the anti-campylobacter jejuni FITC conjugate (dil. 1/100) giving 4+ florescence intensity. Minimal degree of florescence was observed with other conjugates which disappeared when we used 1/160 dil. of the conjugates.

Again the bacteriological examination of 13 faecal samples from chickens were negative for campylobacter bacteria, however, using IFAT one samples reacted positively with the anti campylobacter FITC-conjugates particularly with anti C.jejuni conjugate giving a 3+ fluorescence intensity.

The capability of C.jejuni in causing irritation and pathological changes in the intestinal tract of animals and chickens with subsequent enteritis has been reported by several authors (Grant et al. 1980; Firehammer and Myers, 1981; and Neill et al. 1981).

The isolation of C.jejuni was also successful from one out of 50 buffalo milk samples. Using IFAT, however 3 samples (6 %) reacted positively with the anti C.jejuni FITC conjugate. This finding coincides with those reported by Robinson et al. (1979) and Vogt et al., 1984).

Two C.fetus ss. fetus strains were isolated from 17 aborted sheep foeti. Eight specimens, however, reacted positively in IFAT with 4+ florescence with anti-Campylobacter/ FITC conjugate (1/100 dil.) prepared against C.fetus ss. fetus. Also, the same eight specimens reacted positively with the conjugates prepared against other campylobacter species particularly C. fetus ss. venerealis. Repeating the examination of the 8 positive specimens with 1/160 dilution of the

Table 2:

Comparison between efficacy of IFAT and conventional culturing procedure for detection of campylobacter spieces in different clinical material from various animal species.

Animal species	Type of sample	No. of samples	Detection of C. species by			
			IFAT		Culturing proc.	
			No. of positive	%	No.	%
Calves*	Feces	100	20	20	3	3
Chicken*	Feces	13	1	8	0	0
Buffaloes**	Milk	50	3	6	1	2
Sheep	aborted feti	17	8	47	2	12
Infertile Bulls	Preputial wash	20	4	20	2	10
Total		200	36	18	8	4%

* Animals and birds with diarrhoea.
** Buffaloes showing clinical mastitis.

different conjugates, the reaction was more specific to C.fetus ss. fetus.

With regard to preputial wash samples obtained from 20 infertile bulls, C.fetus ss. venerealis was isolated from two specimens. Using IFAT 4 specimens reacted positively with FITC conjugates prepared against C.fetus ss. fetus and also C.fetus ss. venerealis.

These finding are in agreement with those of Florent (1960), Winkenwerder (1966), Bryner et al. (1972) who recorded that C.fetus ss. venerealis could be detected from preputial wash of bulls and C.fetus ss. fetus from aborted foeti of sheep.

The sensitivity of IFAT in detection of campylobacter microorganisms can be attributed to the fact that IFAT detect the antigenic materils in both dead and living bacterial cells. This conclusion is consistent with those of Ardrey et al. (1972), Maclaren and Wright (1977) and Chang et al. et al. (1984) who reported that IFAT is a satisfactory routine test for laboratory diagnosis of vibriosis. The main disadvantage of IFAT is that various degree of cross-reaction between different campylobacter species have been observed and this make the laboratory diagnosis of campylobacteriosis by IFAT more reliable on the genus level rather than on species level.

SUMMARY

Comparison was made between the direct Immunofluorescent Antibody Technique (IFAT) and the conventional bacteriological procedures in the laboratory diagnosis of campylobacteriosis.

The IFAT test was more sensitive and rapid than the culturing procedure in detection of campylobacter

microorganisms in clinical specimens. However, the IFAT diagnosis was more reliable on the genus level rather than on species level due to the cross-reactivity between various species.

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