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COMPARISON OF CONVENTIONAL STAINING TECHNIQUES AND FITC-LABELED MONOCLONAL ANTIBODY-BASED METHOD FOR DETECTION OF CRYPTOSPORIDIUM OOCYSTS IN NEONATAL CALVES

(With 6 Fig. & One Table)

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(Received at 22/8/1994)

المقارنه بين الصبغات التقليديه وصبغة الفورسنت المقترنه بالاجسام المضاده كطريقة تشخيص حويصلات الكريبتوسبوريديا فى العجول حديثة الولاده

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أجريت هذه الدراسه للتعرف على مدى قابلية أربعة أنواع من الصبغات لاكتشاف طفيل الكريبتوسبوريديا فى براز العجول الرضيعه . وقد تم جمع ٥٥ عينة براز من عجول رضيعه أقل من شهرين ومصابه بالاسهال . وقد تم التعرف على الطفيل فى عينات البراز بجميع أنواع الصبغات ولكن بنسب مختلفه . وجد أن طريقة التركيز بالتعويم عامة تزيد من نسبة الاصابه لانها أدق . ووجد أن أحسن الطرق استخداماً هى صبغة الفلورسنت المقترنه بالاجسام المضاده كطريقة للتشخيص ثم يليها صبغة الزيل نيلسون المعدله . وباقى الصبغات ذات حساسيه أقل سواء بعد أو قبل التركيز بالتعويم ومن هذا نقترح استخدام صبغة الزيل نيلسون والفلورسين معاً وذلك للحصول على نتائج أفضل وذات حساسيه عاليه .

SUMMARY

This study was assessed to discover the ability of four staining techniques, initially, developed for the rapid identification of *Cryptosporidium* species oocysts in faecal samples of 55 neonatal calves less than 2 weeks old. All the stains employed in this study (modified Ziehl-Neelsen, Safranin-methylene blue, Giemsa and FITC-labeled monoclonal antibody as a commercial kit) detected the oocysts with incidence discrepancy. Concentration flotation of the samples raised diagnostic value of all techniques used. FITC-labeled monoclonal antibody was the best in identifing the oocysts from concentrated faecal samples (72.7%) as well as from the unconcentrated one (63.6%). The recovery of oocysts by other methods was variable includes modified Zeihl-Neelsen (45.5%), safranin-methylene blue (36.4%) and Giemsa stain (18.2%). Our findings suggested that FITC-labeled monoclonal antibody as a probex to detect the oocyst-specific epitope even without concentration flotation was highly sensitive and specific than the other traditional techniques. Combination between fluorescent antibody and modified Ziehl-Neelsen stains, where possible, are the currently recommended procedures for thc detection of oocysts.

Keywords: Comparison, conventional staining FITC monoclonal antibody based method, cryptosporidium oocyst, calves

INTRODUCTION

Cryptosporidium species are coccidian parasites of gastro-intestinal and respiratory epithelial cells of many species of vertebrate animals. *Cryptosporidium parvum* is the major species responsible for clinical illness in human and animals *(CURRENT and BLAGBURN, 1990)*. Isolates from animals can initiate infection in man and human isolates can also produce infection in domestic and wild animals *(FAYER and UNGER, 1986 and EL-REFAY, 1992)*. This is uncommon finding among coccidian parasites, which may explain in part its unusual epidemiology and zoonotic significance.

Diagnosis at present is based largely on the identification of cryptosporidial oocysts in faecal smears.

Several techniques have been described by various investigators to improve the speed of diagnosis by devising methods for scanning at low magnification. Some staining techniques were more specific than others, as Wright-Giemsa *(POHLENZ et al., 1978)*, modified Ziehl-Neelsen (MZN) stain *(HENRIKSEN and POHLENZ et al., 1981)*, safranin-methylene blue *(BAXBY et al., 1984)* and FITC-Conjugated monoclonal antibody *(MCLAUGHLIN et al., 1987)*.

The use of immunofluorescent assay may be overlooked because of the small size and paucity of oocysts in some samples *(SNYDER, 1988)*. The use of fluorescent microscopy in conjunction with specific immunological probes was found to be more sensitive and high specific method in detecting oocysts from faecal matter.

The aim of the present study was to assess the relative abilities of some staining techniques in detecting *Cryptosporidium* species oocysts in faecal samples of infected calves.

MATERIAL AND METHODS

Faecal samples:

55 rectal faecal samples were taken from newborn buffalo-calves less than two weeks old. These calves were housed in different private farms for milk production. All animals suffered from yelllowish diarrhea at the time of sampling.

Samples were collected in clean sealable plastic containers and refrigerated at 4°C. The collected samples were subjected to examine both by direct smear and after concentration by flotation using Sheather's sugar solution; the supernatant washed three times by centifugation with dist. water *(REESE et al., 1982)*. The sedemented oocysts were smeared and stained as mentioned below.

Staining of faecal samples:

Faecal samples with and without concentration were stained for the detection of *Cryptosporidium* oocysts by:

1- Giemsa stain *(POHLENZ et al., 1978)*.
2- Modified Ziehl-Neelsen stain (MZN) *(HENRIKSEN and POHLENZ, 1981)*.
3- Safranin-methylene blue *(BAXBY et al., 1984)*.
4- FITC-conjugated monoclonal antibody *(MCLAUGHLIN et al., 1987)* using commercial Kit from Meridian Diagnostics CinCinnati, Ohio. This MERIFLUOR, direct imunofluorescent kit (Fig. 1) was designed for the simultaneous detection of *Cryptosporidium* and *Giardia* catalog # 250050.

The stained faecal smears were examined at X1000 ad X 400 using ordinary and fluorescence microscopes respectively.

Principles and interpretation of the fluorescent assay:

In direct immunofluorescence technique, the detection reagent contains a FITC-labeled monoclonal antibodies directed against cell wall antigens of *Cryptosporidium* oocysts. The monoclonal antibodies attach to *Cryptosporidium* antigens present in the specimen. The slides were freshly rinsed to remove unbound antibodies, a cover slip is affixed with mounting medium and examined under fluorescent microscope for detection of oocysts which were round to slightly oval in shape, apple green in colour and 4-6 micron in diameter. The background material in the sepecimen was counter stained dull yellow to red.

RESULTS

As shown in table (1), cryptosporidial oocysts were detected in faecal samples of examined calves with all staining techniques emploed at a different detection efficiency.

Table 1: Evaluation of different staining techniques for identification of *Cryptosporidium oocysts* from faecal samples of neonatal calves*.

Technique	Result of examination			
	with concentration**		without concentration	
	No. of +ve	% of +ve	No. of +ve	% of +ve
Modified Ziehl-Neelsen	25	45.5	14	25.5
Safranin-methylene blue	20	36.4	9	16.4
Giemsa	10	18.2	3	5.5
FITC-labelled monoclonal	40	72.7	35	63.6

*: Total number was 55 rectal samples of < 2 weeks old.
**: Concentration was performed by Sheather's sugar flotation.

DISCUSSION

The purpose of this investigation was to evaluate the accuracy of some staining techniques in identifing cryptosporidial oocysts in faecal samples of infected calves. In the present study, all the stains used were capable of detecting oocyss with incidence discrepancy from faecal samples (Table 1) including FITC-labeled monoclonal antibody (Fig. 2 & 3), MZN (Fig. 4), safranin-methylene blue (Fig. 5) and Giemsa

(Fig. 6) stains. The chance of recovery of oocysts from faecal samples was inhanced by using Sheather's sugar solution for the concentration technique.

Concerning the traditional staining techniques employed in this study; MZN stain was the best overall in detecting oocysts, a finding which was similar to that previously observed by *SALEM (1989) and MAYSA et al. (1993).*

Safranin-methylene blue and Giemsa stains reported to be both sensitive and rapid but to lesser extent than MZN. These resutls agreed with those recorded by *BADAWY (1989),* Moreover, the use of concentration flotation in combination with MZN, safranin methylene blue and Giemsa stains was found to be more reliable in detecting oocysts (45.5%, 36.4% and 18.2%) than the direct smear (25.5%, 16.5% and 5.5%) respectively (Table 1). This finding was in agreement with that recorded by *CURRENT (1988).*

FITC-labeled monoclonal antibody in the form of a commercial direct immunofluorescent Kit was found to be specific and highly sensitive than other staining techniques either with (72.7%) or without (63.6%) flotation. This finding coincided with the suggestion recorded by *GARCIA et al. (1987); WEBER et al. (1991) and XIAO et al. (1993).* On the other hand, *COZON et al. (1992)* mentioned that, acid fast staining is at least ten times less sensitive than immunofluorescence.

However, screening by immunofluorescent technique was found to be relatively quick to performe. It also offeres the advantage of occysts detection at low magnification. This finding was in agreement with that previously observed by *SMITH et al. (1989).*

All faecal samples examined in this study were watery, so samples were devoid of faecal debris making oocyst detection more easy, consequently false-negatives and false-positives were not considered, this opinion was in agreement with *(WEBER et al., 1991).*

In Egypt, cryptosporidiosis had been recorded in calves by many authors with varying incidence as *ISKANDER (1985)* -9%, *ISKANDER et al. (1987)* -20.7%, *SALEM (1989)* -7.6%, *OTIFY et al. (1990)* -31.15% and *WAHBA (1994)* -8.2%. The discrepancy of infection rates was attributed by the authors to the age of animals and the staining techniques.

Combination between immunofluorescent assay and modified Ziehl-Neelsen stain to identify cryptosporidial oocysts are the currently recommended procedures in the asymptomatic host during epidemiologic or outbreaks investigations. They may eventually provide an improvement in coprodiagnsis because of their higher sensitivity in detecting the infection.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. David allred, associate scientist and Mr. Bigboy Simbi, Department of infectious disease, University of Florida, USA for the fluorescent microscopic examination and photography.

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LEGENDS

Fig. 1: MERIFLUOR *Cryptosporidium/Giardia* Direct immunofluorescent Detection Kit.
A) Mounting Medium, B) Counterstain
C) Detection reagent(FITC labeled monoclonal antibody)
D) Positive control, E) 20 X Wash buffer.,
F) Treated slides. and G) Transfer loops.

Fig. 2-6: *Cryptosporidium* spp. oocysts isolated from calves stained with:

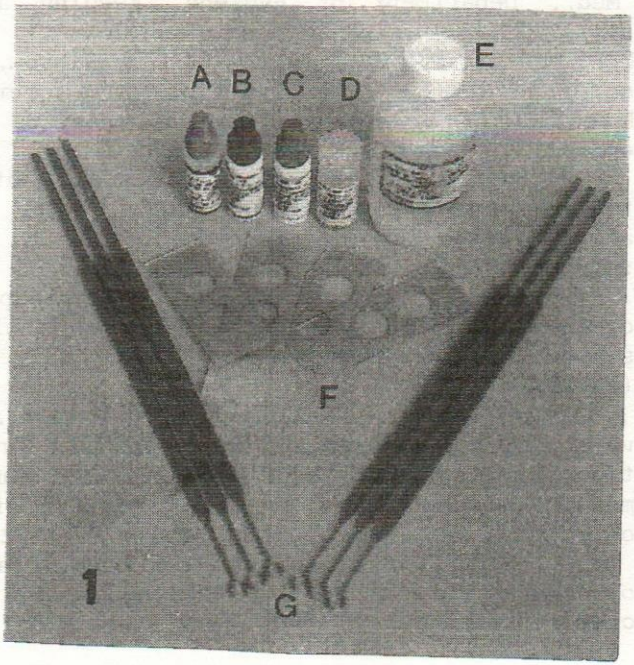
Fig. 2: FITC-labeled monoclonal antibody stain before concentration flotation. X 400.

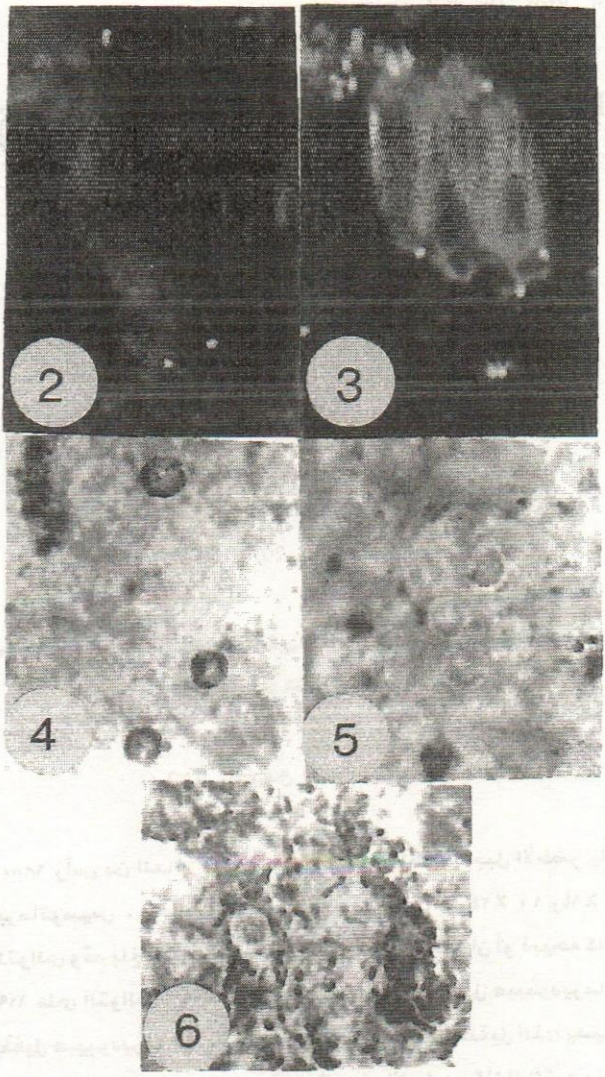
Fig. 3: FITC-labeled monoclonal antibody stain after concentration flotation X 400.

Fig. 4: Modified Ziehl-Neelsen stain after concetration flotation. X 1100.

Fig. 5: Safranin-methylene blue stain after concentration flotation. X 1200.

Fig. 6: Giemsa stain after concentration flotation. X 1000.

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