COMPARISON BETWEEN TWO DIAGNOSTIC METHODS FOR DETECTION OF CRYPTOSPORIDIUM SPP. INFECTING FARM ANIMALS IN KUWAIT

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ABSTRACT

Cryptosporidiosis is an important disease in young farm animals causing diarrhea and consequently leading to economic losses. In addition, the disease is zoonotic transmitted to humans. Accurate and fast diagnosis is needed for improvement hygienic measures as there is no treatment for cryptosporidiosis. Recently, commercial immunochromatographic (IC) assays have appeared in spite of there are some advantages over the conventional methods e.g. like floatation concentration and Ziehl-Neelsen staining (ZN). This study was conducted to compare between immunochromatographic (IC) assay and ZN, which is widely used routinely in laboratories for diagnosis of *Cryptosporidium* infection. The Study revealed that IC was a more sensitive detection method than ZN staining. In a comparison of all 1209 fecal samples collected, *Cryptosporidium* was detected in 12% by IC versus 6.38% by ZN staining (Fishur exact test, P<0.000). Even in each animal species, the number of positive samples detected by IC was higher than those detected by Zn. IC is found to be easy to be performed and its results were easy to be interpret. The overall prevalence of *Cryptosporidium* infection (16.3%) higher than that of other enteropathogens: rotavirus (2%), coronavirus (0.7%) and *E. coli* k99 (7.4%). This finding indicated that *Cryptosporidium* is an important disease agent among farm animals in Kuwait, particularly in cattle and small ruminants.

Keywords: *Cryptosporidium*, cattle, floatation technique, Zoonotic infection, Immunochromatographic (IC), Kuwait

INTRODUCTION

Cryptosporidiosis is one of the most economically important diseases in farm animals, particularly in newborn sand preweaned animals **(De Graaf et al., 1999)**. In addition, the implication of some species of *Cryptosporidium* in zoonotic infection highlights their importance and underline the needs for accurate and rapid diagnosis in routine laboratory work and epidemiological

studies **(De Graff et al., 1999 and Haider et al., 2014)**.
A variety of tests have been development for the diagnosis of *Cryptosporidium* infection. Most of them involve detection of oocysts by microscopy e.g. floatation concentration technique and staining **(Garacia et al., 1983)**. Many staining techniques have been used, but the most common one is modified Ziehl-Neelsen (ZN) staining **(Ramirez et al., 2004)**. New commercial rapid immunoassays have designed to detect *Cryptosporidium* copro-antigen. Immunochromat-ography (IC) is simple and rapid with minimal training; however, debate still continues if its sensitivity is less, equal or higher than conventional methods **(OIE, 2008).**
The aim of this study is to compare between microscopic test (ZN) and immunologic test (IC) for detection of *Cryptosporidium* in different farm animals (cattle, sheep, goats, camels and horses) in Kuwait as well as to screen other 3 enter pathogens (Rotavirus, Coronavirus and *E. Coli* K 99) besides *Cryptosporidium* by using IC to know their relative prevalence and importance in animal farms.

MATERIAL AND METHODS

Sample collection

A total of 1527 samples (400 cattle, 334 sheep, 222 goats, 253 camels and 318 horses) were randomly collected from October 2014-Septemper 2015 during weekly visits to different animal farms in Kuwait. Fresh fecal samples are collected from the rectum of animals or room ground when they were freshly deposited. The samples were placed in clean containers, which were labeled with date, geographical location and animal species, in addition to healthy status and other demographic data. The containers with the samples were placed in ice box and transferred to laboratory investigation.

(1) Ziehl-Nelson (ZN) staining technique:

The technique was performed according to **Casmore (1991)**. Briefly, from each sample, fecal smear was made on a clean slide, which was labeled. The slides with smears from different samples were placed on a multisided carrier. The smears were left to be dried, then fixed with methanol (3 minutes), stained with carbolfuchsin (15 minutes) and lastly stained with ethylene blue (60 seconds). The stained smear was examined under microscope at X1000 oil magnification for detection of stained oocysts, which appeared as red round or slightly ovoid objects against blue background.

(2) Immunochromatographic (IC) assay:

The fecal samples tested for the presence of *Cryptosporidium*, Rotavirus, Corona virus and *Escherichia coli* K99 antigens by a commercial immunochromatography rapid test (BoviD-4 Ag rapid kit, Bionotelnc, Korea), following the instructions of the manufacturer. This kit is qualitative, produced for diagnosis of cryptosporidiosis in cattle, but it was tried to be

used in small ruminants and camels. The device of ach kit has a testing window, which has an invisible T (test) zone and C (control) zone. When a liquefied sample was applied into the sample hole on the devise, the liquid was laterally flow on the surface of the test strip. If the sample was being positive, a red band appeared after 10 minutes. The C band should be appearing red after a sample was applied indicating a valid results.

RESULTS

Table (1) shows the prevalence of *Cryptosporidium* spp. and other enteropathogens in different animal species using IC. The frequency of *Cryptosporidium* infection in animals was higher than that of other enter pathogens. The overall prevalence of *Cryptosporidium* in all animals being 16.3%; *E. coli* ranked the second in prevalence (7.4%). The frequency of *Cryptosporidium* in cattle being the highest and no infection is detected in horses.

The results of the diagnostic techniques used for detection of *Cryptosporidium* in farm animals is shown in Table (2). IC was more sensitive than ZN stain (Fisher's exact test, $P<0.05$).

Table (1): Prevalence of *Cryptosporidium* spp. and various enteropathogens in different animals

Animal species	No of samples collected	No of samples positive with various pathogen (%)			
		Cryptosporidium	*Rota*	*Corona*	*E. coli*
Cattle	400	92 (23)	21(5.3)	4 (1.0)	73 (18.5)
Sheep	334	32 (9.6)	4 (1.2)	6 (1.8)	23 (6.9)
Goats	222	15(6.8)	5 (2.3)	0	9 (4.1)
Camel	253	10 (4.0)	0	1 (0.4)	8 (3.2)
Horse	318	0	0	0	0

Table (2): Results of different diagnostic techniques used for the diagnosis of Cryptosporidium in different animal species

Different diagnostic Technique	Cattle (400)		Sheep (334)		Goats (222)		Camels (253)		Total (1209)	
	Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)
ZN	56	14	14	4.2	8	3.6	3	1.2	81	6.7
1C	92	23	32	9.6	15	6.8	10	4	129	12.3*

* p-value is significant (< 0.05) by Fisher's exact test

DISCUSSION

Detection of *Cryptosporidium* oocysts or *Cryptosporidium* specific antigen in fecal samples is the most appropriate tests for most applications **(OIE, 2008)**. The techniques used for the diagnosis of *Cryptosporidium* infection are either microscopic investigation e.g. flotation concentration and ZN immunologic e.g. ELISA, IC or molecular analysis e.g. PCR.

There is no gold standard technique and recovery of *Cryptosporidium* spp. from specimen with light infection is problematic **(Weber et al., 1991)**. For selection of method to detect *Cryptosporidium*, many criteria should be considered including sensitivity percentage.

In some studies, e.g. **Khurana et al., (2012) and Sharma and Busang, (2015)**, stated that ELISA was found to be more sensitive than ZN; in contrary, **Weitzel et al. (2006)** reported *Cryptosporidium*-antigen assays were less sensitive than conventional microscopic methods. **Al-Megrin (2015)** found no statistical significance between the efficiency of ELISA as immunologic technique and ZN as microscopic method in the detection of *Cryptosporidium*.

In the present study, it was found that the sensitivity of IC was higher as compared with ZN ($P< 0.05$); in addition, IC was ranked higher for other attributes e.g. ease in use and ease for interpretation. ZN stained smear were difficult interpret, requiring frequency examination at x1000 oil magnification to identify the oocyst **(Kehl et al., 1995).**

Moreover, with ZN stains difficult due to poor up take of stain by oocysts as well as sometimes and the discriminating between *Cryptosporidium* oocysts and other spherical objects of similar size (e.g. yeast) staining dull red **(Connelly et al., 2013)**. ZN and IC are adaptable to batch and single test; therefore, they can be used in routine laboratory work as well as in epidemiological studies with larger number of specimens; however, in this study IC was more simple and easier to be performed when compared to ZN staining, which needed several procedures and more time to be performed.

The advantage of ZN stain is that it can roughly measure the intensity of infection by counting the stained oocysts microscopic field. In IC, it is possible to consider the appearance of faint red band is a weak positive result due to light infection, but this is conclusion questionable and needs experimental work.

The present study proved that *Cryptosporidium* is the most important enteropathogen infecting farm animals in Kuwait. This observation should pay the attention of veterinarians and owners to incriminate this parasite as a primary cause of diarrhea in farm animals, particularly in cattle and small ruminants. Also, this study proved immunoassay, BoviD-4 Ag rapid kit, can be used in diagnosis of infection in small ruminants and camels, be sides cattle.

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مقارنة بين طريقتين لتشخيص الإصابة بالكريبتوسبوريديم في حيوانات المزرعة

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يعتبر مرض *الكريبتوسبوريديم* من أهم الأمراض التي تصيب صغار حيوانات المزرعة لما تسببه من خسائر اقتصادية، وذلك لكونه مرض مشترك ينتقل إلي الإنسان، ولاتخاذ الإجراءات الصحية المناسبة يجب أن يكون التشخيص سريعاً ودقيقاً خاصة أن المرض ليس له علاج، وحديثاً مع ظهور اختبار المناعة للوصف اللوني أثير جدلاً حول ما إذا ما كانت هذه الاختبارات لها مزايا تفوق الطرق التقليدية مثل اختبار الصبغة بالزيل نلسن، التي تستخدم عادة في عمل المختبرات الروتيني لتشخيص *الكريبتوسبوريديوم*.

كشفت الدراسة الحالية أن اختبار المناعة للوصف اللوني أكثر حساسية لتشخيص *الكريبتوسبوريديوم* عن الصبغة بالزيل نلسن، حيث وجد أن اختبار (١٢٠٩) عينة براز من جميع حيوانات المزرعة *للكريبتورسبوريديوم* كان منها ١٢% موجب باستخدام اختبار المناعة للوصف اللوني، بينما فقط (٦,٣٨%) بصبغة الزيل نلسن وتم تأكيد هذا الفرق احصائياً باختبار "فيشر" الإحصائي، حتي في كل نوع من الحيوانات على حدة، كانت عدد العينات من البراز التي أعطت نتيجة إيجابية باستخدام الاختبار المناعي للوصف اللوني أكبر مقارنة بعدد العينات الموجبة بصبغة الزيل نلسن.

وكشفت الدراسة الحالية أيضاً أن نسبة الإصابة *بالكريبتوسبوريديوم* (١٦,٣) في حيوانات المزرعة أكثر من المسببات المرضية الأخري، مثل مثال: فيروس الروتا (٢%) وفيروس الكرونا (٠,٧%) والبكتيريا العصوية القولونية (٧,٤%) ، وهذه النتيجة تدل على أهمية *الكريبتوسبوريديوم* كمسبب مرضي بين الحيوانات في دولة الكويت ويجب أن يضعه الأطباء البيطريين والمربين في الاعتبار عند ظهور أعراض الإسهال في المزرعة.