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Comparison Between Different Techniques for Diagnosis of Bovine Paratuberculosis in Dairy Cows in Khartoum State, Sudan.

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ملخص البحث

أظهرت الدراسة أن نسب انتشار بكتيريا المتفطرة الطيريه نوبع نظير الدرن [*Mycobacterium avium* subspecies paratuberculosis (MAP)] فى الأبقارالحلوب الموجبة لإختبار زرع الروث فى ولاية الخرطوم بلغت62.5٪ (8/5) على مستوى القطيع و9.3٪ (215/20) على مستوى الحيوان الفرد. لوحظ أن أدنى نسبة للحالات الموجبة لإختبار زرع الروث كانت بمنطقة سوبا غرب هى 5.4٪ (36/2) وأعلاها بمنطقة السليت 53.3٪ (15/8). الحالات الموجبه لإختبار زرع الروث مع إختلاف نسب إنتشارها رصدت فى كل مناطق الدراسه. سجلت مدينة الخرطوم بحرى أعلى درجة موجبه لإختبار زرع الروث [13.2 (114/15)] بينما سجلت مدينة الخرطوم أدني درجه (69/2)2.9% .

Summary

Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infected diary cows in Khartoum State were reported as 62.5% (5/8) at the herd level and 9.3% (20/215) at the individual dairy cows level. The lowest value of positive faecal culture was 5.4% (2/36) at Soba West whereas the highest was 53.3% (8/15) at Al-Selait. Positive faecal culture was encountered in all localities of Khartoum State with different prevalence rates; Khartoum North showed high positive faecal cultures 13.2% (15/114), whereas it is low in Khartoum 2.9% (2/69). The relationship between of faecal smear, rectal scraping and faecal culture results and clinical signs was discussed.

Introduction

Paratuberculosis is responsible for a high morbidity rate and a significant economic loss in dairy and beef cattle industries (Merkal *et al*, 1975; Buergelt and Duncan, 1980; Jones, 1989; Nordlund *et al*, 1996). In the Sudan, data on the prevalence rate of the disease are meagre and the prevalence rate of sub-clinical infection is unknown. Diagnosis of paratuberculosis represents a major constraint to prevention and control of the disease (Motiwala *et al*, 2005). Faecal smear or tissue imprints stained with Ziehl-Neelsen stain permits the detection of the acid-fast bacilli (AFB). However, microscopic examination cannot differentiate *Mycobacterium avium* subspecies paratuberculosis (MAP) from any saprophytic mycobacteria. In low faecal shedders animals, tissue imprints or faecal smears can also lead to false negative results. Although direct examination is cheap, it is not recommended for diagnosis of paratuberculosis (Lilenbaum *et al*, 2007). Conventional diagnostic methods for paratuberculosis include faecal and tissue cultures and the enzyme-linked immunosorbent assay (ELISA); culture is regarded as the standard for MAP diagnosis (Hulten *et al*, 2001). MAP culture has several disadvantages including long incubation period (up to 16 weeks), the labour-intensive technology (Cocito *et al*, 1994) and false-negative results from sub-clinically infected animals (Collins, 1996). Nevertheless, faecal culture is more specific than serological tests (ELISA, CFT and AGID), and is considered confirmatory for diagnosis of paratuberculosis (Whittington and Sergeant, 2001).

The objectives of this study were to compare the different techniques (faecal smear, rectal scraping and faecal culture) that used for the diagnosis of paratuberculosis and detection of MAP in dairy cows in Khartoum State and to draw the relationship between clinical signs and diagnostic methods in diary cows paratuberculosis

Materials and Methods

Animals:

Two hundred and seventeen crossbred (Friesian x local Butana eco-type), 2-year-old or elder dairy cows, from eight herds, were investigated for the detection of MAP infection.

Sample collection:

Two hundred and fifteen faecal samples were collected, kept in sterile plastic bags, with coded numbers and stored at 4 ºC for 7 days then transferred to -20 ºC until used.

Laboratory examinations:

Culture and isolation:

Inocula from two-hundred and fifteen faecal samples were prepared and cultured according to OIE (2004). All isolates were confirmed as MAP by Ziehl Neelsen- stained smears and mycobactin dependent.

Faecal smear and rectal scrapings preparation:

A direct faecal smear was made onto glass slide from each dairy cow, fixed by passing through benzine flame for three times and stained with Zeihl Neelsen stain.
The rectum was evacuated and the mucous membrane was scraped using two slides. A smear was prepared from the rectal scraping and thenstained as mentioned above.

Results

Faecal smear and rectal scrapings:

Acid-fast bacilli (AFB) appeared in clumps of faecal smears (Fig.1) but not together by a network as observed in culture smear of MAP.

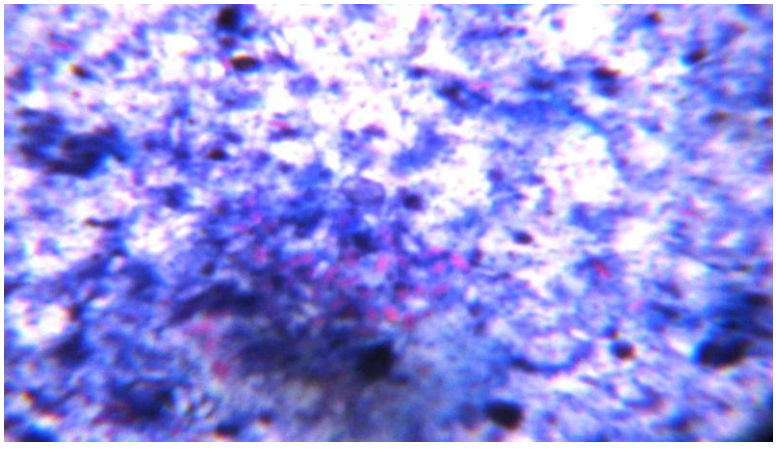

Fig 1: Acid-fast bacilli in a faecal smear: (ZN X 100).

The overall prevalence rate of AFB in faecal smears was 33.6% (73/217) (Table 1) of which only ten were positive by faecal culture (13.7%). Five out of the 73 faecal smear positive diary cows (6.8%) were positive for rectal scraping. AFB was detected in 7 out of the 8 herds (87.5%). The AFB prevalence rates in different areas in Khartoum State are shown in Table 1. The lowest prevalence rate of 6.7 % (2/30) was reported in Hillat Kuku and the highest was 89.42% in El-Kadaro, Khartoum North. AFB were encountered in all locations but with at different prevalence rates. Khartoum North showed the highest prevalence rate of AFB 40.85%; while Omdurman showed the lowest prevalence rate of 9.38% (Table 1). The prevalence rates of AFB in clinical and

sub-clinical cases were 87.5% (7/8) and 31.58% (66/209), respectively (Table 2). Although El-Kadaro herd showed the highest prevalence rate by faecal smear, no AFB were detected by faecal culture and rectal scraping technique.

Table 1: The prevalence rate of AFB in dairy cows in Khartoum State, during the period 2006-2007.

City	Location	Animals tested (No)	AFB +ve No.	Prevalence rate (%)
Khartoum	Soba West	37.00	17.00	45.90
	El-Salama	33.00	07.00	21.20
Khartoum North	Hillat Kuku	30.00	02.00	06.70
	El-Bagair East	50.00	23.00	46.00
	Al-Selait	16.00	04.00	25.00
	El-Kadaro	19.00	17.00	89.50
Omdurman	Omdurman	32.00	03.00	09.40
Total		217.0	73.00	33.64

+ve= positive

Table 2: Prevalence rate of AFB in relation to clinical signs of paratuberculosis in dairy cattle in Khartoum State, Sudan, during the period 2006-2007.

Manifestations	Animals tested (No)	AFB positive (No)	Prevalence rate (%)
Clinical cases	08.00	07.00	87.50
Sub-clinical cases	209.0	66.00	31.58
Total	217.0	73.00	33.60

The overall prevalence rate of AFB-positive rectal scrapings was 14%. The prevalence rate of the disease obtained from rectal scrapings in clinical and sub-clinical cases was 100% and 7.5%, respectively (Table 3).

Table 3: Rectal scraping examination from clinical and sub-clinical cases of paratuberculosis in dairy cattle in Khartoum State, Sudan, during the period 2006-2007.

Manifestations	Animals tested (No)	Positive rectal scraping (No)	Prevalence rate (%)
Clinical cases	06.00	06.00	100.0
Sub-clinical cases	80.00	06.00	07.50
Total	86.00	12.00	14.00

Faecal culture:

Colonies of MAP on Herrold's Egg Yolk Medium (HEYM) slant were small, colourless, translucent and hemispherical or oval. They had round margins and smooth and glistening surfaces (Fig. 2).

The prevalence rate of faecal culture was 9.37% (Table 4). MAP was isolated from 5 out of 8 herds (62.5%). All the 20 MAP isolates were obtained from HEYM slants with mycobactin (Table 4). The frequancy of positive faecal cultures is shown in Table 5. The lowest value of positive faecal culture was 5.6% (2/36) recorded at Soba West and the highest was 53.3% (8/15) at Al-Selait. Different prevalence rates were reported for positive faecal cultures in all locations surveyed. The highest positive faecal culture was reported in Khartoum North [13.2%; 15/114] and the lowest (2.9%) was in Khartoum (Table 5).

Fig 2: Colonies of MAP on Herrold's Egg Yolk Medium.

Table 4: MAP-positive faecal cultures in dairy milk cows in Khartoum State during the period 2006-2007.

Location	positive faecal culture (No)	Incubation period (weeks)	Intensity of growth scraping
Soba West	2	6-8	Dairy Cows Nos 118 and 229 gave +++.
Hillat Kuku	3	7-11	Dairy Cow 198 gave +++ Dairy Cow No 206 gave + Dairy Cow No 215 gave ++.
El-Bagair East	4	10-12	Dairy Cow Nos 4, 62 and 75 gave + Dairy Cow No 90 gave +++.
Al-Selait	8	6-11	Dairy Cow Nos 28, 31, 34, 35, 40, and 41 gave + Dairy Cow Nos 29 and 39 gave +++
Omdurman	3	8-10	Dairy Cow No 186 gave +++ Dairy Cow No 187 gave + Dairy Cow No 197 gave ++

+=1-10 colonies; ++=11-20 colonies; +++= > 20 colonies

The prevalence rates of positive faecal culture from clinical and sub-clinical cases were 87.5% and 6.3%, respectively (Table 6). Dairy cows at Al-Selait herd that gave positive faecal culture and shed AFB in their faeces constituted 53.3% and 25%, prevalence rates, respectively. Animals number 29, 75, 90,118,198 and 229 had positive faecal culture, faecal smear and rectal scraping has manifested clinical signs of paratuberculosis. However, cow No 75 which was positive for faecal culture, faecal smear and rectal scraping showed no clinical signs (Table 7).The first colonies appeared of 6 weeks incubation and this could be attributed to the slow growth of MAP. Nine out of 20 (45%) confirmed by MAP were positive in one bottle of 18 duplicate bottles containing mycobactin. The percentage of light (<10 colonies/bottle), moderate (11-20 colonies/bottle) and heavy (>21 colonies/bottle) MAP shedders were 55% (11/20), 10% (2/20) and 35% (7/20), respectively.

Table 5: The prevalence rate of MAP in faecal samples from dairy cows in Khartoum State during the period 2006-2007.

Source	location	Faecal samples tested (No)	Positive faecal cultures (No)	Prevalence rate (%)
Khartoum	Soba West	36.00	2.000	05.600
	El-Salma	33.00	0.000	00.000
Khartoum North	Kuku	30.00	3.000	10.00
	El-Bagair East	50.00	4.000	80.00
	Al-Selait	15.00	8.000	53.30
	El-Kadaro	19.00	0.000	00.000
Omdurman	Omdurman	32.00	3.000	09.400
	Total	215.0	20.00	09.300

Table 6: Prevalence rate of MAP isolated from faecal culture of dairy cows in relation to clinical manifestation

Clinical manifestation	Animals tested (No)	positive faecal cultures (No)	Prevalence rate (%)
Clinical cases	08	07	87.50
Sub-Clinical cases	207	13	06.28
Total	215	20	09.30

Table 7: Cross tabulation results of faecal smear, rectal scraping and faecal culture tests result of dairy cattle in Khartoum State during the period 2006-2007.

location	No. Animal examined	Faecal smear (result)	Rectal scraping	Faecal culture
Soba West	118	+	+	+
Soba West	229	+	+	+
Kuku	198	+	+	+
Kuku	206	-	ND	+
Kuku	215	-	ND	+
El-Bagair East	51	+	-	+
El-Bagair East	62	-	-	+
El-Bagair East	75	+	+	+
El-Bagair East	90	+	+	+
Al-Selait	28	+	ND	+
Al-Selait	29	+	+	+
Al-Selait	31	-	ND	+
Al-Selait	34	-	ND	+
Al-Selait	35	-	ND	+
Al-Selait	39	+	ND	+
Al-Selait	40	-	ND	+
Al-Selait	41	-	ND	+
Omdurman	186	-	ND	+
Omdurman	187	-	ND	+
Omdurman	197	+	ND	+

ND= not done

Discussion

Paratuberculosis (Johne's disease) is a chronic enteritis of ruminants caused by MAP (OIE, 2004). Apart of Mongash (1989) study on the prevalence rate of bovine paratuberculosis in Khartoum State, no systematic research was conducted in the Sudan. In this study, cases were included based on the clinical manifestations. The faecal samples were microscopically examined and cultured. The study showed that, the overall positive faecal culture frequency is higher compared to that of Mongash (1989).

The prevalence rates of positive faecal culture in clinical and sub-clinical cases were 87.5% and 5.9%, respectively. This indicates a strong positive correlation between faecal culture and clinical disease and is in agreement with Whitlock *et al* (2000), who observed that animals with clinical signs had always been positive on culture and serology. In the current study, 9 out of 20 MAP (45%) of dairy cows were isolated from one HEYM bottle of 18 duplicates containing mycobactin. It is worthmentioning that, the 9 MAP positive animals with the exception of one animal were sub-clinical cases that shed low numbers of MAP. Similar findings were obtained by other investigators (Merkal *et al*., 1968; Whittington and Sergeant, 2001).

It is evident from the results of the current survey, that Al-Selait herd had the highest value of positive faecal culture. This might possibly be attributed to environmental contamination with MAP as a result of increased number of MAP faecal shedder diary cows. In such a herd, replacement heifers have a higher chance of acquiring infection by the oro-faecal route.

In the study of Mongash (1989), the proportion of light (<10 colonies/bottle), moderate (11-20 colonies/bottle) and heavy (>20 colonies/bottle) MAP faecal shedders were 63.6% (7/11), 9% (1/11) and 27.3% (3/11), respectively. Compared with the present study, most of the sampled cattle were either light [55% (11/20)] or heavy [35% (7/20)] MAP faecal shedders, with a few [10% (2/20)] in the moderate category. In Al-Selait herd there was a high proportion of light shedders with increasing MAP percentage. These dairy cows may be carriers of the organism, rather than a consequence of infection. This is in agreement with Sweeney *et al* (1992).

The present study shows that, El-Kadaro herd had the highest value of AFB in faecal smears; but not by faecal culture or rectal scraping. In contrast, Al-Selait herd had the highest value of positive faecal culture, but only 25% of these MAP-infected animals were detected by the faecal smears. This may indicate that faecal smear and rectal scraping tests are not reliable for the diagnosis of Sub-clinical diary cows paratuberculosis in Khartoum state. Faecal smear and rectal scraping tests identified 85% and 100% of clinical cases, respectively, which is in accordance with the report of OIE (1996). However, the current findings indicate that faecal smear is not sensitive for the Sub-Clinical cases; it can be of value in the diagnosis of clinical cases and this coincides with Radostits *et al* (2000) who restricted their usefulness to late clinical stages.

The difference between the findings of faecal smears and faecal cultures necessitates a modification of the faecal smear method. This study indicates that diagnosis of bovine paratuberculosis can not be achieved by a single test. Although faecal culture test can detect clinical cases as well as Sub-Clinical cases and is regarded as "gold standard" and a reference test, it is not suitable as a screening test because it is time-consuming, costly and liable to contamination. Therefore, it is recommended to use faecal culture method in routine diagnosis as a confirmatory test only.

Our study and that of Mongash (1989), revealed MAP-infected dairy cattle at Hillat Kuku, Soba West and El-Bagair East areas. Dairy farms in Khartoum North showed the highest prevalence rate of positive faecal culture.

In conclusion, faecal culture is considered as a "gold standard" technique and that bovine paratuberculosis is widespread in Khartoum State. Further research is warranted for better understanding of the epizootiology of bovine paratuberculosis in Khartoum State. This could be achieved through collaboration with researchers, veterinarians, dairy cattle industry sector and governmental authorities.

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