قســـــــم : الاحياء الدقيقة ـ كلية الطب البيطرى ـ حمـــــاة
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بعض الفحوص المخبرية للكشف عن أمراض التذيفن المعوى فى الأغنام فـــى ســـــوريا

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تم جمع (٢١٥) عينة من محتويات الأمعاء من الحيوانات النافقة حديثا ومن الحيوانـــات المريضة ، فى مخبر استقصاء الأمراض الحيوانية وذلك خلال ٢٫٥ سنة (كانون الثانى ١٩٨٠ - حزيـران ١٩٨٢) .

وكان مصدر معظم العينات من محافظات : حمـاه ـ حمص ـ حلب ـ طرطوس ، والبعض منهـا ارسلت من البادية الســورية .

تم عزل عـترات الكلوستريد يوم وصنفت بالطرق البيولوجية عن طريق استخدام اختبار الحقـن فى الفـئران وبالطرق المصلية باجراء اختبار الحماية الايجابية فى الفئران ، وتوصلنا الـــى حقيقة انتشار ثلاثة أنواع مصليـــة (آ، س، د) من عصيات الكلوستريد يوم ولشاى (الحاطمة)

اما نسبة الاصابات بالتذيفن المعوى نتيجة الاصابة بعصيات الجمبرة الحاطمة (كـــل ولشـــاى) فى (٢١٥) غنمة فقد بلغت ١٨٫٦٠٪ .

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LABORATORY EXAMINATION OF ENTEROTOXAEMIC DISEASES IN SHEEP IN SYRIA

(With 2 Tables)

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SUMMARY

Samples in form of intestinal contents were collected in the Veterinary Investigation Laboratory from recent died and diseased sheeps encountered in a (2,5) years period (January 1980-June 1982), majority of specimens originated from the States of Hams, Homs, Aleppo, Tartus, and some were recieved from Albadia.

Clostridium isolates were obtained and identified biologically by mice inoculation and serologioally by passive mouse protection test into "3" serotypes (A,C,D). The incidence percentage of enterotoxaemia caused by cl. perfringens among "215" sheeps proved to be (18.60%).

INTRODUCTION

Enterotoxaemia is a problem in Syria causing great losses and outbreaks appear despite the use of a toxoid vaccine. Enterotoxaemia is a broad term used for the organisms belonging to the group of enteric intoxication cause a severe and sometimes epidemic diseases of major economic importance.

The aim of this work is to survey the toxigenic types of cl. perfringens at first and later carry out culture work in order to identify the prevalent toxigenic types.

White swiss mice were intravenously inoculated to find out the lethal toxin present in the intestinal contents, and identification of types of cl. perfringens involved was carried out by using toxin-antitoxin neutralization test in mice. Enterotoxaemia in young lamba due to cl. perfringens type, C, was first reported in the United Kingdom in (1968) by FINDALAY and BUNTAIN, they recorded an outbreak of the disease on an upland farm where the lambs were inoculated twice with a multivalent clostridial vaccine.

GREIG, (1974) described an outbreak of Clostridium perfringens infection in a flock of young lambs in South West Scotland and the toxine found to be that of Clostridium perfringens type, C,.

HARBOLA et al. (1975) reported about the incidence of enterotoxaemia disease due to the different types of Clostridium perfringens (A,C,D) in sheep in India.

MATERIAL and METHODS

Morbid marterials in the form of intestinal contents from, 215 sheeps suspected to have died of enterotoxaemia, were recieved from different parts of Syria for detailed laboratory examination. The specific diagnostic antiserum against Clostridium perfringens types. A B C D and E, were recieved from the Wellcome Research Laboratory, Beckenham. Intestinal contents were collected as soon as possible after death.

Several grams were needed or better a piece of affected intestine with contents is ligated on both sides. A smear was made from the contents and stained with Gram stain to observe the unusual numbers of clostridium in the intestinal flora.

Intestinal contents were centrifuged clear at 4000 r.p.m. for 15, minutes and filtrated once or twice if still cloudy and draw out the supernatant fluid, if the contents were very thick or mucillaginous we have diluted with an equal volume of sterile physiological saline.

To find out the major lethal toxin present in the intestinal contents and to identify the type of Clostridium perfringens involved, the pathogenicity test was carried out in the following manner inject 3 mice each I/V with 0.3 ml. intestinal contents supernatant alone (group I). Inject 3 mice each I/V with 0.3 ml. intestinal contents supernatant treated with trypsin and observe mice for death and signs of severs illness for upto 72 hrs after inoculation (group II), if the mice died from either groups proceed with neutralization tests.

Neutralization tests:

If group, I, of pathogenicity test mice died: A) make the following mixtures and incubate mixture at 37, C for. 15, minutes:

Antitoxin serum: A Type B Type C Type D

0.4 ml. 0.4 ml. 0.4 ml. 0.4 ml.

Intest. contents. 1.2 ml. 1.2 ml. 1.2 ml. 1.2 ml.

B) Inject 3, mice each I/V with the following:

I - 0.3 ml. intest. cont. supernatant alone.

II 0.4 ml. intest. cont. supernatant type, A, mixture.

III- 0.4 ml. intest. cont. supernatant type, B, mixture.

VI - 0.4 ml. intest. cont. supernatant type, C, mixture.

V 0.4 ml. intest. cont. supernatant type, D, mixture.

Observe mice for death, if group II, only of pathogenicity test died:

When only trypsin treated intestinal contents kill the mice, the toxine is present in the protoxin epsilon which cane only be activated by trypsin.

After centrifugation the sediment was seeded in ROBERTSONS cooked meat medium and isolation-and identification were done as follow: Inoculate two blood agar plates and incubate aserobically and anaerobically for 48 - 72, hrs. for the anaerobic culture and compare the growth in the two plates and pick up suspicious colonies from the anaerobic plate, make smears and examine microscopically. Fish out single colonies and subculture on ROBERTSON medium. Incubate and centrifuge culture, remove supernatant and trypsinise.

RESULTS and DISCUSSION

A total of 215, intestinal contents smears from 5, areas in Syria was examined and 51, contained large number of CLOSTRIDIUM type. The organisms were Gram positive and bacilli morphologically resembled Clostridium perfringens. This diagnostic method should be also viewed with caution. It is not possible to microscopically distinguish pathogenic Clostridium perfringens from other clostridium perfringens of the normal flora that are frequent in feces, water and elsewhere in the environment.

Nevertheless, microscopic examination of intestinal contents is widely used and is of value to identify shedders in herds known to be infected. The results of the microscopical examination are shown in Table (I). Results given in Table (I), shown that out of 215, samples examined microscopically 51, proved to be positive to Clostridium perfringens, on the other hand, examination of 42, smears of the intestinal contents from ALBADIA area revealed the recognition of 18 positive smears to Clostridium perfringens; (42.85%).

Table (I): Results of bacteriological examination of Clostridium perfringens in sheep

Herd	Number of intestinal contents recieved and examined	Number of smears microscop. positive for the presence of Clostridium perfringens	Percent. %	Number of isolates of Clostridium perfringens	percent. %
HAMA	115	22	19.13	18	15.65
HOMS	36	7	19.44	4	11.11
ALEPPO	17	3	17.64	2	11.76
TARTUS	5	1	20.00	1	20.00
ALBADIA	42	18	42.85	15	35.71
Total	215	51		40	

Isolatin of Clostridium perfringens colonies: All isolates conformed with the known characters of group, and when they were grown on blood agar no type differences were found. Cultures were identified as Clostridium perfringens on the basis of colony formation on blood agar, absence of growth on a corresponding plate incubated aerobically and other easily observed cultural characters, the detailes of isolation of Clostridium perfringens from the intestinal contents are set out in Table (I).

Clostridium perfringens was isolated (18) times from Hama areas sheeps (15.65) and (4) times from Homs areas sheeps (11.11%), (2) times from Aleppo area. (11.76%), and (1) time from Tartus area (20.00) and (15) times from Al-Badia area (35.71%).

The highest prevalence of infection (35.71%) had Al-Badia sheeps, and this is presumptively due to irregularely vaccination of the herds on this territory with local vaccine and due to inadquete ration especially in the Summer and Autum time.

Although there was good correlation between cultured specimens and microscopic examination of intestinal contents.

The results of the prevalence of toxin and isolation of Clostridium perfringens. are set out in Table (II). Results given in Table (II) show that out of '215' intestinal content filtrate the presence of a toxin of Clostridium. perfringens proved to be only from (80) cases (37.20%) and the variation of toxigenic Clostridium perfringens were (A,C,D)

On the other hand examination of '215' intestinal contents revealed the recognition of (40) isolates of Clostridium perfringens (18.60%) and they belong to the types (A,C, and D).

The finding that Clostridium. perfringens. type 'A' predominated over type 'C' presented an important practical implication.

The diagnosis of toxigenic types may easily be missed when only a few single colonies are selected from a relatively uniform-looking growth on blood agar plates.

As shown in Table (I and II) Clostridium perfringens type 'A' was also isolated and identified and could be considered as responsible for mortality in sheep (in Syria). Similar findings go hand in hand with HARBOLA et al. (1975).

The isolated strains of Clostridium perfringens. type 'C' showed characteristics similar to the variety found in neonatal hemmorrhgic enterotoxaemia of calves and lambs in Colorado(NILLO, L. et al. 1974) and KULSHRESTHA et al. (1972) and BROOKS et al. (1957).

On the basis of isolation of the Clostridium perfringens. type (D) bacilli from the intestinal contents, Table (II) showes that the prevalence of this type among the infected sheeps is predominated over all types of Clostridium. perfringens in Syria, and at the second place type 'A' and after that type 'C' and this findings confirms the existence of enterotoxaemia in sheep in Syria.

From the current literature it seems that this is the first time to isolate and to identify Clostridium perfringens in the sheep herds of above nominating areas in the present investigation in Syria.

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Table (II): Datailes of Isolation and serum neutralization test of intestinal filtrate

Herd	Number of sheep tested	Number of clostridium isolates	Number of clostridium isolates confirmed on Serum N.T.					Number of intestinal filtrate positive for the presence of toxin.	Number of different toxigenic variants of cl. perfringens detected by Serum neutralization test.				
			A	B	C	D	E		A	B	C	D	E
HAMA	115	18	6	-	2	10	-	13	4	-	3	24	-
HOMS	36	4	1	-	-	3	-	10	2	-	2	6	-
ALEPPO	17	2	-	-	-	2	-	6	2	-	1	3	-
TARTUS	5	1	1	-	-	-	-	2	-	-	-	2	-
ALBADIA	42	15	3	-	2	10	-	31	8	-	6	17	-
Total	215	40	11	-	4	25	-	80	16	-	12	52	-