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استخدام اختبار بلزمة الدم الغير مباشر لملاحظة وجود اجسام مضادة لميكروب الليستريا مونوسيتوجنس

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استخدم هذا الاختبار للمرة الأولى فى مصر لملاحظة وجود أجسام مضادة لميكروب الليستريا مونوسيتوجنس فى أمصال الأبقار والجاموس . فقد تم تجميع ٢٠٠ عينة من مصل هذه الحيوانات من مزرعة الحواتكة وبنى مر فى محافظة أسيوط. وقد أجرى أختبار بلزمة الدم الغير مباشرة على هذه الامصال مستخدما الأنواع الخمس من محدثات ميكروب الليستريا مونو سيتوجنس. أظهرت نتائج هذا البحث وجود أجسام مضادة لهذا الميكروب فى الحيوانات السليمة مظهريا . وأن هذه الأجسام المضادة أكثر خصوصية لمحدثات النوع المصلى ١أ و ٣ أ من هذا الميكروب . وقد أعتبر المعيار التشخيصى لهذا الميكروب $\frac{1}{160}$.

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THE USE OF INDIRECT HAEMAGGIUTINATION TEST FOR THE DETECTION OF ANTIBODIES AGAINST L. MONCOYTOGENES IN COWS AND BUFFALOES
(WITH 2 TABLES)

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SUMMARY

This test was applied for the first time in Egypt for the detction of L.monocytogenes antibodies in cows and buffaloes. 200 serum samples were collected from adult cows and buffaloes from El-Hawatka and Bani-mor at Assiut Governorate. These sera were subjected to indirect haemagglutination test using five antigenic serotypes of L.monocytogenes. The results of this work shows the presence of L.monocytogenes antibodies in apparently healthy cattle with special increase in the incidence of L.monocytogenes type 1a & 3a in comparson with the other serotypes. The diagnostic titre for listeria infection in animals should be considered at least 1/160.

INTRODUCTION

L.monocytogenes plays an important role in vterinary and medical studies since it affects a wide variety of hosts including man. It was isolated from meningoencephalitis in cattle (JONES and LITTLE,1934; GRY ET AL., 1948). It may also cause bovine abortion (HARBOUR, 1941; BABKIN, 1964), and bovine mastitis (LURAMBY, 1944)and chronic mastitis (DE VRIES and STRIKWARDA 1956).

The organisms was isolated in Egypt from the body cavity of an apparently normal non-gravid buffaloe uterus (BARAKAT, 1965; NASHED and ZAKI, 1968).

Different serological tests were performed for diagnosis of listeria infection including agglutination test (OSEBOLD ET AL, 1960; VANINI ET AL., 1966; DIJKSTRA, 1967; ZHILOV, 1973; SEDDIK, 1978), complement fixation test (WINKENWERDER and ABDALLAH, 1967; SEDDIK, 1978) mobility inhibition test (BERGER, 1970; SEDDIK,1978) and precipitation test (SEDDIK, 1978).

The indirect haemagglutination test has been tried successfully for the diagnosis of some bacterial and parasitic onditions such as tuberculosis, schistosomiasis,Toxoplasmosis,Brucellosis as well as Corynebacterium equi and C.ovis (KAGAN and PELLEGRINO,1961; RIS and PUNGA,1963; RIS,1964; CARTER and HYLTON,1974; ABDEL MEGUID ET AL., 1978; SHIGLDI, 1978).

It was therefore of intrest to try the indirect haemogglutination test as an additional serological test for the detection of antibodies against Listeria monocytogenes in sera of animals.

MATERIALS AND METHODS

- Blood samples were collected from 100 adult cows and 100 adult buffaloes from El-Hawatka and Bani-mor. The serum was separated and kept in the deep freeze until examined.
- Five serotypes of L.monocytogenes (N.C.T.C.) were included as follows: 1 a No. 10357, 2 No. 5348, 3 a No. 5105, 4 a No. 5214 and 6 No. 10889.
- Antigen (s) from the 5 serotypes were prepared as mentioned by SEDDIK (1978).
- Antisera against the five serotypes were prepared in rabbits as described by the same author.

Reagents For The Test:

1- Phosphate buffer saline consisting of 0.15 M $KH_2 PO_4$ and 0.15 M. $Na_2 H PO_4$.
2- Physiological saline 0.85% Nacl.
3- Tannic acid solution 1/20.000 (12.5 mg + 250 ml of buffer).
4- Citrated sheep red cells.
5- Antigen in concentiation of 2 mg/ml in buffer solution.
6- Rabbit or hrose serum inactivated at 56°C for ½ hours.

Method:

1- 20 ml. sheep red cells was washed 3 times, twice with physiological saline and one with phosphate buffer saline in a one ounce universal container then centrifuged at 750 g. for 15 minutes to pack the cells.
2- 0.6 ml. of the packed cells was pipetted into each of the two universal container and 10 ml. of the buffered saline were added to each bottle to resuspend the cells.
3- 10 ml. of the tannic acid solution were added to each continber, shaked and incubated for 15 minutes at 37°C.
4- The bottle was centrifuged at 750 g for 5 mintues and the deposited cells were suspended in 20 ml.of buffer, Centrifuged for a similar period and the supernatant was discarded.
5- One container of cells was kept aside to be used as the source of uncoated cells for absorbing the heterophile agglutinins in the sera to be tested and for various controls.
6- The cells in the other container were resuspended in 10 ml. of buffer and then 10 ml. of buffer containing the antigen to be coated on the cells were added. The mixture was incubated for 30 minutes at 37°C with shaking occasionally.
7- The coated cells were centrifuged for 5 minutes at 750 g and the supernatant was removed.
8- The coated and uncoated cells (step 5) were washed three times with buffered saline, made up to contain 1% normal horse serum (previously inactivated), centrifuged for 5 minutes on each occasion and the supernantant was descarded.
9- Both batches of cells were finally made up to 5 ml. with serumized buffer.

The Test:

1- The tested sera were inactivated for 30 minutes at 45°C for ½ hour.
2- 0.1 ml of each inactivated serum was added to 0.9 ml of the uncoated cell preparation, left on bench for 15 minutes and centrifuged for 15 minute at 750 g to recover the serum diluted than 1/10.
3- Six double fold dilutions of the absorbed serum were prepared in buffered saline in 0.1 ml volumes using WHO plate. 0.1 ml of the coated cells was added to each well and to a control well containing 0.1 buffer only. Controls using uncoated cells with and without serum were also included. The 5 immuner rabit sera were inactivated and each one was tested in a similar manner.

The plates were left on the bench (20°C) for 2 hours then put in the refigerator (4°C) overnight. The end point was taken as the last cup showing a smooth mat of agglutinated cells with a created rim. Doubtful results would appear as a smaller circle of cells having a dark outer rim while a negative result would show as closely packed button of cells. The controls should always be negative.

RESULTS

The result of testing 200 serum samples from cows and buffaloes are shown in Table 1. It is observed that the number of positive reactors decreased with the increase of the dilution.

No positive sera showed the prozone phenomenon known to occur with the agglutination test. Comparing the number of positive reactors at the different dilutions using the 5 types of antigen it is observed from the table that larger numbers were obtained with antigens 1 and 3 a. It is also observed that the number of positive reactors at different dilutions was more with buffaloes than with cows.

The results of the indirect haemagglutination test of the 5 immune seraprepared against the different types of L.monocytogenes antigens are shown in Table 2. It was observed that each antiserum and in particular that against type 3 a reacted with the homologous antigen at a high titre and with the other 4 heterologus antigens at a much lower titre.

DISCUSSION

The result of indirect haemagglutination test of 200 serum samples of cows and buffaloes showed that 24.1% of them were positive at 1/20, 1/7.3% at 1/40, 10.8, at 1/80, 7.9% at 1/160, 2.1% at 1/320 and 0.3% at 1/640.

This means that a good number of sera showed antibodies by the indirect hamemagglutination test. One of the possible explanations of this phenomenon is the presence of shared antigen (s) between L.monocytoges and aother organisms such as staphylococcus aureus, Str.feacalis and E.coli (NETER ET AL., 1960; SEELIGER and SULZBACHER, 1965; MISRA and NILAKANTAN, 1965). The second explanation is the presence of shared antigen in between the 5 types of L.monocytogenes as shown in Table 2.

Tow overcome the problem of shared antigen (s) between Listeria and other organisms SEDDIK (1978) adopted the agglutination and complement fixation test after aborption of the tested sera with the non specific organisms (Staphylococcus aureuse, Str.faecalis and E.coli). By this technique using the agglutintion test he was able to reduce the number of the postitive reactors from 29% at 1/20 to 21.6% and from 24% at 1/40 to 18.2%,and from 17% at 1/80 to 12.5% whereas sera of titreas 1/160 or more were not greatly changed after absorption. It was noted that 7.5% of the sera of cattle were positive at 1/160 which was considered as a diagnostic titre since no changes occured in the titre after absorption with the different shared antigens. The result of the indirect haemagglutination test in this work showed that about 8% of the sera of buffaloes and cows gave a positive reaction at a titre of 1/160. Since such percentage is quite more to that obtained by SEDDIK (1978) which denoted listeria infection in animals. It is concluded that the indirect haemagglutination test can be applied for the diagnosis of listeria infection and that the positive titre should be considered to be at least 1/160.

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TABLE (1)

Indirect haemagglutination test for the detection of anitbodies against L.monocytogenes.

Antigen of type	Kind of animal	Number of serum giving a positive reaction at dilution of:							Number of Sera showing No reaction
		1/20	1/40	1/80	1/160	1/320	1/640	1/1280	
1 a	C	31	20	26	9(2)	2	2	0	69
	B	39	19	11	10(1)	3	1	0	61
2	C	11	8	2	1	0	0	0	89
	B	16	12	5	3	0	0	0	84
3 a	C	35	25	16	11	5	0	0	65
	B	38	30	22	16	9	0	0	62
4 a	C	15	13	10	9	1	0	0	85
	B	21	18	12	11	1	0	0	79
6	C	16	12	5	2	0	0	0	84
	B	19	16	9	4	0	0	0	81

Abbreviations: C = Cows (100 samples) B= Buffaloes (100 samples)

- Number inbetween brackets denotes additional number of sera showing doubtful reaction.

TABLE (2)

Indirect haemagglutination test of the 5 immune rabbit sera against the 5 types of L.monocytogenes.

Antigen of type	End titre of immune serum prepared against				
	1 a	2	3 a	4 a	6
1 a	1/320	1/40	1/80	1/40	1/20
2	1/40	1/320	1/40	1/40	1/20
3 a	1/80	1/40	1/640	1/40	1/20
4 a	1/40	1/40	1/40	1/320	1/40
6	1/40	1/40	1/20	1/40	1/160