Dept. of Animal Medicine,
Fac. Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.F. Raghib.

CRYPTO-LA TEST AS A RAPID DIAGNOSIS FOR OVINE CRYPTOCOCCOSIS

(With 2 Tables)

By

M.I. MOURAD

(Received at 25/5/1992)

إختبار الكربتولا كتشخيص سريع للكربتوكوكوزس في الأغنام

مراد إسماعيل

أستخدم إختبار الكربتولا للتشخيص السريع للإصابة بفطر الكربتوكوكس نيوفورمانز في الأغنام ومقارنة نتائجه بنتائج الفحص المجهري والزرع للعينات المأخوذة من أماكن الإصابة وقد ثبت أن إختبار الكربتولا يتميز عن الطرق التقليدية في التشخيص من حيث سرعة إكتشاف الإصابة وتوفير الوقت للتشخيص . كما تم تسجيل الأعراض الإكلينيكية لمرض الكربتوكوكوزس في الأغنام المصابة طبيعيا .

SUMMARY

Thirty-eight balady ewes (2-3 years age) were included in this investigation to study the clinical signs of ovine cryptococcosis; isolation and identification of the causative agent Cryptococcus neoformans beside trials to evaluate Crypto-LA test as an aid and rapid method for diagnosis of the disease in ewes. Crypto-LA test proved 100% similarity to both the clinical signs of the disease and isolation of its causative fungus. The test was positive for the disease at 1/8 titre or 1+ on slide agglutination test.

INTRODUCTION

Cryptococcosis is a systemic infection caused by the pathogenic Cryptococcus neoformans. The organism gain enterance to the body through causing a brief inflammatory lung infection from which it rapidly disseminates to the central nervous system. The importance of Cryptococcus neoformans lies in the fact that it is usually a secondary invador to tuberculosis. Therapy with corticosteroids, other immunosuppresive agents or broad spectrum antibiotics acts as a predisposing factors to infection with this organism (KAUFMAN and BLUMER, 1968; 1975).

The organsim have been isolated from the udder of cattle with mastitis; following cases of meningitis in the horse; pulmonary abscesses in horses and goats; localized lesions of the oral and nasal mucosa of dogs and cats and lastly from generalized infection of these species (COLES, 1986).

EMMONS et al. (1970) suggested the propability that Cryptococcosis begins as a pulmonary disease with secondary haematogenous spread to skin, bones, abdominal

Tab. (3): Serum analysis of sheep infected with D. Filaria (Gr.I)

No. Samp.	Na mEqu/L	K mEqu/L	Cl mEq/L	T.P. gm %	Alb. gm %	Glob. gm %	A/G
1-	122.4	3.6	114	8.9	3.3	5.7	0.6
2-	122.4	3.5	114	7.8	4.3	3.5	1.2
3-	122.4	3.5	109	7.6	3.6	4.0	0.9
4-	120.0	3.5	111	8.6	3.7	4.9	0.8
5-	120.0	3.3	129	8.0	3.8	4.2	0.9
6-	120.0	3.5	108	8.9	4.0	5.0	0.8
7-	120.6	3.0	124	7.6	3.9	3.7	1.0
8-	127.2	3.5	110	8.4	4.5	3.9	1.2
9-	120.0	3.3	108	7.0	4.2	2.9	1.5
10-	127.2	3.3	115	6.1	3.8	2.3	1.7
$\bar{X}$	122.2	3.8	113.7	7.9	3.9	4.0	1.1
SD	2.8	0.3	7.2	0.9	0.4	1.0	1.1

Tab. (4): Serum analysis in control healthy sheep (Gr.II)

No. SAmp.	Na mEqu/L	K mEqu/L	Cl mEqu/L	T.P. gm %	Alb. gm %	Glob. gm %	A/G
1-	118	3.8	118	6.8	3.6	3.2	1.1
2-	118	3.8	110	6.8	3.8	3.0	1.3
3-	123	3.6	110	6.4	3.6	2.8	1.3
4-	127	3.4	112	7.8	4.7	3.1	1.5
5-	120	3.8	120	6.6	3.8	2.8	1.4
6-	120	2.8	116	7.2	4.0	3.2	1.3
7-	123	3.6	124	6.8	3.6	3.2	1.1
8-	118	3.8	110	7.8	4.7	3.1	1.5
9-	133	3.2	116	7.2	3.8	3.4	1.1
10-	120	3.4	110	6.8	4.2	2.6	1.6
$\bar{X}$	122	3.5	113.6	7.0	4.0	3.0	1.3
SD	1.5	0.1	1.6	0.2	0.1	0.1	0.1

viscera and specially the central nervous system.

Diagnosis of Cryptococcosis can be performed by direct microscopical detection of the causative fungus from suspected lesions and or culturing infected material on Sabauroud's dextrose agar media. Such techniques are somewhat difficult especially when the lesions are not avaliable from living animals as in lung and CNS lesions.

This work aimed to evaluate the accuracy of using Crypto-LA test kits as an aid and rapid serological method for diagnosis of ovine Cryptococcosis. The clinical signs of diseased ewes were also reported.

MATERIAL and METHODS

Animals: 38 Balady ewes (2-3 years age) belonging to Manqabad Military farm at Assiut Governorte were choosen for the study. Animals were classified into 3 groups (A,B and C) according to the reported clinical signs (Table 1).

Crypto-LA test*: (Latex agglutination test) for the qualitative and quantitative determination of Cryptococcus neoformans antigen in serum of infected animals. The principle of the test is the agglutination of C.neoformans antigen in animal serum or cerebrospinal fluid with the sensitized latex particles of Anticryptoccocal rabbit globulin.

Blood serum: was obtained from each animal of all groups for application of Crypto-LA test.

Sabauroud's dextrose agar: media was used for isolation and identification of Cryptococcus neoformans from the nodular lesions.

RESULTS

Clinical manifestations of ovine Cryptococcosis were illustrated in table 1.

Crypto-LA test (table 2) for both diseased groups (A&B) reacted positively from 1+ to 3+ by slide agglutination test. The reading was 0+ for group C (Control). On application of two fold dilution agglutination test group B showed 1/8 titre while rather higher titres (1/8 to 1/32) were specific for group A. On the opposite group C (Control) showed no agglutinations.

Following 2-5 weeks after application of Crypto-LA test. Animals in group B expressed visible nodules on the udder that spreaded to the inner aspect of the tail. Lesions appeared firstly as red spots then nodules containing soft material. During spontaneous rupturing creamy pus was discharged.

Cryptococus neoformans was isolated and identified from the contents of the nodules of all infected animals (groups A&B). The organism appeared microscopically as Gram positive spherical or ovoid budding, double walled yeast like cells. On culturing on Sabauroud's dextrose agar the colonies were white, glistening and mucoid in nature.

*: Kindly supplied by International biological laboratories, inc. dist. subsidiary of Carter-Wallance, inc. Cranburry, NJ 08512.

Table 1: Total number andclinical signs of ovine cryptococcosis in A, B & C groups.

group	total number	Clinical signs on the infected animals during application of crypto-LA test
A	25	Diminished appetite, intermittent fever, emaciation and presence of painless round nodules of various sizes and numbers on the udder and under the tail. Some of these nodules were spontaneously opened discharging creamy exudate
B	8	They have the same clinical signs of group A except abscence of visible nodules on the animal body
C	5	Clinically healthy and served as control

Table 2: Results of slide and two fold dilution aggtutination tests in animal groups A,B&C.

Animal group	Total No	Serial No.	Slide aggl. test	Two fold dil aggl test
A	twenty-five	1	3+	1:32
		2	1+	1:8
		3	1+	1:8
		4	2+	1:16
		5	2+	1:16
		6	1+	1:8
		7	2+	1:16
		8	2+	1:16
		9	3+	1:32
		10	2+	1:16
		11	1+	1:8
		12	2+	1:16
		13	3+	1:32
		14	3+	1:32
		15	2+	1:16
		16	2+	1:16
		17	1+	1:8
		18	1+	1:8
		19	2+	1:16
		20	3+	1:32
		21	2+	1:16
		22	2+	1:16
		23	2+	1:16
		24	1+	1:8
		25	1+	1:8
B	Eight	1	1+	1:8
		2	1+	1:8
		3	1+	1:8
		4	1+	1:8
		5	1+	1:8
		6	1+	1:8
		7	1+	1:8
		8	1+	1:8
C	Five	1	0+	-
		2	0+	-
		3	0+	-
		4	0+	-
		5	0+	-

0+:No agglutination. 1+:Slight agglutination. 2+:Moderate agglutination. 3+:Highly agglutination.

DISCUSSION and CONCLUSION

The observed clinical signs of Cryptococcsis in ewes were generally similar to that mentioned by MONGA et al. (1970); FRANSWORTH (1977) on cattle and SHARMA et al. (1977) on buffaloes.

The character of C.neoformans either on direct microscopical examination and or on culturing on Sabauroud's dextrose agar coincided with that reported by COLES (1986).

There is no avaliable literature discussing the evaluation of using Crypto-LA test as an aid and rapid test for diagnosis of sheep Cryptococcosis, however KAUFMAN and BLUMER (1968) and GOODMAN et al. (1971) reported that Crypto-LA test provides an immunochemical technique for detecting the polysaccharide antigen of C.neoformans in serum or Cerebrospinal fluids. The technique gives more rapid results than culturing of C.neoformans from serum or cerebrospinal fluids, and occasionally detects antigen in patients from whom no organism can be isolated. On the other hand NOXON et al. (1986) Cited that the latex agglutination test for Cryptococcal antigen was monitored in both feline and canine Cryptococcosis to evaluate the length of Ketoconazole therapy.

Finally as a conclusion, Crypto-LA test can be used as an aid and rapid technique in the diagnosis of ovine Cryptococcosis especially to those with no avaliable lesions for isolation and identification of the agent.

Positive Crypto-LA tests were evident even before appearing of visible skin nodules by 2-5 weeks (group B). Crypto-LA test thus reduces the time of ovine Cryptococcal diagnosis than using the culture method.

REFERENCES

Coles, E.H. (1986): Vet. Clinical Pathology: 4th Ed. W.B. Sounders Company Phladelphia, London.

Emmons, C.W.; Binford, C.H. and Utz, J.P. (1970): Med. Mycology, 2nd Edition Philadelphia, Lea & Febiger.

Fransworth, R.J. (1977): Cryptococcosis in cattle. J. of American Vet. Med., Asso. 170: P. 1173.

Goodman, J.; Kaufman, L. and Koenig, M.G. (1971): Diagnosis of Cryptococcus meningitis: Value of immunological detection of Cryptococcal antigen N. Eng. J. Med., 285: 434-436.

Kaufman, L. and Blumer, S. (1968): Value and interpretation of serological test for diagnosis of Cryptococcosis: Appl. Microbiology 16: 1907-1912.

Kaufman, L. and Blumer, S. (1975): The latex agglutination test for Cryptococcosis, from laboratory diagnosis by serological methods. U.S.Dept. of HEW, Public health service, center for disease control. Bureau of labortories, laboratory training section Dan F. Palmer and staff.

Monga, D.P.; Mohapatra, L.N. and Kalra, D.S. (1970): Cryptococcosis in cattle. Indian J. and Med. Res. 58: P. 1023.

Noxon, J.O.; Monroe, W.E. and Chinn, D.R. (1986): Ketoconazole therapy in canine and feline Cryptococcosis. J. of the American Animal Hospital Association 22: 178-183.

Sharma, S.D.; Pal, P. and Saxena, S.G. (1977): Cryptococcosis in buffaloes Indian Vet. J. 54: P. 284.