Assiut Vet. Med. J. Vol. 39 No. 78, July 1998.

Animal Reproduction Research Inst.
Giza, Egypt.

BACTERIOLOGICAL AND SEROLOGICAL EVIDENCE OF CAMPYLOBACTER IN SHEEP

(With 7 Tables and One Figure)

By

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(Received at 13/6/1998)

دليل بكتريولوجى وسيريولوجى على ميكروبات الكامبيلوباكتر فى الاغنام

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تم تجميع عدد ١٥٨٨ عينة شملت (١١٣٠ عينة سيرم ٢٦ جنين مجهض, ١٢ مشيمة , ٩٠ افرازات مهبلية من نعاج مجهضة , ٢١٥ حويصلة مرارية من السلخانات , ١١٥ عينة براز) جمعت هذه العينات من حالات تعانى من الاضطرابات التناسلية مثل الاجهاضات وقلة الخصوبة لسلالات مختلفة من الاغنام بمصر. وقد زرعت عدد ٤٥٨ عينة فى المزارع البكتيرية الخاصة بالكامبيلوباكتر لمدة ٢٤-٧٢ ساعة عند درجة حرارة ٣٧°م لعزل الانواع المختلفة للكامبيلوباكتر. اما عينات السيرم والافرازات المهبلية فقد تم فحصها سيريولوجيا ومناعيا لاكتشاف الاجسام المضادة للكامبيلوباكتر بواسطة اختبار التجمع البطئ والاليزا. ومن هذه الدراسة تم عزل عدد ٥٧ (٤٥ر١٢%) عترة للكامبيلوباكتر من عدد ٤٥٨ عينة وشملت العينات الموجبة عدد ٣٢ (٩٩ر٦%) عينة موجبة للكامبيلوباكتر الجنينى المعوى وعدد ٢٥ (٤٦ر٥%) عينة موجبة للكامبيلوباكتر القولونى. وكانت اعلى نسبة للعزل فى عينات الاجنة المجهضة (٦٢ر٣٤%) ثم عينات البراز (٣٩ر١٧%). وكانت نتيجة الفحص السيريلوجى والمناعى لعدد ١١٣٠ عينة سيرم و٩٠ عينة افرازات مهبلية بواسطة استخدام اختبار التجمع البطئ ايجابية لعدد ٣٨ (٣٦ر٣%) للسيرم و١٣ (٢٤ر٤%) للافرازات المهبلية مع انتجين الكامبيلوباكتر الجنينى المعوى. وايجابية لعدد ٣٦ (١٩ر٣%) للسيرم و٤ (٤٤ر٤%) للافرازات المهبلية لانتجين الكامبيلوباكتر القولونى. بالمقارنة بنتائج الاليزا التى كانت ايجابية لعدد ١٠٨ (٥٥ر٩%) فى السيرم و١٩ (١١ر٢١%) للافرازات المهبلية مع انتجين الكامبيلوباكتر الجنينى المعوى و ايجابية لعدد ٩٢ (١٤ر٨%) للسيرم و٧ (٧٧ر٧%) للافرازات المهبلية لانتجين الكامبيلوباكتر القولونى. وقد وجدت حالات تفاعل مزدوج بين الكامبيلوباكتر الجنينى المعوى والكامبيلوباكتر القولونى فى عدد ٧ حالات (٨٩ر٩%)

بواسطة استخدام اختبار التجمع البطئ عند تخفيف ١/١٦٠> ولكن نسبة هذا التفاعل كـــانت اقل ما يمكن فى اختبار الاليزا ٢ (٨٨ر٠%) عند ١/١٠٠ ولم توجد فى التخفيف الاعلى. وقد اوضح لنا هذا العمل اهمية استخدام الاليزا لكفائته وسهولة استخدامه وسرعته ودقة نتائجه فى التشخيص المبكر لميكروبات الكامبيلوباكتر للاغنام.

SUMMARY

A total of 1588 samples including (1130 serum samples, 26 aborted foeti, 12 faetal membranes, 90 vaginal discharges from aborted ewes, 215 gall bladder from slaughterhouses and 115 fecal swabs) was collected from different breed of sheep in Egyptian farms suffered from reproductive disorders as abortion and /or infertility. Out of these samples 450 were inoculated into thiol media for 24-72 hours at 37°C for detection of Campylobacter organisms. The specimens of ewe serum samples and vaginal swabs were subjected to serological and immunological tests by using tube agglutination test and ELISA. In this study, Campylobacters were isolated in 57 (12.45%) from 458 samples represented as 32 (6.99%) C.f.ss. fetus and 25 (5.46%) C. jejuni. The highest isolation rate of Campylobacters were presented in aborted foeti (34.62%) then fecal swabs (17.39%). Out of 1130 serum samples and 90 vaginal discharge samples examined by tube agglutination test , and ELISA: Tube agglutination test results were positive in 38 (3.36%) for serum samples and 13 (14.14%) in vaginal discharges with C.f.ss. fetus antigen and positive in 36 (3.19%) of serum and 4 (4.44%) in vaginal discharge with C. jejuni antigen. In comparing to results of ELISA test which they were positive for 108 (9.55%) in serum and 19 (21.11%) in vaginal discharges with ss. fetus antigen and positive in 92 (8.14%) of serum and 7 (7.77%) in vaginal discharges with C. jejuni respectively. The cross reactions between C. f.ss. fetus and C. jejuni were demonstrated by serological tests which were 7 (9.89%) by tube agglutination test > 1/160 titer but failed to give homologus titer.The cross reactivity minimized by ELISA 2(0.88%) at 1/100 but no correlation occured at the higher dilution. This work is monitoring the effectiveness of ELISA as a; simple, more sensitive and rapid technique for early diagnosis of Campylobacters in sheep.

INTRODUCTION

Sheep are considered as one of the very important sources in animal production all over the world for their useful products as wool, meat, milk and plant fertilizer. Also, sheep can give two reproductive cycles around the year and this make very quick money round under optimum condition of climate, food and management in comparison to cattle and buffalo. Due to failure of ewe to carry her off spring for full period of gestation, premature birth or perinatal lamb loss caused economic losses in sheep flocks (Kholeaf, et al., 1977; Firehammer and Myers, 1981 and Khalil, 1997).

Campylobacters are the third most common cause of abortion in sheep after chlamydia and Toxoplasma (Kimberling, 1988). The disease is characterized by abortion during late stage of pregnancy, still births and weak lambs. The outbreaks nearly occur in flocks which had not the disease before. The losses are usually about 15-20% and may reach 70% of ewe flock (Dennis, 1990).

Campylobacter fetus ss. fetus and C. jejuni are the causative agent of the disease. Both organisms are common inhabitants in the intestines of healthy sheep, in certain circumstance become virulent, however,that abortions follow when new strains gain entry to the flock (Duffell and Skirrow,1978). Susceptible ewes may acquire infection through ingestion of forage contaminated with fetal material or uterine discharge. Other sources of infection may include feces of carrier sheep and other mammals. Venereal transmission has not been documented in sheep. Human beings working with sheep may acquire the infection from aborting ewes then develop enteritis (Delong , et al., 1996).

The two subspecies of Campylobacters can be isolated from the placenta which shows characteristic pale necrotic lesions, also the organisms are present in large numbers in the stomach content of aborted foeti, on the surface of liver, in the intestine and the bloody fluid in the abdominal and thoracic cavities. The organisms can be isolated from fecal samples of diarrhoeic sheep and bile of infected ones (Bird, et al., 1984 and Kirkbride, 1993).

Ewes develope life long immunity after abortion; however, there is no cross protection between C. jejuni and C.f.ss. fetus. There are antigenic variance between strains and there is no laboratory tests which have been devised to distinguish abortion strains from normal intestinal strains. Such differentiation would be a major step toward a better using

of serological test for diagnosis of the disease (Patton, et al., 1991 and Varga, 1991).

The objective of this study to identify the different Campylobacter species associated with abortion in Egyptian sheep by isolation and identification methods beside application of serological tests for diagnosis of campylobacteriosis.

MATERILAS and METHODS

A total of 1588 samples were collected from different breed of sheep in Egyptian farms suffered from reproductive disorders as abortion and / or infertility. These includs 1130 serum ewe samples, 26 aborted foeti, 12 faetal membranes, 90 vaginal discharges from aborted ewes, 215 gall bladder from slaughterhouses and 115 fecal swabs (Table, 1).

Table (1): Samples taken from sheep at different localities in Egypt.

Sources	Samples collected						Total
	Serum	Aborted foeti	Placenta	Vaginal swabs	G. bladder	Fecal swabs	
Cairo	55	3	1	27	115	20	221
Giza	300	8	2	18	100	35	463
Fayoum	-	4	2	-	-	20	26
Beni-Suef	-	3	2	31	-	17	53
K.Elshaikh	75	6	4	14	-	23	122
El-Behera	300	-	-	-	-	-	300
Alex.	400	-	-	-	-	-	400
Assuit	-	2	1	-	-	-	3
Total	1130	26	12	90	215	115	1588

All samples except serum samples were inoculated into thiol media for 24-72 hours at 37oC for detection of Campylobacter organisms. The isolation of Campylobacters was done by the conventional methods (Smibert, 1984) and identified biochemically (Holt,et al., 1994).

The specimens of ewe serum samples (1130) and vaginal swabs (90) were subjected to serological and immunological tests by using tube agglutination test Foreyt, et al., (1983) and ELISA Hedstrom, et al., (1989). Antigen preparation was made from 2 reference strains of C.f.ss.fetus and C. jejuni obtained from Ames, IOWA, USA. In addition to 2 reference antiserum used as control positive. The formalized antigens

used for tube agglutination test was prepared according to Yoshida, et al., (1987) and soluble antigens used for ELISA was prepared according to Blobel, (1983). The negative and positive controls were included in both tests.

RESULTS

Table (2): Campylobacter species isolated from the different samples of sheep by bacteriological examination.

Site of isolation	No. of samples	No. of isolates	C.f.ss. fetus	C. jejuni
Aborted foeti	26	9 (34.62%)	9	-
Faetal memb.	12	2 (16.67%)	2	-
Vaginal disch.	90	7 (7.78%)	2	5
G. bladder	215	19 (8.84%)	19	-
Fecal swabs	115	20 (17.39)	-	20
Total	458	57 (12.45%)	32 (6.99%)	25 (5.46%)

Table (3): The biochemical characters of Campylobacter species isolated from sheep.

Campylobacter species	Biochemical tests							
	Catalase	H2S*	Hippurate Hydrolysis	Temp. Growth 25o	37o	42o	Susceptibility@ Nalidixicacid	Cephalothin
C.f.ss. fetus	+	+	-	+	+	-	R#	Ss
C. jejuni	+	+	+	-	+	+	S	R

*: Hydrogen sulphide with lead acetate strips.
@: 30 ug/Disk
R: Resistant S: Sensitive.

Table (4): Results of Tube Agglutination Test for detection of Campylobacter antibodies in sheep.

Type of samples	No. of samples	Antigen	Results +ve	Results -ve	Serial dilution of serum and mucus 1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Serum	1130	C.f.ss.fetus	38 (3.36)	1092 (96.64)	44	36	15	10	8	3	1	1
Vaginal disch.	90		13 (14.14)	77 (85.55)	14	9	8	3	1	1	-	-
Serum	1130	C. jejuni	36 (3.19)	1094 (96.81)	30	18	14	12	3	2	3	2
Vaginal disch.	90		4 (4.44)	86 (95.55)	5	2	3	1	-	-	-	-

*: Positive results are considered at agglutination titer of <1/40

Table (5): Results of ELISA for detection of Campylobacter antibodies in ewes.

Type of samples	No. of samples	Antigen	Results +ve	Results -ve	Serial dilution of serum & mucus 1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Serum	1130	C.f.ss.fetus	108 (9.55)	1022 (90.44)	52	24	13	9	6	4	-
vaginal disch.	90		19 (21.11)	74 (78.88)	8	5	3	2	1	1	-
Serum	1130	C. jejuni	92 (8.14)	1038 (91.86)	44	25	19	2	2	-	-
vaginal disch.	90		7 (7.78)	83 (92.22)	3	2	1	1	-	-	-

Table (6): The comparison between results of tube agglutination test and ELISA for detection of Campylobacter antibodies.

Type of samples	No. of samples	Type of antigen	Tube agg. test	ELISA
Serum	1130	C.f.ss. fetus	38 (3.36)	108 (9.55)
Vaginal disch.	90		13 (14.14)	19 (21.11)
Serum	1130	C. jejuni	36 (3.16)	92 (8.14)
Vaginal disch.	90		4 (4.44)	7 (7.77)

Table (7): The correlation between C. f.ss. fetus and C. jejuni antigens in serological tests.

Type of samples	Test	No. of positive	Antigenic groups		
			+ ve with ss. fetus	+ve with c. jejuni	+ve with both@
Serum	Tube agg. test	74	38	36	7
	ELISA	200	108	92	2
Vaginal disch.	Tube agg. test	17	13	4	2
	ELISA	26	19	7	0

@: The crossreactive occured only in the low titer not more than >1/160.

DISCUSSION

In Egypt, sheep have taken good position in animal production for their ability to living in green and desert land with production of meat, milk and wool even with bad condition (Khalil, 1997).

Campylobacter is causing reproductive disorders characterized by late abortions, still births and weak lambs. It has major importance to sheep industry and often cause sever financial waste to individual enterprises (Clean, 1988 and Delong, et al., 1996).

Little is known about strain differences of Campylobacters associated with abortions in different breeds of sheep. In this study, Campylobacters were isolated from 57 (12.45%) of 458 samples represented as 32 (6.99%) C.f.ss. fetus and 25 (5.46%) C. jejuni (Table,2). From table (2) we found that C.f.ss. fetus was associated with ovine abortion and C. jejuni considered as the abortifacient agent in the recent survey Adesiyun, et al., (1992); Cabrita, et al., (1992) and Kirkbride, (1993).

The highest isolation rate of Campylobacters were presented in aborted foeti (34.62%) then fecal swabs (17.39%). This finding indicated that the invasive ability of enteric Campylobacter to pregnant ewes developed bacterimia, the placenta became infected and the lamb aborted (Manser and Dalziel, 1985 and Lander, 1988).

Campylobacter organisms are often difficult to isolate from clinical samples and once they are isolated, the identification methods by biochemical test take long time. Therefore, we applied the serological tests to reach a rapid and a precise conclusion to the actual states of low campylobacter reactors to prevent the infection from place to place in

flocks,but not in the individual cases (Dennis, 1990 and Patton, et al 1991).

Out of 1130 ewes serum samples and 90 vaginal discharge samples examined by tube agglutination test and ELISA using reference strains of C.f. ss. fetus and C. jejuni antigens to'detect campylobacter antibodies of the infected flocks. Tube agglutination test results were 38 (3.36%) in serum samples and 13 (14.14%) in vaginal discharges with C.f.ss. fetus antigen and 36 (3.19%) in serum and 4 (4.44%) in vaginal discharge with C. jejuni antigen. In comparing to results of ELISA test these were 108 (9.55%) in serum and 19 (21.11%) in vaginal discharges with ss. fetus antigen and 92 (8.14%) in serum and 7 (7.78%) in vaginal discharges with C. jejuni respectively (Tables, 4, 5 & 6).

The highest positive rate demonstrated by ELISA test than tube agglutination test could be attributed to the presence of low titer by tube agglutination test which was negative results, could be detected by ELISA which captured nanograms of antibodies to screen for C. antigens with defined specificity (Fig,1).

Furthermore, the cross reactions between C. f.ss. fetus and C. jejuni were demonstrated by serological tests which were 7 (9.89%) by tube agglutination test > 1/160 titer and failed to give homologus titer.The cross reactivity minimized by ELISA 2(0.88%) at 1/100 but no correlation at the higher dilution (Table,7) (Eaglesome and Garcia, 1992 and Hum, et al., 1994).

Finally, this work monitoring the special consideration for the effectiveness of ELISA which is simple, more sensitive and rapid technique for early diagnosis of Campylobacters in sheep. On the other hand, we need further work to prepare national multivalent vaccine used as a prophylactic and control measures of the disease.

REFERENCES

Adesiyum, A.A.; Kaminjolo, J.S. and Loregnard, R (1992): Campylobacter infection in calves, Piglets, Iambs and Kids in Trinidad Br. Vet. J.; 148:547-556.

Bird, M.M.E.; Stephans, D.J.; Wall, E.P.; Lisle, G.W. and Delisle, G.W. (1984): Serology of C.f.ss. fetus strains from four outbreaks of ovine abortion. New Zeland, Vet. J.; 32:112-114.

Cabrita, J; Rodriques, J. and Braganca, F. (1992): Prevalance, biotypes, plasmid profile and antimicrobial resistance of

Campylobacter isolated from wild and domestic animals from northeast Portugal. J. Appl. Bacterial. 73:279-285.

Clean, V.K. (1988): Jensen and Swift,s diseases of sheep. 3rd Ed. Lee & Febiger, philadelphia, USA.

Delong, W .J.; Jaworski, M.D and Ward, A.C.S (1996): Antigenic and restricition enzyme analysis of Campylobacter spp. associated with abortion in sheep. AJVR, Vol.57, no.2, 163-167.

Dennis, S.M. (1990): Campylobacter abortion in sheep: In Kirkbride, Ed. Laboratory diagnosis of livstock abortion. 3rd. Ed. Ames, IOWA State Univ. Press, 82-85.

Duffell, S.J. and Skirrow, M.B. (1978): Shepherd's scours and ovine Campylobacter abortion- a new zoonosis (Lett). Vet. Rec; 103:144.

Eaglesome, M.D. and Garcia, M.M. (1992): Microbial agents assoicated with bovine genital tract infections and semen. Part.I. Brucella abortus, Leptospira, Campylobacter fetus and trichomonas fetus. vet. Bull., 62:8, 743-775.

Firehammer, B.D. and Myers, L.L. (1981): Campylobacter fetus subsp. jejuni. Its possible significance in enteric disease of calves and Iambs. Am.J. Vet.Res., Vol. 42, No.6P. 918-922.

Foreyt, W.J.; Smith, T.C.; Evermann, J.F. and Heiwer, W.E. (1983): Hematologic serum chemistry and serologic values of Dall's sheep (Ovis dalli dalli) Aiaska.J. Wildl0 19 (2):13639.

Hedstrom, O.; Sonn, R.; Dearing, P.; Synder, S.P. and Lass, E.P. (1989): Measurement of IgG concentration in ovine fetal fluids a useful diagostic test. Boll Ist. Sieroter Milan,; 67(5-6): 363-368.

Holt, J.H.; Krieg, N.R.and Sneath, P.H.A. (1994): Bergey's Manual of determenative Bacteriology, 9th Ed. Williams and Wilkins, Baltimore.

Hum, S.; Quinn, C and Kennedy, D. (1994): Daignosis of bovine venereal Campylobacteriosis by ELISA. Aust. Vet.J. 71:140-143.

Khalil, M.A. (1997): The economic importance of sheep in Egypt. Bovine and Ovine Middle East and North Africa. 3rd Year-No.10P. 48-49.

Kholeaf, Z.M.; Farag, Y.A.; Rakha, A.M. and Abul-Fadle, W. (1977): A preliminary study on the bacteria causing abortion in locl sheep.Egypt.J.Vet. Sci., 4,No.2,141-148.

Kimberling, C.V. (1988): Diseases causing abortin. Kimbrling, C.V. Ed. Jensen and Swift's diseases of sheep 3rd. Philadelphia: Lea Febiber, 57.

Kikrkbride, C.A. (1993): Diagnosis in 1,784 ovine abortions and still births. J.Vet. Diagn. Invest. 5:398-402.

Lander,K.P. (1988): Campylobacters in sheep. Fertililty and infertility in Veteinary Practice, 4 th Ed. by Laing M.M. pp.226-227.

Manser, P.A. and Dalziel, R.W. (1985): A survey of Camplobacter in animals. J.Hyg. Comb., 95, 15-21.

Monfort, J.D.; Bech-Nielsen and Still, H.F. (1994): Detection of flagellar antigen of Campylobacter jejuni and C. coli in canine feces with ELIþþSA new prespects for diagnosis. Vet. Res. Comm 18:2,85-92.

Patton, C.M.; Wachsmuth, I.K. and Evins, G.M.(1991): Evaluation of 10 methods to distingush epidemic associated Campylobacter strains. J.Clin. Microbic. 29:680-688.

Smibert, R.M. (1984): Campylobacter. In Bergey;s Manual of Determinative Bacteriology, 9th Ed. J.G. Holt, pp. 111 Baltimore, Williams & Wilkins.

Varga,J. (1991): Comparison of surface antigens of some Campylobacter fetus subsp. fetus strains of ovine origin by polyacromide gel electrophorsis and immunoblotting.Zbl. Vet Med (B); 38:497-504.

Yoshida, S.I.; Kancko, K.I.; Ogawa, and Takizawa, T. (1987): Serum agglutiatio titers against somatic and flagellar antigens of Campylobacter jejuni and isolation of Campylobacter spp. in chickens. Am.J.Vet.Res., Vol. 48, No.5.801-803.

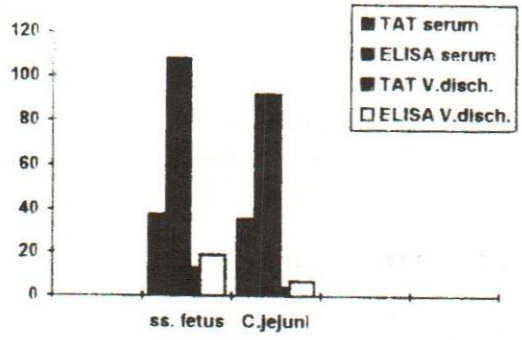

Fig.1 The comparison between results of tube agglutination test and ELISA for detection of Campylobacter antibodies.