Dept. of Animal Hygiene and Zoonoses,
Fac. Vet. Med., Assiut University.

PREVALENCE OF *COXIELLA BURNETII* INFECTION AMONG DOGS AND HUMANS IN UPPER EGYPT

(With 6 Tables and 1 Figure)

By

AMAL, S. M. SAYED; ASMAA, A. A. HUSSEIN;
A. A. ISMAIL; S. E. M. NASR;
M. YASUKAZU*; H. UENO* and C. MORITA*

*: Vet. Public Health Dept. Rakuno-Gakuen Univ. Hokkaido, Japan

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مدى إنتشار الإصابة بالكوكسيلا برنيتى بين الكلاب والإنسان فى صعيد مصر

أمل سيد محمد سيد ، أسماء عبد الناصر حسين ،
عبد المعز أحمد اسماعيل ، سعد الدين محمد نصر ،
مورماتسو ياسوكاز ، هيروشى أوينو ، شيهارو موريتا

تلعب الكلاب دور هام في نقل الكثير من الأمراض المشتركة للإنسان لذلك فقد أجريت هذه الدراسة لتحديد مدى إنتشار مرض الحمى المجهولة في كل من الكلاب والإنسان ولذلك فقد تم جمع عينات دم من عدد 150 كلب ، 150 مريض مصاب بحمى غير معروفة المسبب (PUO) هذا بالإضافة إلى 50 عينة دم أخذت من أشخاص أصحاء ظاهرياً. ولتقدير مستوى الأجسام المضادة المناعية لميكروب الكوكسيلا برنيتى بإستخدام الإختبار الفلورسينتى الغير المباشر (IFA)، فقد وجد أن معدل إنتشار الكوكسيلا برنيتى بين الكلاب المفحوصة 22.7% وذلك بنسبة 24.7% للذكور و 19.7% للإناث. وكان معدل إنتشار الأجسام المضادة المناعية لميكروب الكوكسيلا برنيتى 37.3% فى عينات المرضى و 30% في عينات دم الأفراد الأصحاء ظاهرياً. وقد تم التشخيص الجينى للكشف عن وجود جين *com-1* الخاص بالكوكسيلا برنيتى بإستخدام تفاعل البلمرة المتسلسل (PCR) وتم التعرف عليه في كل من 6% ، 3.3% و8% في عينات دم كل من الكلاب والمرضى والأفراد الأصحاء ظاهرياً على التوالي. وقد تمت مناقشة نتائج كل من الأختبار الفلورسينتى الغير مباشر وتفاعل البلمرة المتسلسل والتوصية بالطريقة المناسبة لتشخيص مرضى الحمى المجهولة حسب الطور المرضى هذا بالإضافة إلى التوصية بوضع مرض الحمى المجهولة ضمن قائمة أمراض التشخيص المقارن للمرضى المصابين بإرتفاع في درجة الحرارة وغير معروف المسبب المرضى وقد تمت مناقشة الخطورة الصحية لمرض الحمى المجهولة مع مراعاة العلاج المبكر لها قبل الدخول في طورها المزمن الذي يحتاج إلى فترة طويلة في العلاج.

SUMMARY

To estimate the seroprevalence of antibodies to *Coxiella burnetii* among dogs and humans in Upper Egypt in the preiod of 1998-1999 by using indirect immunofluorescence assay, blood samples were collected from 150 dogs and 150 patients with pyrexia of unknown origin. The frequency of occurrence of *C.burnetii* antibodies in 50 blood samples collected from apparently healthy humans was studied. Nested polymerase chain reaction was used to detect the *com-1* gene (genetic target of *C.burnetii*) encoding a 27-kDa outer membrane protein in the examined samples. *C.burnetii* antibodies were detected in 22.7%, 37.3% and 30% of the examined dogs, patients and apparently healthy humans, respectively. IgM antibodies against *C.burnetii* were detected in 1(0.67%) of the examined patients and IgG antibodies were detected in 24 (28%) and 52 (34.7%) of the examined apparently healthy humans and patients, respectively. Both IgM and IgG antibodies were detected in 1(2%) and 3(2%) of the examined apparently healthy humans and patients, respectively. *Com-1* gene fragment was amplified from 6%, 3.3% and 8% of the examined dogs, patients and apparently healthy humans, respectively. It was concluded that dogs are good indicators for monitoring the prevalence of Q fever in a community as the prevalence of Q fever obtained in both dogs and humans was parallel to each other. Based on the obtained results of both IFA and PCR methods, the suitable method of Q fever diagnosis according to the suspected stage of infection was proposed.

Key words: *C.burnetii Indirect immunofluorescence assay, Polymersase chain reaction, Dogs, humans.*

INTRODUCTION

Q fever is an endemic worldwide zoonosis except in Sweden, Norway, Iceland and New Zealand where reports of the disease are rare (Criag and Edward, 1998). Q fever is caused by *Coxiella burnetii*; a highly pleomorphic Gram negative coccobacillus (Thomas, 1998). *C.burnetii* has a wide host range including cattle, sheep, goats, pigs, horses, camels, buffaloes, water buffaloes, dogs, cats, pigeons, ducks, geese, fowls and turkeys (Babudieri, 1959; Marrie *et al.*, 1993 and Jim and Vanessa, 1999). In addition wild animals and their ectoparasites plays an important role in the sylvan cycle (Jim and Vanessa, 1999). Typically, animals are subclinically infected and shed environmentally resistant organisms in their milk, urine, feces and parturient discharges

acting as a potential source for human infection (Scott and Williams, 1990). Occasionally, infections are associated with abortion in sheep, goats and to a lesser degree in cattle (Criag and Edward, 1998). Dogs are considered as sentinel animals for monitoring the prevalence of *C.burnetii* in surrounding household environment, live stock areas and stray wild life which was documented by previous studies (Martinov *et al.*, 1989; Htwe *et al.*, 1992; Kelly *et al.*, 1993; Bacellar *et al.*, 1995 and Boni *et al.*, 1998). Although *C.burnetii* infects both rural and urban residents (Schaal, 1985 and Raoult and Marrie, 1995), the high risk of infection is occupationally acquired among abattoir workers, wool sorters, tanners, farm workers (Shepherds; dairy workers), veterinary and laboratory personnel from livestock (Criag and Edward, 1998). Q fever in man is represented either as acute self-limiting flu-like illness which is easily treated with antibiotics (Baca and Paretsky, 1983 and Zhang *et al.*, 1998) or as a chronic form represented either by pneumonia (Schulze *et al.*, 1996; Stoilova *et al.*, 1996; To *et al.*, 1996 and Lim *et al.*, 2001); hepatitis (Yale *et al.*, 1994 and Wu *et al.*, 1995) or endocarditis (Stchepinsky *et al.*, 1995; Lupoglazoff *et al.*, 1997 and Shovman *et al.*, 1997) which requires prolonged therapy. It has been reported that Q fever may lead to miscarriage, delivery of abnormal children (Ellis *et al.*, 1983 and Dindinaud *et al.*, 1991) and sometimes may lead to spontaneous abortion in women (Raoult and Marrie, 1995 and Rey *et al.*, 2000).

In this study we attempted to monitor the prevalence of *C.burnetii* antibodies in both dogs and humans in Upper Egypt by indirect immunofluorescent assay (IFA), which is sensitive and highly specific method. Polymerase chain reaction is sensitive and highly specific genetically based method, it was used to detect *C.burnetii* genome in the examined samples.

MATERIAL and METHODS

1- Samples collection:

Blood samples of 150 patients with pyrexia of unknown origin (PUO) and 50 apparently healthy individuals were collected from different districts in Upper Egypt in the period from 1998-1999. Blood samples were also collected from 150 dogs as a guided random.

Blood samples were collected using blood sampling filter paper (Toyo-Roshi, Tokyo) as previously described (Ima *et al.*, 1999). The blood was eluted with 0.6ml of phosphate buffer solution pH 7.5 (PBS).

Samples were collected in Egypt and the laboratory work was performed in the Vet. Public Health Dept. Rakuno-Gakuen Univ. Hokkaido, Japan.

2-Indirect Immunofluorescence Assay:

C.burnetii Nine Mile strain phase II (ATCC VR-616) were maintained on BGM (Buffalo Green Monkey) cells at 35°C and used to prepare the assay slide glass as previously described by Yanase *et al.*, 1997 and Yanase *et al.*, 1998. Two fold serial dilutions of each sample were prepared using PBS. IFA was performed as described by Morita *et al.*, 1994 and Yanase *et al.*, 1997 to detect IgM and IgG antibodies against *C.burnetii*. Titer 64 was considered as the cut off point.

3- Preparation of Samples for Polymerase Chain Reaction:

Extraction and rapid purification of DNA from the blood samples was performed using QIA amp blood kit (QIAGEN, Funakoshi, Japan) in accordance with the manufacture's instructions.

4-DNA amplification:

Nested PCR was performed using OMP1-OMP2 primer set and OMP3-OMP4 primer set designed from the nucleotide sequence of the *com-1* (*C.burnetii* genetic target) encoding a 27-kDa outer membrane protein (Zhang *et al.*, 1998). OMP1-OMP2 primer set and OMP3-OMP4 primer set amplify a region of 501 bp and 438 bp fragments, respectively (Zhang *et al.*, 1998). The first amplification was performed in a total volume of 50 µl containing 10 µl of DNA sample, 2.5 units of *Taq* DNA polymerase (Life Technologies, USA) and final concentration of 50 mM KCL, 10mM Tris-HCL (pH 8.3), 2mM $MgCl_2$, dATP, dCTP, dGTP and dTTP at a concentration of 200 µM each and the primers at a concentration of 0.8 µM each. Each sample was subjected to 35 cycles of amplification in a thermal cycler. Each cycle consist of denaturation, annealing and extention at 93°C, 56°C and 72°C, respectively performed for 1 min. Five microliters of the first amplification product was then subjected to the second amplification with OMP3-OMP4 primer set. Amplification was performed in 35 cycles each consist of denaturation at 93°C for 40 sec, annealing at 53°C for 40 sec and extention at 72°C for 1 min. A positive control with 5 pg of *C.burnetii* DNA as a template and a negative control without DNA template were included in each PCR run.

5- Detection of PCR products:

The amplified product of PCR were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide (0.5 µg /ml),

visualized under UV illumination (TM-20; UVP, Inc) at 320 nm and photographed (Zhang *et al.*, 1998).

RESULTS

The obtained results are recorded in Tables 1-6 and Figure 1.

DISCUSSION

Dogs play an important role in the transmission chain of *C.burnetti* and act as a potential source for human infection, moreover the role of dogs cannot be ignored in disseminating the organism in the environmental surrounding through their infected secretion, excretion and uterovaginal discharges (Babudieri, 1959; Criag and Edward, 1998 and Jim and Vanessa, 1999). Based on the obtained results, it is concluded that dogs are good indicators for monitoring the prevalence of *C.burnetii* in a community as the prevalence of Q fever infection in both dogs and humans is parallel to each other. The overall prevalence of *C.burnetii* phase II antibodies recovered in dog's sera was 22.7% (Table 1). The obtained prevalence was higher than that detected by Kelly *et al.*, 1993 and Bacellar *et al.*, 1995 who estimated a prevalence rate of 15% and 4.8%, respectively, but the obtained prevalence was lower than that detected previously by Martinov *et al.*, 1989 and Metzler *et al.*, 1993 who estimated a prevalence rate of 59.25% and 31.4%, respectively. It was apparent that gender of dogs does not act as a risk factor in acquiring Q fever infection as the prevalence variation between males (24.7%) and females (19.7%) (Table 1) does not reflect a true sex difference and this result was previously documented by Criag and Edward, 1998 and Jim and Vanessa, 1999.

C.burnetii antibodies were recovered from 30% of the examined apparently healthy human (Table 2). Previous studies reported the detection of *C.burnetii* antibodies in clinically healthy individuals (Marrie and Fraser, 1985 and Akesson *et al.*, 1991). In addition *C.burnetii* antibodies were detected in 37.3% of the examined patients with pyrexia of unknown origin (Table 2). *C.burnetii* antibodies were recovered from patients with variable complains (Baca and Paretsky, 1983; Hoen *et al.*, 1995 and Zhang *et al.*, 1998). Specific *C.burnetii* IgM antibodies were detected in 1(0.67%) of the examined patients, moreover IgG antibodies were detected in 34.7% and 28% of the examined patients and apparently healthy humans, respectively (Table 2). However both IgM and IgG antibodies were detected in 3(2%) and 1(2%) of the

examined patients and apparently healthy humans, respectively (Table 2).

Although diagnosis of Q fever has been based mainly on serological analysis, it does not allow diagnosis before the second week of infection because both IgM and IgG antibodies cannot be detected before 2 weeks of infection (Vene, 1989 and Stein and Raoult, 1992). On the contrary genetically based methods predominantly polymerase chain reaction have been widely used to detect *C.burnetii* genome in the early stage of infection before antibodies appearance (Shibata *et al.*, 1988; Stein and Raoult, 1992 and Zhang *et al.*, 1998).

Com-1 gene of *C.burnetii* was recovered from 9(6%), 5(3.3%) and 4(8%) of the examined dogs, patients and apparently healthy humans, respectively (Table 3 and Figure 1). The inability to detect *C.burnetii* genome in the rest of the IFA positive samples may be explained by the relatively short period of rickettsaemia of *C.burnetii* before the entrance to macrophage cells (Oda and Yoshiie, 1989), hence most of the blood samples were drawn out after the end of rickettsaemic period. This result agrees with that obtained previously (Zhang *et al.*, 1998) in which *Com-1* gene was detected in 87% of 155 positive IFA human sera.

The inability to detect the humoral response in all of the nine positive PCR cases of dogs, patients no.s 2,3 and 5 and the four positive cases of apparently healthy humans were referred to the early stage of infection before the formation of antibodies (Tables 4,5 and 6). This result agrees with that obtained previously (Zhang *et al.*, 1998) in which *Com-1* gene was recovered in 11% of negative IFA humans sera. Only IgM antibodies against *C.burnetii* phase II antigen was detected at a low titer in patient no.4 and thus reflecting the relatively early stage of infection (Table 5). However both IgM and IgG antibodies were detected at a titer of 32 and 128, respectively in patient no.1 (Table 5). Although the temperature of patients no.s 3 and 5 was 37°C (Table 5) when blood samples were drawn out, *C.burnetii* organisms were still circulating in the blood. (The patients were given antipyretic drugs). The existence of *C.burnetii* genome in 4(8%) (Table 3) of the examined apparently healthy humans seems to be a latent danger which either suddenly arise clinically with non specific symptoms or silently passes and runs a chronic protracted course requiring prolonged therapy (Bental *et al.*, 1995).

From a medical stand point, the danger caused by Q fever should attract the attention of physicians to consider it in differential diagnosis

of patients suffering from pyrexia of unknown origin, hence rapid diagnosis of the disease is made and appropriate antibiotic treatment may lead to a better prognosis for the infected patients.

Based on the obtained results of both IFA and PCR methods, it is concluded that early diagnosis of Q fever infection must be made by PCR because indirect immunofluoresence assay or any other serological tests is useless in diagnosis before the formation of antibodies. Although PCR is able to detect *C.burnetii* genome in the early stage of infection, it can not detect it after the invasion of the organisms to the cells, so at that time the negative result of PCR does not preclude the presence of infection. In this case the rising titer of any serodiagnostic method is confirmatory for the presence of infection.

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Table 1: Sero-prevalence of *Coxiella burnetii* antibodies in male and female dogs.

Sex	No. of samples	Seropositive samples
Male	89	22(24.7%)
Female	61	12(19.7%)
Total	150	34(22.7%)

Table 2: Prevalence of *Coxiella burnetii* antibodies (IgM & IgG) in humans sera.

		Type of antibodies			
Source of samples	No. of samples	IgM	IgG	IgMG *	Total
Patients (P.U.O)	150	1(0.67%)	52(34.7%)	3(2%)	56(37.3%)
Apparently healthy humans	50	-	14(28%)	1(2%)	15(30%)
Total	200	1(0.5%)	66(33%)	4(2%)	71(35.5%)

Cut off point 64. * Positive for both IgM and IgG

(P.U.O) Patients with pyrexia of unknown origin

Table 3: Detection of *C.burnetii* genome in the examined samples.

Source of samples	No. of samples	*C.burnetii* genome
Dogs	150	9 (6%)
Patients (PUO)	150	5 (3.3%)
Apparently healthy humans	50	4 (8 %)
Total	350	18 (5.14%)

(PUO): Patients with pyrixia of unknown origin

Table 4: Correlation between detection of *C.burnetii* genome and IFA antibodies in dogs sera.

Sample No.	Sex	Type	Polyvalent *C. burnetii* antibodies	*C.burnetii* genome
1	Male	Pet	-	+
2	Female	Stray	-	+
3	Male	Stray	-	+
4	Female	Stray	-	+
5	Male	Stray	-	+
6	Male	Stray	-	+
7	Male	Stray	-	+
8	Female	Stray	-	+

Table 5: Correlation between detection of *C.burnetii* genome and IFA antibodies in patients with pyrexia of unknown origin.

Sample No.	Sex	Age	Occupation	Temperature	*C. burnetii* antibodies		*C.burnetii* genome
					IgM	IgG	
1	Female	25 yrs	Housewife	40 °C	32*	128	+
2	Female	30 yrs	Housewife	38 °C	-	-	+
3	Female	30 yrs	Housewife	37 °C	-	-	+
4	Female	52 yrs	Housewife	39.7 °C	16*	-	+
5	Male	65 yrs	Farmer	37 °C	-	-	+

* Although the cut off point was considered as 64, the low titer of IgM antibodies in patients no.s 1 & 2 were described only to show the level of IgM antibodies in the positive cases of PCR.

Table 6: Correlation between detection of *C.burnetii* genome and IFA antibodies in apparently healthy humans sera.

Sample No.	Sex	Age	Occupation	*C. burnetii* antibodies		*C.burnetii* genome
				IgM	IgG	
1	Male	14 yrs	Student	-	-	+
2	Male	25 yrs	Workless	-	-	+
3	Male	33 yrs	Worker	-	-	+
4	Female	50 yrs	Housewife	-	-	+

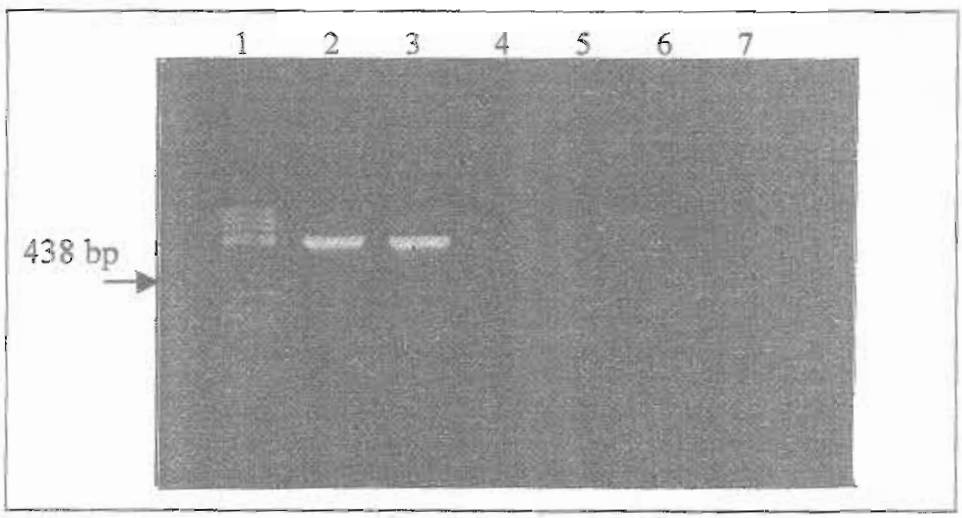

Fig. 1. Detection of *C. burnetii* DNA in human sera by nested PCR with primers OMP1-OMP2 and OMP3-OMP4. An agarose gel electrophoretogram of amplified DNA after the nested PCR and ethidium bromide staining. Lane 1: molecular size standard marker, Lane 2: Nine Mile phase II strain (positive control), Lane 3: positive sample, lanes 4,5 and 6: negative samples. Lane 7: negative control.