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Epizootic of *Chlamydia psittaci* infection in goats in Taiwan

Y. K. Liao,* C. Y. Chain, Y. S. Lu, N. J. Li, H. J. Tsai[1] and P. P. Liou

Taiwan Animal Health Research Institute.
[1)]Depatrment of Veterinary Medicine National Taiwan University

ABSTRACT Epizootic abortion in goats has been frequently reported in Taiwan since 1993. The outbroken flocks were found in most of districts in Taiwan. No apparent clinical signs were found in aborted doe. The typical abortion occurred in the last two months of pregnancy. The incidence of abortion was from 10 % to 87 % in outbroken farms in 1993, and a total of 976 out of 2,130 pregnancies (46 %) were found abortion in our investigation. Gross lesions in aborted fetuses included generalized haemorrhage and swollen liver. *Chlamydia psittaci* was isolated from tissues of aborted fetuses and from vaginal swabs of aborted does. Chlamydial antibodies were detected among 67 % to 100 % of aborted does from epizootic flocks. The *C. psittaci* was diagnosed as the causal agent in enzootic abortion. This is the first report on chlamydial isolation and antibody surveys in epizootic abortion in goats in Taiwan.

Key words: Chlamydia psittaci , Epizootiology , goat

INTRODUCTION

C. psittaci was recognized as a causal agent in abortion in ewes (HERRING 1993, LEONARD *et al.* 1993, SOURIAU *et al.* 1986), goats, cattle (DOMEIKA *et al.* 1994, SEAMAN *et al.* 1986) and swine (OKADA *et al.* 1992) in many countries. The pathogenesis of disease favours the development of endemic infections, and an initial epidemic may result in one-third of ewes abortion, losses of 5 to 10 % in production can be expected when the disease becomes endemic (LEONARD *et al.* 1993). The disease is therefore responsible for an important reduction in the efficiency of goat production. *C. psittaci* infetion was accounted for three main conditions in sheep and goat : abortion, polyarthritis and conjunctivitis (BUZON-GATEL *et al.* 1989, RODOLAKIS *et al.* 1989). *C. psittaci* isolates had been divided into two serotypes on the basis of a plaque reduction test, serotype 1 was associated with abortion and inapparent infection of the intestinal tract whereas serotype 2 was associated with polyarthritis and conjunctivitis. Most of the strains isolated from abortion cases are invasive in mice following subcutaneous inoculation, whereas strains isolated from feces of apparently healthy ewes are not invasive in mice (RODOLAKIS *et al.* 1989). *C. psittaci* are obligate intracellular pathogens with a unique biophasic lifecycle. The cycle begins when the metabolically inert, spore-like elementary body (EB) meets and adheres to a suitable host cell (HERRING 1993). Fertility is usually normal in pregnancies subsequent to the abortion, though some feel that immunity decrease after 3 years and chlamydial abortion may then recur.

*Corresponding author
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Taiwan Animal Health Research Institute. Taiwan, R. O. C.

Enzootic abortion in goats associated with *Chlamydia* has not previously been reported in Taiwan. The clinical and pathological features of goat abortion are different from those reported in epizootic bovine abortion in Taiwan (LIAO *et al.* 1996). This is the first report of the isolation of *C. psittaci* from aborted fetuses and does. and an epizootic investigation in Taiwan is also described in this paper.

MATERIALS AND METHODS

Clinical and epizootic feature of natural infection :

Between 1993 and 1995, many abortion cases of goat farms were reported in several districts in Taiwan. Total of 23 aborted cases were sent to our laboratory for diagnosis in that period. The epizootic investigation and pathogen isolation were conducted. During these episodes, none of aborted does had shown any other clinical signs.

Etiological examination :

Samples of liver, spleen, lung, kidney, heart blood, stomach contents and brain from aborted fetus were inoculated onto 5 % blood agar and incubated at 37 ℃ under microaerophilic condition. Otherwise, a 10 % homogenate of fetues tissue and collected placenta were made with EAGLE'S MEM (GIBCO CO.) comtaining streptomycin (0.1 mg / ml) and gentamycin (0.05 mg / ml). After centrifugation at 3,000 rpm at 4 ℃ for 20 minutes, the supernatant of homogenate was respectively collected and stored at − 70 ℃ for etiological examination. Furthermore, the heparinized blood of affected does were collected and prepared for viral isolation as previouly described (LIAO *et al.* 1996).

The vaginal swabs of affected does were collected and then were put into transport medium as previously described by SPENCER *et al.* (1983). After transported to laboratory, the swab with transport medium was homogenated and then the swab was depleted, the homogenate was centrifuged at 3,000 rpm at 4 ℃ for 20 minutes. After centrifugation, the supernatant of homogenate were respectively collected and stored at − 70 ℃ for etiological examination.

After frozen and thawed, the suspended blood liquid and supernatant of tissues homogenate were inoculated into cell culture for virus isolation. The HmLu-1 (Hamster lung continuous cell), BHK-21 (Baby hamster kidney cell) and MDBK cells were used for virus isolation. The cultures were maintained at 37 ℃ in 5 % CO_2 incubator. They were observed daily for cytopathic effect (CPE) until 7 days after infection. Each culture fluid was passaged on these cells at least three times. Isolation was scored positive or no based on the appearence of CPE in culture or more subcultures.

The tissues homogenate of fetuses and vaginal swabs of does were also inoculated into the yolk sacs of 7-day-old specific-pathogen-free (SPF) embryonated eggs. The infected eggs were candled twice a day post inoculation. The yolk asc fluid (YSF) was collected for organism detection from dead eggs. Seven days after inoculation, the YSF of survival eggs were collected, and they were reinoculated into another 7-day-old embryonated eggs for subculture, respectively. This culture process was repeated at least three times. Positive isolation was determined by the death of eggs and organism detection in YSF. SPF embryonated eggs from a SPF flock maintained at The Branch Institute of Animal Drugs Inspection-Taiwan Animal Health Research Institute were used.

For organisms identification, the speciments were scaned by the electronmicroscope after CPE appeared in cell cultures of YSF of dead eggs. The samples collected from cell cultures or YSF were firstly centrifuged at 3,000 rpm for 20 minutes. The supernatant was respectively collected for ultracentrifugation by Airfuge (BECKMAN Co.) at 90, 000 rpm for 10 minutes. The sediments were mounted on carbon-coated 400-mesh grids, stained with 2 % potassium phosphtungstic acid, and examined with a transmission electron microscope (Hitachi model H600).

Serological examination :

Sera of aborted and clinical healthy goats in epizootic farms were collected, and a total of 455 heads of goat were randomly sampled from 23 farms between 1993 and 1995, respectively. The neutralization test and enzyme immunoassay of *C. psittaci* were

conducted for detecting antibody in collected sera.

The Akabane virus and Chuzan virus isolated from calves in Taiwan were used as antigen for neutralization tests. These two viruses were etiological agents of abortion and deformities in cattle in Taiwan and Japan (GOTO *et al.* 1988, LIAO *et al.* 1994). For neutralization test, all serum samples were inactivated at 56 ℃ for 30 minutes. The test wsa performed by the constant virus-diluted serum method described previously (LIAO *et al.* 1994). The HmLu-1 cell was used for all neutralization test. The antibody titer was expressed as the reciprocal of the highest serum dilution which inhibited CPE of indicator virus. The titer wsa 4 fold or higher to be considered as positive in test.

The enzyme immunoassay was employed commercial kit for the detection of antibodies against *C. psittaci* or *Toxoplasma gondii*. The ImmunoComb kit (Biogal Galed Labs., Isreal) was used in this study. The test procedure was operated as list introduction of kits.

Results

Epizootic investigation

The clinical features of abortion were similar in most of outbroken farms. The typical abortion occurred in the last two months of pregnancy. Gross finding in aborted fetuses included generalized haemorrhage and swollen liver. No other clinical signs were found in aborted does. The prevalence of abortion was from 10 % to 87 % in outbroken farms in 1993 (Table 1). A total of 976 out of 2,130 pregnancies (47 %) was found abortion in our investigation. It was all outbroken suddenly, no predictive sign could be observed in aborted does and no deformities was found in epizootic farms. In addition, some newborn kids were weak and usually died with in 2 weeks. Furthermore, nine farms were confirmed to be infected with *C. psittaci* by etiological examination (Table 1).

Etiologic examination

No bacterium of significance was detected in the tissues of aborted fetuses, and no virus was isolated from prepared samples by cell cultures.

For isolation, the inoculated eggs were usually died in 5 days post infection or more subculture. The chiamydial elementary bodies were found in YSF from dead eggs under electronmicroscopy (Fig. 1). An antigen prepared from the infected yolk sacs reacted strongly to the complement fixaion (CF) test with chlamydial antiserum by Dr. IKUO TAKASHIMA (Department of Veterinary Public Health, Facculty of Veterinary Medicine, Hokkaido University, Japan). These criteria confirm the organisms to be *C. psittaci*.

A total of 147 strains of *C. psittaci* were isolated from aborted fetus and from vaginal swabs of aborted does between 1993 and 1995 (Table 2). Most isolates were isolated from liver (43.8 %) and spleen (43.8 %) of aborted fetuses. The other isolates were from lung (26. 3 %), kidney (22.8 %), brain (10.5 %) and placenta (23.8 %). In addition, 58 strains of *C. psittaci* were also isolated from 233 samples of vaginal swabs of aborted does.

Serological surveys

The sera neutralization test of Akabane disease and Chuzan disease were conducted in aborted does. The antibody titer was not significant difference in pair sera of aborted does. The antibody positive rate was ranged from 0 % to 30 % in tested flocks (Table 3). The immunoassay with commercial kits were used for detection of Chlamydial and Toxoplasmal infection in affected farms. The results of tests were ranged from 67 % to 100 % to be Chlamydial infection, and they were from 10 % to 40 % to be Toxoplasmal infection in does of outbroken farms between 1993 and 1995 (Table 3).

From the results of isolation and serological examination, *C. psittaci* infection of goats was diagnosed as causal agent in epizootic abortion in Taiwan.

Table 1 The incidence of abortion in epizootic goat farms in 1993

Farm	District	Prevalence of abortion			Isolation of Chlamydia
		No. of pregnancy*	No. of abortion	Abortion Rate	
A	Nantou	100	10	10 %	ND[a]
B	Nantou	50	15	30 %	Negative
C	Nantou	40	30	75 %	Positive
D	Taoyuan	120	36	30 %	Positive
E	Taoyuan	185	50	27 %	Positive
F	Kaohsiung	90	60	67 %	Positive
G	Kaohsiung	80	70	87 %	Positive
H	Kaohsiung	55	30	55 %	ND
I	Kaohsiung	250	90	36 %	Positive
J	Tainan	450	320	71 %	ND
K	Tainan	130	40	30 %	ND
L	Miaoli	200	80	40 %	Positive
M	Miaoli	160	80	50 %	Positive
N	Taichung	190	65	34 %	Positive
Total 14		2130	976	46 %	9 / 14

* : The total number of pregnancy in epizootic farm

a : No sample was collected for detection

Fig. 1 The morphology of elementary bodies of isolated *C. psittaci* under electronmicroscopy. (Bar = 200 nm)

Table 2 The results of isolation of *C. psittaci* from aborted fetuses and vaginal swabs of aborted does in epizooitc cases between 1993 and 1995

Sample	No. of head	No. of isolate	Rate of isolation
Liver	57	25	43.8 %
Spleen	57	25	43.8 %
Lung	57	15	26.3 %
Kidney	57	13	22.8 %
Brain	57	6	10.5 %
Placenta	21	5	23.8 %
Swabs	233	58	24.8 %
Total	539	147	27.3 %

Table 3 The results of serological investigation of aborted does in epizootic cases.

case	No. of serum	Akabane*	Chuzan*	Toxoplasma[a]	Chlamydia[a]
1	15	2 (13 %)[b]	0 (0 %)	2 (13 %)	15 (100 %)
2	30	3 (10 %)	0 (0 %)	6 (20 %)	24 (80 %)
3	20	4 (20 %)	0 (0 %)	4 (20 %)	14 (70 %)
4	25	5 (20 %)	0 (0 %)	ND	ND
5	30	4 (12 %)	0 (0 %)	7 (24 %)	24 (80 %)
6	40	4 (10 %)	1 (2.5 %)	ND	ND
7	40	4 (10 %)	0 (0 %)	ND	ND
8	20	4 (20 %)	1 (5 %)	5 (25 %)	19 (95 %)
9	10	1 (10 %)	0 (0 %)	4 (40 %)	8 (80 %)
10	10	3 (30 %)	0 (0 %)	ND	ND
11	20	6 (30 %)	0 (0 %)	ND	ND
12	25	3 (12 %)	0 (0 %)	5 (20 %)	18 (72 %)
13	15	3 (20 %)	1 (6.6 %)	2 (20 %)	10 (67 %)
14	20	6 (30 %)	2 (10 %)	8 (40 %)	18 (90 %)
15	20	5 (25 %)	1 (5 %)	3 (15 %)	14 (70 %)
16	20	5 (20 %)	0 (0 %)	ND	ND
17	10	2 (20 %)	1 (10 %)	1 (10 %)	7 (70 %)
18	10	2 (20 %)	1 (10 %)	2 (20 %)	10 (100 %)
19	20	0 (0 %)	0 (0 %)	4 (20 %)	20 (100 %)
20	10	3 (30 %)	0 (0 %)	2 (20 %)	10 (100 %)
21	10	2 (20 %)	0 (0 %)	2 (20 %)	7 (70 %)
22	15	2 (13 %)	0 (0 %)	5 (33 %)	10 (67 %)
23	20	2 (10 %)	1 (5 %)	8 (40 %)	18 (90 %)
Total	455	75/455 (16.5 %)	9 / 455 (1.9 %)	71/300 (23.7 %)	246/300 (82 %)

*= The results of neutralization tests.
a= The results of enzyme immunoassay of ImminoComb
b= Percentage was described as : No. of positive / No. of tested

Dicscussion

According to the results, *C. psittaci* was isolated from most of aborted cases. The liver and spleen were the prominant tissues of isolated in aborted fetus. In addition, *C. psittaci* was also isolated from vaginal swabs of aborted does. Serological examination of aborted does suggested that abortions in does was not associated with infection of Akabane disease virus, Chuzan disease virus and *Toxoplasma gondii* (Table 3). On the other hand, chlamydial antibodies were detected among aborted does and clinical healthy does, and the prevalence of antibody was from 67 % to 100 % in affected flocks. This we diagnosed *C. psittaci* was the causal agent of epizootic abortion in goat in Taiwan. This disease is therefore responsible for an important reduction in the efficiency of goat production. Although drug's treatment could be effective, they might have significant costs for the producer.

However, the risk of *C. psittaci* has not been investigated, and no isolation of *C. psittaci* was reported in Taiwan. Transmission of *C. psittaci* in goats was thought to be predominately via ingestion of food contaminated with aborted material and genital discharge (BROWN *et al.* 1988). A suspected venereal involvement was also reported previously (McCAULEY and TIEKEN 1968). The experimental findings of RODOLAKIS *et al.* (1984) indicated that *C. psittaci* could isolated from the vagina for 9 days prior to abortion and for 12 days following abortion. As farmers expended production scales of goats, a lot of breeder goats were imported from United States, Canada or Australia in recent year. The chlamydial abortion had been reported in many countries (BROWN *et al.* 1988, HERRING 1993). Nervertheless, the examination of chlamydial antigen in breeders was not listed in quarantine test. Because the antibody against *C. psittaci* was detectable in imported breeders (LIAO 1995), the transmission of *C. psittaci* may occur by natural or artificial insemination (BOWEN *et al.* 1978) from foreign breeders. It is suspected that the presence of carrier breeders induced an epizootic infection in Taiwan. The high prevalence of chlamydial antibody in aborted does suggested the high infection rate in Taiwan, as no vaccine was ever used in farms. More tests in quarantine of imported animals is suggested.

Diagnosis of chlamydial infection in animals has traditionally been made by serological investigation or the isolation of *C. psittaci* in embryonated eggs or in cell cultures. More recently, the detection of chlamydial antigen by direct immunofluorescence assay and by enzyme immunoassays has been reported (THOMAS *et al.* 1990). Nucleic acid techniques using gene amplification such as polymerase chain reaction (PCR) have also been employed in chlamydial diagnosis (DOMEIKI *et al.* 1994). PCR is comparatively simple to perform and takes less time when dealed with a large number of samples. We do strongly recomment to develope a rapid diagnosis method for the quarantine examination in the future.

C. psittaci infection is an important zoonosis. Organisms usually are transmitted to human by inhalation or by contact with infected animals. The disease is considered an occupational harzard of poultry raisers, veterinarians, zoo workers and private owners of animals. Abortion of women after contact with sheep infected with *C. psittaci* had been reported (BUXTON 1986). For public health and disease control in Taiwan, we recomment to setup the *C. psittaci* free goats flocks by rapid detection and eradication of carriers in farms.

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本省山羊披衣菌 (*Chlamydia psittaci*) 感染之疫情調查

廖永剛* 張秋燕 呂榮修 黎南榮
蔡向榮 劉培柏

台灣省家畜衛生試驗所

摘要 本省自 1993 年以來在各縣市的山羊養殖户時常傳出流產疫情，所發生的山羊都沒有明顯的臨床症狀，因此農民往往在無預警的情況下就發現母山羊流產，母山羊的流產往往是發生在懷孕後期，畜主所敘述的發生率從 10 % 到 87 % 都有，故造成的損失非常大。而我們調查結果是，1993 年時在所有懷孕母山羊 2,130 頭中有 976 頭發生流產。流產胎兒的外觀是有些呈現出血或是紅腫，解剖的觀察則只在肝臟呈腫大現象。我們收集胎兒臟器、流產胎的胎盤以及流產母羊的陰道棉棒拭子進行病原分離，結果在大部份病材都能分離出披衣菌。因此進行山羊披衣菌抗體的調查，結果抗體陽性率在發生流產疫情的羊群是 64 % 到 100 % 之間。而其它懷疑的病毒性病原在這些病材中都沒分離出，因而由抗體調查及病原分離的結果可證實披衣菌是造成母山羊流產疫情的病因。這是本省首次對山羊流產疫情分離出披衣菌及抗體調查的研究報告。

關鍵詞：披衣菌，流行病學，山羊

*抽印本索取作者
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