Prevalence of *Chlamydia abortus* Infection in Domesticated Ruminants in Taiwan

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**ABSTRACT.** This study is to (1) investigate the prevalence of *Chlamydia abortus* infection in cows and goats in Taiwan, and (2) compare the genetic properties of Taiwanese isolates with abortion strains from other sources. Approximately 71% of aborted cows and 58% of aborted does had IgG against *C. abortus* in their sera. The seroprevalence rate in cows may be overestimated, because a certain degree of cross-reactivity with *C. pecorum* cannot be ruled out. Only 22.7% (from aborted cows) and 33.3% (from aborted does) of vaginal swabs that tested positive by polymerase chain reaction led to successful isolation of *C. abortus* by inoculation into chicken embryos, equivalent to 7.1% and 7.9% of isolation rates, respectively. The major outer membrane protein gene of 15 Taiwanese abortion isolates was compared with that of various strains by restriction fragment length polymorphism (RFLP) and nucleotide sequencing. Restriction enzyme *Cjol* was able to distinguish Taiwanese ruminant isolates, which have identical RFLP patterns, from *C. felis* (feline) and *C. psittaci* (avian) strains. Taiwanese isolates had 98.8–100% homology with known ruminant abortion strains and were phylogenetically closest to bovine LW508 strain.

**KEY WORDS:** bovine, caprine, *Chlamydia abortus* infection, major outer membrane protein gene, RFLP.

Abortion has been a serious economic problem for domesticated ruminants worldwide [1, 6, 8, 10, 14, 16, 17]. In the past 5 years, dairy herds in Taiwan have experienced increasing incidence of abortion rising from 11% to 25% (by herds), based on interviews and questionnaire surveys of 115 herd owners out of a total of 868 cow herds in Taiwan. The epizootic of *C. abortus* (formerly called *Chlamydia psittaci*) infection in goats has been described [10]. Here, one of the frequently suspected ruminant abortigenic agents is *C. abortus* [10]. This is based on the clinical history, therapeutic effects of tetracycline, and a preliminary serological survey showing >37.5% seropositives among dairy cows and goats that tested positive by polymerase chain reaction (PCR) with primers CTU [3, 5], CHOMP371 [9], CPI and PSI selected from one of the conserved regions of the gene. Primer CTU and CHOMP371 were used for primary amplification, while primer CPI and PSI were used in nested amplification, if necessary. The sequence of CTU primer was 5’-ATGAAAAAAACTCTTTGAAATCCGG-3’ (1 to 22), that of CHOMP371 was 5’-TTAGAAIC(GT)GAATTGIGC(AG)TTTA(GT)GIGCICG-3’ (1177 to 1209), that of CPI 5’-TTACAAGCCTTGCTTGG-3’ (58 to...
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77), and that of PSI 5'-CCACAAGCTTTTCTAGA-3' (1100 to 1084). In primary PCR, 14.6 µl of DNA was mixed with 5.4 µl of buffer containing 5 mM KCl, 7.5 mM Tris-HCl (pH 9.0), 0.1 mM EDTA, 0.2 mM MgCl2, 2 mM (NH4)2SO4, 0.2 mM dNTP, 0.25 µM primer and 1 unit Taq DNA polymerase (Biotools-Biotechnological & Medical Laboratories, U.S.A.). The cycling conditions were 94 °C for 2 min, followed by 30 cycles at 94 °C for 1 min, 48 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. In nested PCR, 3 µl of primary PCR product was mixed with 97 µl buffer containing similar concentrations of components with 2 units of Taq polymerase. The cycling conditions were 94 °C for 2 min, followed by 25 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 80 sec. Negative controls consisting of DNA extracted from normal specific-pathogen-free (SPF) yolk sac fluid and distilled water were included in all amplifications. The PCR product was loaded in 2% agarose gel in 0.5x TAE buffer (0.02 M Tris-acetate, 0.5 mM EDTA, pH 8.0), stained with ethidium bromide, and specimens with approximately 1209 or 1027 basepair product were classified as positive. PCR product was cloned into the pcDNA3.1/V5-His-TOPO vector in a TA cloning ® kit (Invitrogen, CA, U.S.A.). Nucleotide sequencing was performed by using reagents provided in ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit by using an automated ABI PRISM 377–96 DNA Sequencer (Perkin-Elmer, CA, U.S.A.). Upon complete restriction, CfoI should yield three fragments of 1025, 154 and 34 basepairs (bp), HaeIII and StuI two fragments of 842 and 367 bp, NdeI two fragments of 865 and 344 bp, and AluI 14 fragments of 411, 282, 102, 94, 85, 72, 41, 33, 32, 18, 16, 12, 7, and 4 bp, based on the C. psittaci 6BC strain sequence [4]. Approximately 1–2 µg DNA was incubated with 5 µl of restriction enzyme for 2 hr at 37°C, and the digested products were loaded in 2% agarose gel, photographed, and analyzed with Bio-Profile software Bio-ID Version 99 (Vilber Lourmat, France).

RESULTS

Table 1. The seropositive rate, the PCR detection and the isolation of Chlamydia abortus from cows and does

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cows (non-aborted)</th>
<th>Aborted</th>
<th>Healthy (non-aborted)</th>
<th>Aborted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgG</td>
<td>345/672 (*)</td>
<td>45/63</td>
<td>4/24</td>
<td>65/112</td>
</tr>
<tr>
<td>Vaginalswab PCR</td>
<td>14/51(*)</td>
<td>22/63</td>
<td>7/18</td>
<td>24/112</td>
</tr>
<tr>
<td>Vaginalswab (Isolate)</td>
<td>0/14(*)</td>
<td>5/22</td>
<td>0/7</td>
<td>8/24</td>
</tr>
<tr>
<td>Aborted fetus (PCR)</td>
<td>NA(*)</td>
<td>1/12</td>
<td>NA</td>
<td>3/9</td>
</tr>
<tr>
<td>PCR</td>
<td>8.3%</td>
<td>33.3%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The serology and PCR detection of Chlamydia abortus from aborted cows and does

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aborted Cows (N=63)</th>
<th>Aborted Does (N=112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgG positive</td>
<td>20/63(*)</td>
<td>14/112</td>
</tr>
<tr>
<td>PCR positive</td>
<td>31.7%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Serum IgG positive</td>
<td>25/63</td>
<td>51/112</td>
</tr>
<tr>
<td>PCR negative</td>
<td>39.7%</td>
<td>45.5%</td>
</tr>
<tr>
<td>Serum IgG negative</td>
<td>2/63</td>
<td>10/112</td>
</tr>
<tr>
<td>PCR positive</td>
<td>3.17%</td>
<td>8.93%</td>
</tr>
<tr>
<td>Serum IgG negative</td>
<td>16/63</td>
<td>37/112</td>
</tr>
<tr>
<td>PCR negative</td>
<td>25.4%</td>
<td>33.0%</td>
</tr>
</tbody>
</table>

a) Because not all serologically tested healthy animals (Table 1) were swabbed and screened by PCR, the construction of a similar table is impossible for healthy animals.
b) Number positives / Total number of samples tested.

Agent isolation: Vaginal swabs and aborted fetuses were first screened for the MOMP gene by PCR, and PCR-positive samples were inoculated into the yolk sacs of 6 to 7-day-old SPF embryonated eggs. Inoculated eggs were candled twice a day, and yolk sac fluid was harvested from dead ones. On day 7 postinoculation, the yolk sac of surviving eggs was collected, and subcultured twice before being harvested.

Restriction fragment length polymorphism (RFLP) of MOMP gene: Chlamydia abortus strains and isolates used for RFLP are listed in Table 4. PCR products were analyzed with CfoI, HaeIII, NdeI, Stul and AluI (Promega, WI, U.S.A.). Upon complete restriction, CfoI should yield three fragments of 1025, 154 and 34 basepairs (bp), HaeIII and Stul two fragments of 842 and 367 bp, NdeI two fragments of 865 and 344 bp, and AluI 14 fragments of 411, 282, 102, 94, 85, 72, 41, 33, 32, 18, 16, 12, 7, and 4 bp, based on the C. psittaci 6BC strain sequence [4]. Approximately 1–2 µg DNA was incubated with 5 µl of restriction enzyme for 2 hr at 37°C, and the digested products were loaded in 2% agarose gel, photographed, and analyzed with Bio-Profile software Bio-ID Version 99 (Vilber Lourmat, France).

RESULTS

Table 1 summarizes the results of serology, PCR detection of agent from vaginal swabs and aborted fetuses, and agent isolation. A total of 735 sera from healthy (N=672) or aborted (N=63) cows, primarily Holstein, from 72 herds
in nine districts were collected from March 1999 through February 2000. A total of 136 sera from healthy (N=24) and aborted (N=112) does, primarily (Nubian × Saanen), of 11 flocks in four districts were collected during the same period. All herds (for cows)/flocks (for goats) in this study were seropositive. The seropositive rate was 51.3% for healthy and 71.4% for aborted cows; 16.7% for healthy and 58% for aborted does. Vaginal swabs were sampled from all aborted cows and does and from a portion of healthy animals, and screened with PCR for the presence of Chlamyphila abortus MOMP gene. The PCR detection rate was 45.2% for healthy and 34.9% for aborted cows; 38.9% for healthy and 21.4% for aborted does. PCR-positive vaginal swabs that were inoculated into chicken embryos, and the agent isola-
tion rates were 22.7 and 33.3% for aborted cows and does respectively, equivalent to isolation rates of 7.9 and 7.1% for aborted cows and does respectively. No isolate was obtained from healthy but PCR-positive individuals. The PCR detection rate for aborted fetuses was 8.3% for cattle obtained from healthy but PCR-positive individuals. The isolation rates were 22.7 and 33.3% for aborted cows and does respectively, equivalent to isolation rates of 7.9 and 7.1% for aborted cows and does respectively. No isolate was obtained from healthy but PCR-positive individuals. The PCR detection rate for aborted fetuses was 8.3% for cattle and 33.3% for goat.

The majority of aborted animals tested positive for either serum IgG or PCR (Table 2). Only 31.7% of aborted cows and 12.5% of aborted does tested positive for both serum IgG and PCR. However, 25.4% of aborted cows and 33.0% of aborted does tested negative for stat ELISA and PCR, suggestive of a multiple infection.

For a study of multiple infection (Table 3), sera from 20 healthy and 28 aborted does from two flocks with recent abortions in two districts were tested for IgG against abortigenic agents C. abortus, T. gondii, and N. caninum. Results showed that about 4.2% (2/48, row 1) of does had triple infections and other 14.6% (7/48, rows 2–4) of does had dual infections. N. caninum infection was found in four does, which were also coinfected with either C. abortus or T. gondii (rows 1 and 3). C. abortus was likely to be responsible for abortion in four does (row 5), and T. gondii apparently responsible for the abortion in 18 does in one flock (row 6). Some does were seronegative to all three agents (row 8).

Chlamydophila strains and isolates from different animal species (Table 4) showed distinct RFLP patterns when analyzed with CfoI (Fig. 1) and HaeIII, but not with NdeI, Stul, or Alul. CfoI yielded four basepair (bp) fragments of 401, 390, 234 and 184 for both cow and goat abortion isolates; a 1209 bp fragment for C. felis FEPN strain; and three bp fragments of 1025, 154 and 30 for C. psittaci avian strain as expected [4]. The RFLP patterns of C. pneumoniae TWAR were indistinguishable from those of C. psittaci avian strains when analyzed with all five enzymes. The CfoI restriction pattern of five additional cow isolates and eight goat abortion isolates (Table 1) was identical to that shown in Fig. 1.

The MOMP gene sequences of Taiwanese isolates were 98.9–100% homologous among themselves. Comparison of Taiwanese isolates with 12 abortion strains from ovine, bovine, and swine also revealed a 98.8–100% homology, wherein two consistent point variations found in all Taiwanese isolates and bovine LW508 strain clearly distinguished the Taiwanese isolates from a majority of other abortion strains (Table 5 and Fig. 2). One consistent variation at nt 343 from GCC to ACC, which changed the 115th amino acid from alanine to threonine, was located in a variable domain I. There were also 14 inconsistent point variations, six of which led to amino acid divergence (Table 5). Phylogenetic analysis (Fig. 2) showed that all C. abortus strains and isolates clustered in a group with four subgroups, distinct from the C. psittaci avian strains and C. felis FEPN strain.

**DISCUSSION**

A preliminary serological survey conducted by Dr. Fong at National Chung-Hsing University, showed the C. abortus seroprevalence rate among dairy cows to be >37.5%, suggesting that sampling of six to nine heads per herd is 99% confident for detecting at least one case [11]. This study confirmed the relatively high seroprevalence of C. abortus in healthy non-aborted (51%) and aborted (71%) cows. Some IgG may be induced by natural infection with C. abortus since a vaccine has not been used in this country. However, due to known cross-reactivity, some IgG detected may have been induced by C. pecorum [18] and thus the seroprevalence rate reported herein should have been overestimated. So far there has been no investigation on the situations surrounding the incidence of the C. pecorum infection in Taiwan’s ruminant population, it was thus difficult to estimate how much of the seroprevalence rate was contributed by C. pecorum. However, because 31.7% of aborted cows tested positive for both serum IgG and PCR (Table 2), and if this is used as a strict criterion for making etiologic diagnosis, it can be speculated that <68.3% of the seroprevalence may have been contributed by C. pecorum, and after correction with this percentile, the bona fide C. abortus seroprevalence rate should have been at least 22.6%. The seroprevalence rate of 16.7% (healthy non-aborted) and 58% (aborted) in does suggested that the C. pecorum coinfection in does was not as widespread as in cows.

It was interesting to see that the PCR detection rates, in both aborted cows and does, were lower than those in their healthy counterparts, while their agent isolate rates were higher (rows 2, 4, and 6 in Table 1). The reasons for these findings were not clear. However, it was suggested that the PCR detection was not necessarily correlated to agent isolation. The 7.1–7.9% isolation rate was roughly equivalent to stat 8.8% rate found in sheep in natural infection [13].

It was clear that triple or dual infection occurred in
aborted does (Table 3). However, for does, *T. gondii* appeared to be a stronger abortive agent than *C. abortus* (compared rows 5 with row 6) and *N. caninum* was coinfected and less prevalent (rows 1 and 3, Table 3). In a few cases, the causative agent was unknown. The multiple infection in cows was not clear, however, *N. caninum* infection of cows in Taiwan had been confirmed [15].

Previous RFLP studies indicated the power of *Alu* and *Hae*III discriminating *C. psittaci* isolates [3, 9]. Our study showed that *Cfo*I was most useful, while *Alu*I was not ideal because of the presence of too many fragments to be resolved in mini agarose gel.

The MOMP gene of the ruminant abortion isolates is similar worldwide, consistent with the results obtained in other studies [3, 7]. It was evident that Taiwanese isolates were phylogenetically closest to the *C. abortus* bovine LW508 strain and clustered within the same subgroup, while other abortion strains were clustered in the other three subgroups (Fig. 2). It was less likely that the abortion cases studied herein were transmitted from birds, because only 76–85% homology and a distinguishable RFLP pattern were found with *C. psittaci* avian strains (Figs. 1, 2), although an avian serovar strain of *C. psittaci* had been isolated from bovine abortion cases [2]. It seems unnecessary to prevent the contact between birds and ruminants for disease control.

In conclusion, the prevalence of *C. abortus* infection in domesticated ruminants in Taiwan is relatively high, and the MOMP gene of Taiwanese abortion isolates are phylogenetically closest to bovine abortion strain LW508. In aborted flocks, multiple infections with at least three agents, i.e. *C. psittaci*, *T. gondii* and *N. caninum*, occurred.

**REFERENCES**