# Serological Assays and Seroprevalence of Ruminant *Anaplasma Marginale* Infection in Taiwan

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**SUMMARY** *Anaplasma marginale,* and infectious agent, was successfully isolated from naturally infected cattle originating from a farm in northern Taiwan. Three serological tests, including complement-fixation test ( CF ), enzyme-linked immunosorbent assay ( ELISA ) and indirect fluorescent test ( IFA ) were developed to detect antibodies to *A. marginale* in ruminants. *A. marginale* organisms isolated from an infected calf were used as the CF, ELISA, and IFA antigens. There were no significant cross reactions between the antigens and the cattle hyperimmune sera produced by *Bebesia bigemina, Theileria sergenti,* and *Eperythrozoon wenyoni.* Comparative testiog among the 3 serological tests showed that the CF had the highest sensitivity and the IFA the least. The ELISA, as compared to CF, was found to have relative agreement and sensitivity of 94.4 and 89.1 %, respectively, whereas the relative agreement and sensitivity between the CF and IFA were 87.9 and 73.9 %, respectively. A serological survey was carried out to determine the prevalence of antibodies to *A. marginale* in cattle and goats in Taiwan using CF, ELISA, and IFA tests. The results indicate that the three tests had a high degree of agreement. Of the 915 cattle serum samples from 8 prefectures tested, 283 ( 30.9 % ) were positive. Collectively, all 8 prefectures had *A. marginale* antibodies. Among these, Taipei prefecture had the highest prevalence ( 62.3 % ), followed by Hsinchu ( 47.5 % ), Taoyuen ( 43.2 % ), Miaoli ( 35.8 % ), Taichung ( 16 % ), Pingtung ( 12.5 % ), Tainan ( 11.8 % ), and Taitung ( 2.7 % ). In goats, only two of the 4 prefectures submitting serum samples had *A. marginale* antibodies with prevalence of 7 and 4 %, respectively. [* Su JF, Hsu FS. Serological assays and seroprevalence of ruminant *Anaplasma marginale* infection in Taiwan. J Chin Soc Vet Sci 19 (2) : 79-89, 1993. * Corresponding author TEL : 02-6212111, FAX : 02-6225345 ]

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## INTRODUCTION

Anaplasmosis ( Ana ) is an infectious and transmissible disease of cattle by the intra-erythrocytic rickettsia, *Anaplasma spp.*[9,17] The disease has a worldwide distrbuttion, having been reported from Africa, South America, Central America, North America, the Middle East, Most European Countries, Australia, New Zealand, and Southeast Asia. The disease

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was not recorded in Taiwan of the Republic of Chian until 1942, when Ana was diagnosed on a small cattle farm. Since then, the Prevalence of Ana among cattle has markedly increased in many areas of Taiwan and has caused severe economic losses.[3] However, the exact morbidity and mortality of Ana infection in Taiwan still remain unknown; therefore, an island-wide survey of the antibodies to *A. marginale* is of value.

The disease is characterized by anemia, icterus, abortion, reduced milk production, temporary infertility in bulls, profound weight loss, and death.[9].

The genus *Anaplasma* comprises three species, *A. marginale, A. centrale* and *A. ovis. A. marginales* and *A. centrale* are infectious for cattle, *A. ovis* for sheep and goats. The organisms are gram-negative and appear as dense, round dark-blue structures 0.3-1.0 $\mu$m in diameter lying near the margin ( *A. marginale* and *A. ovis* ) or in the center ( *A. centrale* ) of red blood cells.[7,17] Cattle are refractory to *A. ovis* as sheep are to *A. marginale.*

Anaplasmosis may be transmitted by ticks such as *Dermacentor* and *Boophilus microplus,* blood sucking flies, needles and other instruments contaminated with the infected blood[9,17]. Young calves are susceptible to infection but relatively resistant to the disease, while increasing severity occurs in a dult cattle.[9]. All breeds and types of cattle are susceptible.[9].

The complement fixation test ( CF )[3,5], an immunoglobulin G ( IgG ) enzyme-linked immunosorbent assay ( ELISA )[6,8,14,16,18,19], and the indirect fluorescent antibody test ( IFA ) have been used for the serodiagnosis of *A. marginale* infection.

It was our purpose to isolate *A. marginale,* to develop the three serological tests mentioned above in measuring antibodies against *A. marginale,* to compare the relative specificity and sensitivity of these 3 procedures and to determine the incidence of *A. marginale* illness in cattle and goats in Taiwan.

## MATERIALS AND METHODS

**Animals** The calves, 4-6 months old, were purchased locally for this study. These animals were negative for Ana by clinical and blood smear examinations. They were splenectomized 2 weeks before inoculation with *A. marginale*-infected blood and kept in an isolate during the period of the study.

**Isolation and purification of *A. marginale*** Twenty whole blood samples were collected from a herd in Hsinchu prefecture. Infection of these animals had been previously demonstrated by CF and IFA tests and by microscopic examination of a thin blood smear stained with Giemsa. All blood samples were placed into bottles containing Alsever's anticoagulant solution. The blood was colled with ice packs and transported 120 km to an infectious disease transmission facility. Each animal received 250 mL of whole blood. Observations were made at least once a day after transfusion. Records were kept each day of rectal temperature, food consumption and general appearance. Blood samples were collected daily from each calf for hematological analysis and serological examination. Blood smears were stained by the Giemsa's method for determining percentage of *A. marginale* infection.

After an incubation period of 9 days, the infected calves were febrile and had a parasetimia with combined *Anaplasma sp, Theileria sp,* and *Eperythrozoon sp* infection in cattle.

At the peak of parasitemia, 200 mL of blood was obtained from the jugular vein of the infected calves in citrate phosphate dextrose ( ACD ) solution. The blood was inoculated intravenously into 2 goats for purification of *A. marginale* as previously described.[7] After 14 days, another 2 calves were subsequently transfused from the goats.

**Identification of *A. marginale* isolates.** *Anaplasma* isolates were determined by the CF procedures.[3,5] The reference antigen ( *A. marginale* ) was obtained from Dr. Tetsuo Minami ( Japan ).

**Complement fixation ( CF ) test** An *A. margi-*

*nale* isolate from this study was used. The method for preparation of antigen was similar to that described previously.[3,5]. Splenectomized calves were inoculated intravenously with *A. marginale*- infected blood, and at the peak of parasitemia, blood was collected in a sodium citrite solution. The plasma and buffy coat were removed after centrifugation at 600 Xg for 10 min at room temperature. The packed RBC were suspended in phosphate buffered saline ( PBS, 0.01 M, pH 7.2 ), washed and centrifuged at 3500-4000 rpm, at 25 ℃ for 10 min in PBS dilutent. This was repeated 3 times. The washed RBC were resuspended in PBS to the original whole blood volume. The RBC suspension was lysed by the nitrogen cavitation method cell disruption bomb; parrinstrument, USA ). The hemolysed solution was diluted with PBS ( pH 7.2 ), washed and centrifuged at 8000 rpm for 30 min at 4 ℃ three times. Te supernatant was aspirated and discarded. The pellet was resuspended with PBS ( pH 7.2 ), sonicated ( at 20 kc ) for 10 min and centrifuged at 8000 rpm for 30 min and the pellet discarded. The supernatant fluid was used as the CF antigen and was stored at −70 ℃.

A microplate CF test was performed by a modified method[3,5], using 2 units of antigen ( 1 : 5 ). The test serum was diluted 1 to 5 with VBS ( veronal buffer soluton, pH 7.2 ) and inactivated at 56 ℃ for 30 min. Twenty-five μL of the test serum was transferred to a microtiter plate. Twenty-five μL of the 2 units of antigen and 50 μL of the 2 units of guinea pig complement were added to each well containing the test serum. They were mixed and incubated at 4 ℃ for 16-20 h. This was followed by the addition of 50 μL of hemolysins ( 2 units of hemolysin and an equal volume of 2.8 % sheep RBC ). The solution was mixed and incubated at 37℃ for 30 min. Visual reading of over 75 % preventing hemolysis at 1 : 4 was considered positive in this test.

**Enzyme-linked immunosorbent assay ( ELISA )**

The method for preparing *A. marginale* antigen was similar to that reported previously[16,18,20]. At the peak of parasitemia ( > 30 % RBC infected with *A. marginale* ), 24 mL of the infected blood was collected and placed into beakers containing 6 mL of acid citric dextrose ( ACD ) solution. The plasma and buffy coat were removed after centrifugation at 200 rpm for 10 min at room temperature. the packed RBC were suspended in 9 mL of potassium-free buffer saline ( PBS, pH 7.2 ) in one centrifuge tube, washed, and centrifuged at 2000 rpm at 25℃ for 10 min in PBS dilutent. This was repeated 3 times. The washed RBC were suspended in PBS to the original whole blood volume. The RBC suspension was then mixed with twice its volume ( 30 mL ) of distilled water to lyse the RBC. The preparation was made isotonic by adding 15 mL of 5-time concentrated PBS solution. The suspension was centrifuged at 8000 rpm at 4 ℃ for 30 min. The supernatant was aspirated and discarded. The pellet was sonicated at 20 kc and resuspended in PBS. The suspension was used as an antigen in the ELISA. Frozen antigen was thawed at 37℃ and centrifuged at 11,000 rpm at 4 ℃ for 30 min. The supernatant was diluted 800 times swith coating buffer ( 0.015 M $Na_2$ $CO_3$, 0.003 M $NaHCO_3$ [ pH 9.4 ] ) immediately before use.

A commercial conjugate of horseradish peroxide-conjugated goat anti-swine IgG, heavy and light chains ( Jackson immuno research laboratories ), was used in the detection of cattle *A. marginale* antibodies. The conjugate was stored at − 20 ℃ and was diluted with PBS immediately before use. The optimal dilutions of conjugate were determined by the checkerboard titration method. A 1 : 8000 dilution of the conjugate was used. The substrate used in the ELISA was a commercial ( ophenylene-diamine, Sigma P-1256 ) chromogen ( OPD ) system.

The ELISA procedure was conducted as described previously[8,16,18,20]. ELISA 96-well microtiter plates ( Nunc Laboratories, Roskilde, Denmark ) were coated with the partially purified *A. marginale* antigen. Coating was achieved by adding 100 μL of antigen dilued to 1 : 800 in coating buffer to each well. The plates were covered with cellophane tape and incubated at 4℃ in a humidified atmosphere for 16 h.

The plates were emptied by shaking them over a sink and washing them 3 times with phosphate buffered saline plus 0.005 % Tween 20 ( PBS-Tween 20, Merck laboratory, pH 7.2 ). The plates were emptied and 250 μL of a 0.5 % bovine serum albumin ( BSA ) PBS was added to each well. The plates were then incubated at 37℃ for 1 h.

The test serum had been inactivated and treated with glutaraldehyde-treated RBC ( GTE ). Three successive rinses of the wells with 0.5 % PBS-Tween 20 were followed by the addition of 100 μL of a 1 : 100 dilution of test sera to the *A. marginale* antigen-coated wells and incubated at 37℃ for 1 h. The microplate wells were again washed 3 times with PBS-Tween 20, and 100 μL of horseradish peroxidase-conjugated goat anticattle IgG, diluted at 1 : 8000 in PBS, was added to each well.

Plates were incubated at 37℃ for 1 h and washed with PBS-Tween 20 as previously described. A total of 100 μL volume of OPD substrate was added after 30 min at room temperature and in darkness with 50 μL 2 N-$H_2$ $SO_4$; then the preparation in each well was measured at A490 nm by a Multislan ELISA plate reader ( Dynatech laboratiries ).

**Indirect fouorescent antibody ( IFA ) test** The IHA test was conducted according to that described by Sonia[19]. blood was collected when the calf developed 10 % parasitemia and the blood placed in an ACD solution. The infected RBC were washed twice in 0.1 M glycine buffer ( pH 3.0; centrifuged at 1000 xg for 15 min at 4 ℃ ) to remove adherent antibodies. The erythrocytes subsequently were washed once in PBS ( pH 7.4 ). The buffer coat was removed after each wash. Cold PBS containing 1.75 % bovine serum albumin ( BSA ) was then added in a volume equal to that of the washed packed cells. Thin blood films made on precleaned glass slides were air dried and fixed with acetone for 25 min. These were stored at −70℃ until use.

The technique was conducted according to that described by Sonia[19]. Serrial twofold dilutions of test sera in PBS starting at 1 : 40 were made in 96-well microtiter plates. Ten ul of each dilution was added to each well coated with *A. marginale*. The slides were incubated in a humidified 37℃ incubator for 30 min and rinsed 3 times in PBS containing 0.002 % Tween 20. After the slides were blotted to near dryness, 10 μL of flourescent-conjugated goat anti-bovine IgG diluted 1 : 200 in PBS was added to each well. the slides were incubated. washed and blotted as previously described, and a small drop of mounting fluid ( 70 % glycerol in PBS pH 8.9 ) was placed in each well. Cover slips were added, and the slides were viewed with a fluorescent microscope.

**Comparison of CF, ELISA and IFA tests** Forty-six field sera from infected herd were tested by the CF, ELISA and IFA procedures.

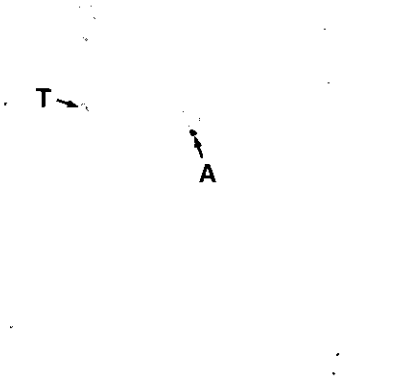
Specificity of the CF, ELISA and IFA tests Sera obtained from cattle infected with the following organism : *B. bigemina, T. sergenti* and *A. marginale* provided by Japan[7]. were tested for cross reactions using CF, ELISA and IFA antigens prepared in this study.

Serological survey on the prevalence of *A. marginale* infection in Taiwan.

Detection of *A. marginale* antibodies was accomplished by CF, ELISA and FA. A total of 915 sera were obtained from abult cattle in 8 prefectures including Taipei, Taoyuan, Miaoli, Taichung, Tainan, Pingtung, and Taitung; an additional 500 sera were obtained from adult goats in 4 prefectures including Taipei, Taoyuan, Pingtung, and Taitung. These sera were stored at −70℃ for serological tests.

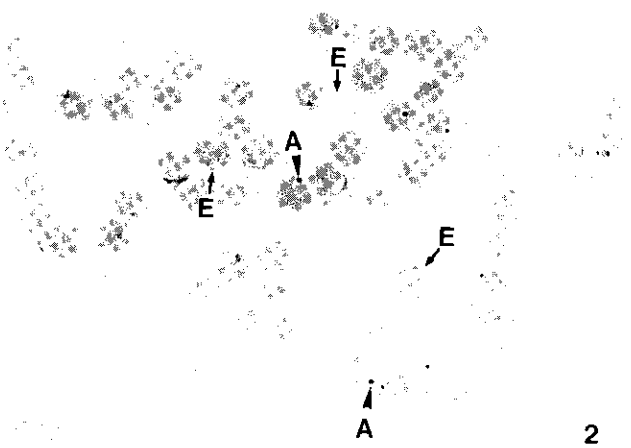
## RESULTS

**Isolation and identification of *A. marginale*** *A. marginale* was successfully isolated from a naturally infected cattle herd located in Hsinchu prefecture. However, a combined *A. marginale, Theilera sergenti* and *Eperythrozoon sp* infection in adult cattle was commonly observed ( Figs 1, 2 and 3 ), when the blood from a combined infection was inoculated intravenously into 2 goats and subsequently transfused to calves, a pure *A. marginale* organism (Fig 4) was isolated from intravenously inoculated



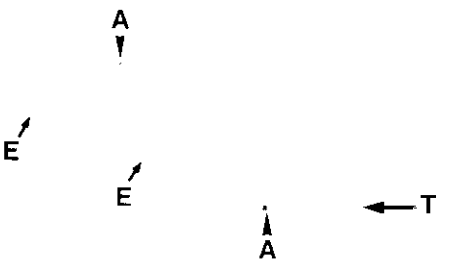

1

Fig 1 A blood film obtained from a splenectomized calf that was inoculated with infected blood collected from a herd in Hsinchu prefecture. Two typical parasites, *A marginale* and *T. sergenti*. were seen in red blood cells. 8 days postinfection, Giemsa staining, × 1000.



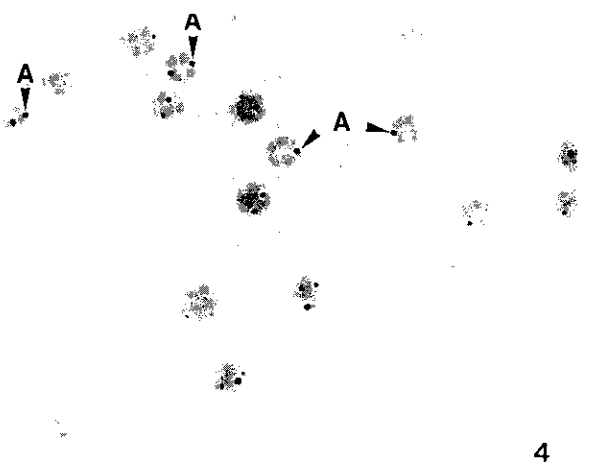

2

Fig 2 A blood film obtained from a splenectomized calf that was inoculated with infected blood collected from a berd. Two typical parasles, *A. marginale* and *Eperythrozoon sp,* were seen in red blood cells. 23 days postinfection, Giemsa staining, ×1000.




3

Fig 3 A blood film obtained from a splenectomized calf that was inoculated with infected blood collected from a herd, Three typical parasites. *A.marginale, T. sergenti* and *Eperythrozoon sp.* were seen in red blood cells. 26 days postinfection Giemsa staining × 1000.




4

Fig 4 Pure *A.marginale* was obtained when the blood from a combined *A. marginale. T. sergenti* and *Eperythrozoon sp* infection was inoculated intravenously into 2 goats and subsequently transfused to a calf.

calves 6 weeks postinoculation ( PI ).

The organisms isolated from field cases were identified as *A. marginale* by CF procedures and by microscopic examination. It was Gram negative and appeared as a dense round dark-blue structure 0.3 − 1.0 $\mu$ m in diameter lying near the margin of RBC.

**Specificity of the CF, ELISA and IFA tests** Cross-reactivity was not observed between the CF, ELISA and IFA *A. marginale* antigens and antisera to *B. bigemina, T. sergenti,* and *E. wenyoni.* ( Table 1 ) Additionally, no antibody against *A. marginale* was detected in the serum samples from the 20 cattle that were negative for *A. marginale* by the CF and IF tests. However, these antigens reacted with the reference antiserum provided by Dr. T. Minami.

**Comparison of the CF, ELISA and IFA tests** Of the 46 sera from cattle with various degrees of parasitemia tested ( Table 2 ), 37 ( 80.4 % ), 36 ( 78.3 % ), and 33 ( 71.7 % ) were positive by the CF, ELISA and IHA procedures, respectively.

When the CF test was considered standard, the ELISA and IFA had a relative sensitivity of 94.4 and 87.6 %, relative specificity of 70 and 38.5 %, and relative agreement of 89.1 and 73.9 %, respectively.

**Serological survey for the prevalence of *A. marginale* infection in Taiwan** The prevalence of *A. marginale* infection in cattle and goats is shown in Table 3. Table 3 indicates that the three tests had a high degree of agreement. Of the 915 serum samples from 8 prefectured tested by CF procedure, 283 ( 30.9 % ) were positive. Collectively, all 8 prefectured had *A. marginale* antibodies. Among these, Taipei prefecture had the highest prevalence ( 62.3 % ), followed by Hsinchu ( 47.5 % ), Taoyuen ( 43.2 % ), Miaoli ( 35.8 % ), Taichuug ( 16 % ), Pintung ( 12.5 % ), Tainan ( 11.8 % ) and Taitung ( 2.7 % ). In goats, only 2 of the 4 prefctures submitting serum samples had *A. marginale* antibodies with prevalence of 7 and 4 %, respectively.

# DISCUSSION

The morphology and antigenicity of the isolated organism, *A. marginale,* were identical to those reported previously[1,11,16].

On many occasions, a mixed *A. marginale, T. sergenti* and *E. wenyoni* infection in cattle was observed. The relationships among the 3 intra-erythrocytic parasites are still unknown. There are conflicting reports of interference with experimentally induced *Babesia* and *Eperythrozoon* infections[12,13].

According to established criteria for determining sensitivity and specificity, the CF test had the highest sensitivity and specificity and the IFA the least. The results are somewhat different from those erported previously[6,16]. Both Liu[6] and Nakamura[16] reported that ELISA was more sensitive than the CF or IFA tests.

It is knowm that *A. marginale* and *A. centrale* mutually cross react in CF, ELISA and IFA tests. It would be possible to distinguish the infections with *A. marginale* and *A. centrale* by CF and ELISA utilizing both *Anaplasma* antigens[16].

The cross-reactivity between *Anaplasma* and *Eperythrozoon* as reported previously [14,16] might be due to antibodies against erythrocyte material in the *A. marginale* antigen, or there are some common antigens in the 3 intraerythrocytic parasites.

The prevalence of *A. marginale*-infected cattle in Taiwan in this study was relatively lower than that reported previously[3,5]. Previous reports indicated prevalence rates of 34.2 % in 1966[1], 72-80 % in 1984[3] and 65 % in 1987[5]. The decrease in prevalence of Ana infected cattle might be due to the changes of farming system. This is supported by the present study that the majority of cattle in northern Taiwan were kept in pastures, while those in the southwestern area were confined in feedlots. The incidence of *A. marginale*-infected goats in the present study was relatively low; this might be due to the result of lower infection in goats, or goats might be resistant to *A. marginale.*

**Table 1 Specificity of the CF, ELISA and IFA antigens against various hyperimmune sera to 4 intraerythrocytic parasites of cattle**

| Antigen-test | Hyperimmune sera to[a] | | | | |
|---|---|---|---|---|---|
| | Am | Bb | Ep | Ts | C |
| CF ( T ) | 80 | < 5 | < 5 | < 5 | < 5 |
| ELISA | 0.783 | 0.212 | 0.207 | 0.207 | 0.078 |
| IFA | +++ | ± | ± | ± | ± |
| CF ( J ) | 80 | < 5 | < 5 | < 5 | < 5 |

[a] Am = A. *marginale*, Bb = *B. bigemina*, Ep = *Eperythrozoon sp* Ts = T. *sergenti*, C = Control
[b] CF (T) = CF antigen, using the isolated A. *marginale*
ELISA = ELISA antigen, using the isolated A. *marginale*
IFA = IFA antigen, using the isolated A. *marginale*
CF = CF antigen provided by Dr. T. Minami (Japan)
[c] ELISA OD values
[d] The CF titers of > 5 were considered positive

**Table 2 Comparison of the CF, ELISA and IFA tests in the detection of antibodies to A. *marginle*[a]**

| | | | ELISA | | IFA | |
|---|---|---|---|---|---|---|
| | | | + | − | + | − |
| CF | + | 37 | 34 | 3 | 29 | 8 |
| | − | 9 | 2 | 7 | 4 | 5 |
| | Subtotal | 46 | 36 | 10 | 33 | 13 |

Calculation :
Sensitivity %, 34 / 36 = 94.4 29 / 33 = 87.9
Specificity %, 7 / 10 = 70.0 5 / 13 = 38.3
Agreement %, 41 / 46 = 89.1 34 / 46 = 73.9

[a] The CF, ELISA, ane IFA antigen, using the isolated A. *marginale*, were used

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**Table 3 Prevalence of antibodies detected by CF in cattle serum in Taiwan**

| Animal | Prefecture | No. of sera examined | No (%) of sera positive | | |
|---|---|---|---|---|---|
| | | | CF (%) | ELISA (%) | IFA (%) |
| Cattle | Taipei | 117 | 73 ( 62.3 ) | 66 ( 56.4 ) | 53 ( 45.3 ) |
| | Hsinchu | 120 | 56 ( 47.5 ) | 50 ( 41.6 ) | 41 ( 34.3 ) |
| | Taoyuen | 148 | 64 ( 43.2 ) | 54 ( 36.4 ) | 47 ( 31.7 ) |
| | Miaoli | 120 | 43 ( 35.8 ) | 39 ( 32.5 ) | 31 ( 25.8 ) |
| | Taichung | 100 | 16 ( 16.0 ) | 18 ( 18.0 ) | 11 ( 11.0 ) |
| | Pingtung | 120 | 15 ( 12.0 ) | 14 ( 11.6 ) | 11 ( 9.1 ) |
| | Tainan | 110 | 13 ( 11.8 ) | 2 ( 19.1 ) | 9 ( 8.2 ) |
| | Taitung | 80 | 3 ( 2.5 ) | 2 ( 2.5 ) | 2 ( 2.5 ) |
| | Total | 915 | 183 ( 30.9 ) | 264 ( 28.8 ) | 205 ( 22.4 ) |
| Goat | Taipei | 200 | 14 ( 7.0 ) | 0 | 0 |
| | Taoyuen | 100 | 4 ( 4.0 ) | 0 | 0 |
| | Pingtung | 100 | 0 | 0 | 0 |
| | Taitung | 100 | 0 | 0 | 0 |
| | Total | 500 | 18 ( 0.04 ) | 0 | 0 |


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# 反芻動物邊蟲病之血清診斷技術與血清疫情調查

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**摘要** 本研究旨在分離與鑑定牛邊蟲 ( *Anaplasma marginale* )，開發血清診斷法，包括補體結合反應 ( CF )，間接螢光抗體法 ( IFA ) 與間接酵素結合免疫吸附法 ( ELISA )，以及應用上述方法調查台灣地區牛與羊邊蟲病之感染情形。本研究自台灣地區疑邊蟲病患牛血液中，成功地分離到牛邊蟲。該分離到之病原體，經標準血清鑑定證實爲 *A. marginale*。應用分離到之 *A. marginale*，分別研製 CF、ELISA 與 IFA 等三種抗原，該等抗原經與 *A. marginale* 標準血清，*B. bigemina*, *T. sergenti* 與 *E. wenyoni*陽性血清，以及標準陽性血清進行交叉試驗，證實均具高度特異性。爲瞭解上述 CF、ELISA與 IFA 等三種方法之敏感性與一致性，應用牛邊蟲污染場之血清，進行測試。其結果爲 CF 對 ELISA 之相對敏感性與一致性，分別爲 94.4 與 89.1 %；另 CF 對 IFA 之相對敏感性與一致性，分別爲 87.9 與 73.9 %。本試驗以 CF、ELISA 與 IFA 法調查台灣地區牛隻邊蟲病之感染情形，顯示上述三種方法測試之結果大致相似。以 CF 法測試結果如下：牛邊蟲病之陽性爲 30.9 %，所有 8 個受檢縣，均見牛邊蟲之污染，8 個陽性縣中，以台北縣之陽性率爲最高 ( 62.3 % )，其他依次爲新竹縣 ( 47.5 % )，桃園縣 ( 43.2 % )，苗栗縣 ( 35.8 % )，台中縣 ( 16 % )，屏東縣 ( 12.5 % )，台南縣 ( 11.8 % ) 與台東縣 (2.7 % )。另調查台北、桃園、屏東與台東等四縣羊隻 500 頭，結果 CF 陽性縣有台北 ( 7 % ) 與桃園 ( 4 % ) 等二縣。〔*蘇杰夫、徐興鎔。反芻動物邊蟲病之血清診斷技術與血清疫情調查。中華獸醫誌 19 (2) : 79-89, 1993. *聯絡人 TEL 02-6212111 , FAX 02-6225345〕

***關鍵詞：反芻動物，邊蟲病，血清診斷技術，血清疫學調查***