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2012 年臺灣馬匹馬傳染性貧血之血清盛行率調查

Seroprevalence of Equine Infectious Anemia in Horses of Taiwan in 2012

駱君樺

Chun-Hua Luo

指導教授：吳應寧 博士

Advisor: Ying-Ling Wu, Ph.D.

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中文摘要

馬傳染性貧血(EIA)是由反轉錄病毒科(Retroviridae)慢病毒亞科(Lentivirinae)之病毒所引起的馬科動物傳染病。受感染的馬匹會終身帶原，因此血清學檢驗結果為陽性的馬匹就認定為已受感染。此病為 OIE listed disease，典型臨床症狀會出現高熱、貧血、體重喪失。於懷孕母馬可能會造成流產，且病毒也能經由胎盤傳染給胎兒。大多數感染此病的馬匹可能呈現無臨床症狀或臨床症狀不明顯的隱性帶原或慢性感染狀態。但都有可能會反覆的發生嚴重臨床症狀甚至導致死亡。此病自 2001 年在台灣進行 EIA 的調查後，10 年來台灣地區未曾再進行過相關調查。因此本研究將針對台灣地區馬匹 EIA 進行調查，以期彌補十年來相關研究不足之憾。本實驗中所採集的 217 個血清樣本中，分別為臺灣北部(台北、桃園、新竹)121 匹、中部(苗栗、台中)41 匹、南部(高雄、屏東)35 匹和東部(花蓮、台東)20 匹，採集全臺馬場共 23 場。以酵素免疫分析法(Enzyme-linked immunosorbent assay，ELISA)檢測血清樣本之 EIA 抗體，217 匹皆為陰性反應。自 1992 年的調查報告全台有 2.1%的血清陽性率後，已連續 20 年調查無陽性結果。可知我國在馬傳染性貧血檢疫上極成功，但未來還是需要持續的監控與嚴格檢疫才能保持成果。

關鍵字:臺灣、流行病學、馬、傳染性貧血、血清盛行率

ABSTRACT

The Equine infectious anemia (EIA) is an infectious disease caused by the Equine infectious anemia virus, a lentivirus belonging to the Retroviridae family. Infected horses will become carriers for life; therefore any horse with a positive result of serological test will be identified as infected. It's typical clinical signs include high fever, anemia, and weight loss. Abortion may occur if pregnant mares are infected, and the virus can infect the fetus via the placenta. Most horses are infected with EIA with no obvious clinical signs as latent carriers, or in chronic stage with mild clinical signs. Both of latent carriers and chronically infected horses can show repeated severe clinical signs, even ending up with death. EIA serological test is necessary in the import and export of horses. There has been no investigation of EIA in Taiwan in the past ten years ever since 2001. In this study we took all 217 blood samples from the northern region (Taipei and Taoyuan, Hsinchu), the central region (Miaoli, and Taichung), the southern region (Kaohsiung and Pingtung), and the eastern region (Hualien and Taitung). Samples were tested by enzyme-linked immunosorbent assay (ELISA), and there is no positive result. It's been 20 years we have no positive result in Taiwan since 1992. It can be seen as a huge success of our EIA elimination strategies, but still need to continuously investigate in the future.

Key words: Taiwan, Epidemiology, Horse, Equine infectious anemia, Seroprevalence

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Chapter 1 Introduction

Equine infectious anemia (EIA), also known as swamp fever, has been documented in numerous geographical areas and is considered a worldwide disease that occurs only in the family Equidae including horses[32]. Equine Infectious Anemia Virus (EIAV) is an RNA virus that is a member of the Retroviridae family and the lentivirus genus [37]. Equine Infectious Anemia was first described in France by veterinarians in 1843 as an infectious disease of horses [24]. The causative agent of EIA was first identified as a 'filterable agent' in 1904, making EIA one of the first animal diseases to be assigned a viral etiology[11]. For years, information regarding the molecular biology of EIAV was limited by the lack of a tissue culture system [27]. Development of in vitro systems and production of viral particles led to the classification of EIAV as a member of the Retroviridae family and enabled biochemical and molecular studies [6].

The major focus of measures to control EIA has been the development of regulatory policies that involve the identification and elimination of EIAV-infected horses [32] Equine infectious anemia is mechanically transmitted by arthropod vectors or unsterile medical instrument. The main route of transmission is by hematophagous arthropod of the Tabanidae family. The virus is carried on the mouthparts of the horsefly or deerfly[16].

Currently, there is no specific treatment for EIA. Although there are some vaccines used in China, no USDA approved vaccine exists for EIA. Therefore, the control program in place is to limit EIA prevalence by detecting infectious horses and removing them[32].

Equine infectious anemia is an OIE-listed disease that must be tested when a horse

is imported or exported. Even though considered by some less economically important than cows, pigs, and chickens in Taiwan, horses are still animals of economical importance, and therefore, we analyzed the epidemiology of EIA among horses in the areas. This study is the first time to carry out on the large serological study of EIA prevalence in Taiwan in the past decade.

Chapter 2 Literature Review

2.1 Etiology

Equine infectious anemia virus is a single stranded, positive-sense RNA virus that belongs to Retroviridae family and is closely related to maedi visna virus (MVV), caprine arthritis-enceohalitis virus (CAEV), bovine immunodeficiency virus (BIV), feline immunodeficiency virus (FIV) simian immunodeficiency virus (SIV), and human immunodeficiency virus (HIV)[32].

The virions are irregular-spherical and enveloped with glycoprotein, which make the surface appear rough. The nucleoids are isometric, concentric and rod-shaped. The viral RNA contains about 8200 base pair, the EIAV RNA genome is the smallest and genetically simplest lentiviral genome. The genome contains three major genes to make the structural proteins (gag, pol, env) [30,34], and three open reading frames encoding the tat and rev proteins generally present in lentiviruses and the S2 protein[24].

Virus attaches to the target cells by the specific interaction of the viral envelope glycoproteins and cellular receptor proteins contained in the plasma membrane [38]. After getting inside the target cell, the virus reverse transcript the single-stranded RNA genome to double-stranded DNA, then transported to the nucleus of the cell. After getting inside the nucleus of the cell, the proviruses DNA incorporate into cellular chromosomes. The proviruses DNA express, transcript, assemble into a virus, then budding out to shed the virus [33].

2.2 Epidemiology

EIAV has been diagnosed in many areas of the world and is considered a

worldwide disease of Equidae. The incidence of EIAV-infected horses is the highest in tropical and sub- tropical climates, presumably due to the longer warm seasons and more abundant populations of insect vectors that may transmit EIAV among horses [20].

During the past 20 years, the EIA infection rate reported by the USDA has dropped from about 4% to less than 0.2%. But these testing results do not reflect the general horse population. There are less than 10% of the horses in the United States are tested for EIA [32]. In Brazil, the infection rate in Pantanal's wild horse herds has been as high as 30% [14]. A report of Netherlands is 0.066% in 2011[36], and another report of Sultanate of Oman is 0.67% in 2011[4]. In France, the incidence of EIAV infection is low, with only 11 cases being detected between 1988 and 1992 but several outbreaks have developed recently. In 1993 and 1994, a total of 59 animals coming from 17 herds were teste seropositive for EIAV [17]. In Taiwan EIA had been investigated in 1990, and the prevalence is 2.1% among 751 horses[2]. Another investigation in 2001 the results are all negative of all 726 samples, and at least 595 horse serum samples were gathered in 1999. The total population in Taiwan is 624 horses in 1998[1].

Although the probability of EIAV infection by insect vectors is greatest during seasons that are warm, infections can occur throughout the year via mechanical transfers of blood by hypodermic needles and other veterinary instruments. Sexual transmission of EIAV has not been demonstrated to date [32].

In 1970, a reliable serological test based on agar gel immunodiffusion (AGID) using the p26 antigen of EIAV was developed [9]. The AGID test is an efficient way of detecting infected animals. It has been qualified as the standard diagnostic for EIAV infection by the United States Department of Agriculture (USDA) and AGID is the only test officially recognized by the Office International des Epizooties (OIE). It's the gold stander of EIA diagnosis [24].

2.3 Clinical Signs

The clinical response of horses following artificial inoculation or natural exposure to EIAV is variable and depends in part on host resistance factors, viral virulence factors, environmental factors, the virus strain, and the dose [22].

Clinical signs may be severer in horses than in donkeys and mules. While the infection, donkeys do not develop clinical EIA, and lower amounts of plasma viral nucleic acids are observed in donkeys compared to ponies infected with the same strain of EIAV. Viral RNA levels were observed in ponies 10,000 fold higher than in the donkeys infected with the same strain during the first 20 days of infection [10].

In general, EIAV infections can be apparent with distinctive clinical symptoms or inapparent without any clinical signs of EIA. Although the distinctions are not absolute the clinical disease is typically described as three different stages acute, chronic, or asymptomatic [18].

Initial exposure to a virulent strain usually results in an acute disease characterized by hyperthermia with a severe decrease of the platelet number. The animal may die because of the clinical signs of the acute or chronic disease, but in many cases these signs are relatively mild that will be overlooked. The acute episode usually ends within a few days, and then the animal enters the chronic stage of disease characterized by the recurrence of clinical cycles. Acute EIA signs include fever, thrombocytopenia, lethargy, and inappetence, and small percentage of horses can have a severe and fatal form of disease characterized by persistent viremia, severe anemia, and high loads of virus in most organs[12]. Chronic EIA is characterized by recurrence of signs including anemia, thrombocytopenia, weight loss and dependent edema.

Pale mucous membranes, petechiation, icterus, and epistaxis can be observed with

severe hemolytic and thrombocytopenia[32]. Neurologic signs like ataxia, and encephalitis occasionally develop[28].

There are more than one pathogeneses of thrombocytopenia and anemia in EIA infection. In experimentally infected horses suggests an increase in platelet-bound antibody, and immune-mediated erythrocyte destruction episode of thrombocytopenia and anemia. RBC life span is also reduced in EIAV-infected animals [32].

2.4 Diagnosis

Equine infectious anemia should be suspected in horses with compatible clinical signs that reside in an area of virus activity, and insect vector existing. According to clinical signs, there are many pathogens or causes should be differential from Equine infectious anemia, and list of differential diagnoses depends on the predominant clinical signs[23].

Anemia: Chronic infections or inflammation, Streptococcal infection, Corynebacterium pseudotuberculosis, ehrlichiosis, babesiosis, dourine, surra, trypanosomiasis, gastrointestinal parasitism. Thrombocytopenia: Equine viral enteritis, Africa horse sickness, endotoxemia, salmonellosis, ehrlichiosis, Potomac horse fever, enteric clostridiosis, babesiosis, trypanosomiasis. Ventral body part edema: Pleuritis, pericarditis, thrombophlebitis, bacterial endocarditis, equine herpes virus, equine viral arteritis, purpura hemorrhagica, Corynebacterium pseudotuberculosis, anthrax, mycobacterial infection, endotoxemia, ehrlichiosis, piroplasmosis, dourine, surra, trypanosomiasis, gastrointestinal parasitism. Petechial hemorrhages: equine viral arteritis, purpura hemorrhagica, endotoxemia, septicemia, bacteremia, salmonellosis, ehrlichiosis, Potomac horse fever, piroplasmosis, trypanosomiasis, surra[23,32].

Diagnostic confirmatory tests are made by serologic assays such as agar gel

immunodiffusion (AGID), and competitive enzyme-linked immunosorbent assay (cELISA)[31]. The AGID test was developed in 1970s, and been widely accepted. This test has become the “gold standard” for the diagnosis of EIAV infections [19]. The ELISA was a more sensitive assay and it decreased the disease eradication period. In an outbreak of EIA that occurred in Ireland in 2006, ELISA and immunoblotting were found to be more sensitive than AGID in detecting acute cases of EIA[13].

Test that detect and quantitate EIAV in blood are not generally used clinically, but in research settings. These tests are sensitive and specific, including animal inoculation, virus titration, and real-time RT-PCR[32].

2.5 Treatment

There is no specific treatment for Equine infectious anemia and treatment is supportive; with assistance given for minimizing stress, providing good nursing care, and giving nonsteroidal anti-inflammatory drugs (NSAIDs) leg wraps and hydrotherapy for dependent edema, and blood transfution for severe anemia and thrombocytopenia[32]. Because they will exacerbate viremia and clinical disease, corticosteroids are contraindicated[35].

EIA does not cause immunodeficiency, but high levels of viremia can induce transient immunosuppression[29]. Therefore antibiotics would be used to prevent secondary bacterial infection and decrease the mortality of Equine infectious anemia during febrile episodes[32].

2.6 Prevention

There is currently no effective vaccine for the prevention of EIAV infection. The primary challenge in developing an effective EIAV vaccine is overcoming the antigenic

diversity intrinsic to this virus. The development of an efficacious vaccine against lentiviral infections remains a high priority in human and veterinary medicine[24].

An attenuated live EIAV vaccine with a reported protection efficacy of about 70% has been used in China since the early 1980s, but the effectiveness of this vaccine remains to be confirmed outside of that country[26]

The transmission of EIAV infection has been controlled by improving animal husbandry techniques to prevent the spread of infected blood, by reducing the horsefly population in the vicinity of herds, never reusing needles, and good biosecurity with any medical instrument[26]. EIA is a World Organisation for Animal Health (OIE) listed disease. The United States Department of Agriculture (USDA) control program of EIA is designed to maintain low prevalence by detecting EIAV infected horses. They test horses in following situations, imported, entered into exhibitions or competitive events, moved interstate, changed ownership, entered auction or sales markets[32]. In Taiwan, there is no regular detect. There are few requests for horses imported into Taiwan to prevent EIA; the horse imported must come from a farm without EIA outbreak for at least one year, the horse should take the AGID test and tested negative 15days before import.

Chapter 3 Material and Methods

3.1 Sample Size and Selection of Horses

There are approximately 30 registered riding schools and facilities that raise horses in Taiwan; in total, approximately 1000 horses are housed. Samples were obtained from horses from 23 chosen horse fields from March to July, 2012, which represented approximately 500 horses. Sample size was calculated using the following formula:

$$N = Z^2(P)(1-P)/E^2$$

where N was the required sample size, Z was the critical value for the desired confidence level (1.96), P was the expected prevalence of test results (0.5), and E was the maximum tolerable error for the confidence interval (0.05). The confidence interval was set at 95%. A sample size of 384 horses was calculated.

Because this large sample size was impractical to use, a second formula was used with the known population to narrow the sample size. The new sample size was calculated by the following formula:

$$New\ SS = SS/[\ 1+[(SS-1)/POP]]$$

where New SS was the new sample size, SS was the sample size calculated before, and POP was the population. A new sample size of 217 horses was calculated.

A randomized sampling method based on the number of horses per field was used. Horses with a recent history of untreated infectious disease or outward signs of clinical disease were excluded.

3.2 Blood Samples

Sera were collected from the jugular vein from 217 horses, each from 1 of the 4 pre-defined geographic regions: the northern region (Taipei, Hsinchu and Taoyuan, 121 samples), the central region (Miaoli, and Taichung, 41 samples), the southern region (Kaohsiung and Pingtung, 35 samples), and the eastern region (Hualien and Taitung, 20 samples). Blood samples were stored in blank blood collection tubes, contain EDTA. All of the samples were stored at -20℃ until ELISA test processing.

3.3 Putative Risk Factors

Putative risk factors were assessed using questionnaires for all horses, including questions on origin, breed, gender, age, and past medical or transport history. Horses were classified by age (0–5 years, 5–10 years, 10–15 years, 15–20 years, and >20 years), origin (place of birth or importation, such as Europe, America, Australia/New Zealand, or Taiwan), and type of activity (indoor vs. outdoor pleasure riding). Other risk factors for the horses were also recorded, including frequency of grooming and inspection, contact with other non-equine animals, management of the facilities (including frequency of environmental disinfection).

3.4 Equine Infectious Anemia ELISA

3.4.1 General Information

The IDEXX cELISA EIA test (IDEXX Laboratories, Inc., Maine, USA) was used to detect antibodies to EIAV. Specifically, the IDEXX cELISA EIA test can detect exposure in any species that generates an antibody response to the nucleoprotein, regardless of the EIAV strain or antigenic variance between species. The IDEXX

cELISA EIA test with purified EIA antigen and monoclonal antibodies to p26 was used to reduce nonspecific reactions commonly found in ELISA tests. Importantly, the correlation between the IDEXX EIA cELISA and the EIA agar gel immunodiffusion (AGID) assay was greater than 99 %.

3.4.2 Descriptions and Principle

The assay was performed on 96-well plates that were coated with the monoclonal antibody specific for p26, which is a major core protein and the major group-specific antigen of EIAV. The p26 antigen was conjugated to horseradish peroxidase (HRPO). Upon incubation of the test sample with the HRPO-conjugated p26 antigen in the coated wells, serum antibodies specific for p26 form a complex with the coated antigen, and compete with the microtiter plate-bound anti-p26 monoclonal antibodies for the enzyme–linked purified p26 antigen. After washing away unbound material, peroxidase substrate is added to each well. When antibodies to EIAV p26 antigen are present in a serum sample, the HRPO-linked p26 antigen is blocked from binding to the precoated monoclonal antibody on the wells, and little or no color in the positive sample wells results. In negative samples, the HRPO-linked p26 antigen is free to bind to the monoclonal antibody on the plastic wells, and a strong blue color is displayed. Color is inversely related to the levels of antibody to EIAV. The colors in the test sample wells were compared to the positive control wells to yield a final assay interpretation.

3.4.3 Instruments

The IDEXX EIA cELISA is mainly applied to horses to examine whether they are infected with EIA. However, it can also be used on other Equidae animals to detect the existence of antibody for EIAV (as mentioned above). The following materials were

used:

One EIAV p26 antigen coated 96-well plate; one bottle EIA positive control preserved with sodium azide; one bottle EIA negative control preserved with sodium azide; one bottle HRPO-linked EIAV p26 antigen preserved with thimerosal; one bottle TMB Substrate; one bottle Stop Solution; distilled or deionized water for plate washing; precision pipettes and tips suitable for delivering 50- and 100-µL aliquots for loading samples and controls; wash bottle or 96-well plate washer for plate washing; 37℃ incubator; 96-well plate reader equipped with 620–650 nm filters.

3.4.4 Preparation of Sample

Frozen samples were thawed and thoroughly mixed prior to use by inverting the tube several times. The presence of turbidity or visible indication of bacterial growth (clear serum turned cloudy) can interfere with the performance and accuracy of the test. Hemolyzed, lipemic, or bacterially contaminated serum can cause erroneous results.

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3.4.5 Procedure of ELISA

Reagents were used at room temperature (18℃–25℃) and mixed by inverting and swirling. The procedures of the ELISA are as following:

The antigen-coated plate was obtained, and the sample position was recorded on a worksheet. Some wells were reserved for the positive and negative controls. Initially, 100 µL of undiluted Positive Control or Negative Control was dispensed into the reserved wells. Then, 100 µL of serum sample was dispensed into the appropriate wells. The EIAV antigen conjugate (50 µL) was aliquoted into all the wells and the plate was

incubated for 30 min (±2 min) at 37℃. Each well was thoroughly washed by hands or plate washer, with approximately 350 μL of deionized water 3–5 times. Improper washing can produce nonspecific color development and no color difference between the negative and positive controls due to sample pollution. The TMB substrate solution (100 μL) was then dispensed into each well and thoroughly mixed by gently tapping the holder 10 times. The plate was then incubated for 15 min (±1 min) at room temperature (18℃–25℃). Finally, the stop substrate solution (100 μL) was dispensed into each well to stop the reaction, and the absorbance values at wavelength 650 nm were measured.

3.5 Statistical Analyses

Statistical analyses were performed using SAS version 9.1 (SAS Institute, NC, US). Relationships between the risk factors and ELISA results were analyzed using the PROC frequency procedure. Chi-square tests were used to determine influence of putative risk factors on the seroprevalence of EIAer. The level of significance was set at $P < 0.05$.

Chapter 4 Results

According to the manufacturer's instructions for the IDEXX EIA cELISA assay, the controls must appear as follows: negative control should be dark blue and the positive control should be light blue. The positive control must have an optical density (O.D.) at a wavelength 650 nm (OD650) of ≥0.150. The positive control must be ≤70% of the negative control OD650. The O.D. of the positive samples must be less than the positive control and the O.D. of the negative samples must be greater than the positive control.

Little or no color change in the test sample indicates the presence of antibodies to EIAV in the serum. Positive test results need to be verified by an EIA AGID assay.

All of samples analyzed were negative for the EIA antibody (Table 1).

The sampled horses included geldings, mares, and stallions. Some horses were imported from Europe, America, Australia, or New Zealand, while some were born in Taiwan. The characteristics of the sampled horses are summarized in Table 2 and Table 3.

Table 1. Results of EIA test in Taiwan measured by ELISA

	Northern	Central	Southern	Eastern
Sample size	110	52	35	20
Positive numbers	0	0	0	0
Positive rate (%)	0	0	0	0

Table 2. Horse demographics

	Gelding	Mare	Stallion
Numbers of horses	161	44	12

Table 3. Age of horses

Age	<5	5–10	10–15	15–20	>20
Numbers of horses	9	46	78	59	25

Chapter 5 Discussion and Conclusion

Horses have long been among the most economically important animals to humans. Although their importance has declined as a result of mechanization, they are still involved in human lives worldwide in various ways. However, in Taiwan, horses are less economically important compared to other animals such as cows, pigs, and chickens. As a result, horse populations are comparatively small in Taiwan; there were less than 1200 horses in Taiwan in 2011 according to the Council of Agriculture, Executive Yuan's investigation. In Taiwan, horses are not seen as production animal, and therefore, they are often overlooked in epidemic prevention and investigation. Most horses in Taiwan were imported from other countries. Imported horses must come from a farm that has not had an EIA outbreak for at least 1 year and must take the AGID test and be negative 15 days before import. However, after the horse has passed customs there is no regular detection of any horse diseases. The main purpose of this study was to determine the EIA prevalence rate to aid government institutions in epidemic prevention policy formulation.

The most common method to diagnose EIA involves a serological test. Other tests that detect and quantities EIAV in blood are not generally used clinically, but in research settings. These tests are sensitive and specific, including animal inoculation, virus titration, and real-time RT-PCR[32] Once a horse is infected with EIA, it remains infectious for the remainder of its life [7] and therefore, the best way to control EIA is to detect and remove the carrier. There are many ways to detect the antibody of EIAV, such as AGID, ELISA, and western blot. Nowadays, the most common methods to diagnose EIA are AGID and ELISA. The AGID test and ELISA are accurate, reliable tests of EIA

detection in horses [25], except when used to analyze horses in the early stages of infection and foals infected by dams. Other rare situation that lead to false results may occur when the level of virus in circulation during an acute episode of the disease is sufficient to bind most, but not all, of the available antibody and if the initial antibody levels never raise high enough to be detectable [21]. The ELISA detects antibodies earlier and at lower concentrations than the AGID test. However, the AGID test is the gold standard of EIA diagnosis, and therefore, when ELISAs are positive they need to be confirmed using the AGID test because false-positive results have been noted with ELISAs [3].

The occurrence of EIA is known worldwide [3]. , and it was documented in Hong Kong in 1972 with a prevalence rate of 23%. After disease control measures were implemented, the spread of infection was reduced, and in 1976, the prevalence of EIA was believed to be sufficiently low [8]. In Taiwan, EIA was investigated in 1990, and the prevalence rate was 2.1% among 751 horses [2]. An investigation in 2001 identified no positive results in the 726 samples [1]. In the 1990s, EIA was appears to have been eradicated in Taiwan. However, all the previous investigations were performed using AGID tests, and the imported horses are only tested by AGID tests. Furthermore, a long time has passed since the previous investigation, and therefore, this study is of importance to the current health of horses in Taiwan. In addition, the ELISA detects antibodies earlier and at lower concentrations than AGID tests, and therefore, we used ELISAs in this investigation.

In Brazil, from 1992 to 1993 [15]. , a high incidence of EIA was detected in a herd using AGID and ELISA tests. Subsequently, the horses that had a positive result from the AGID test were removed. A total of 470 sera were tested by ELISA and AGID during the study from a herd of 86 horses. In 96.6% of the cases, the results from both

methods agreed. All of the 28 animals that had positive results in both methods were immediately sacrificed. The 16 animals that were positive only by ELISA were isolated and became positive in both tests when retested a month later. No animal was positive by AGID only. When all surviving animals gave negative results twice by both methods, the herd was regarded as free of infection and the study was discontinued [15]. The study highlights the ability of ELISAs to detect EIA infection much earlier than the AGID test, and no false-positive ELISA results were obtained.

Another study in Romania in 2008 showed that the serological test results of EIA may differ from time to time because the asymptomatic stage of disease can decrease the levels of antibodies [5]. Therefore, ELISAs can increase the likelihood of identifying carriers that may have been missed since the investigation in Taiwan in 2001. In the previous study, 23 horses that were detected as EIA infected by AGID test between 1 and 7.5 years ago were retested by ELISA. The other 5 horses that were never infected by EIA were used as negative controls. In the retest, 3 horses were negative, excluding the 5 controls. The first horse was identified as negative in a serological test at 7.5 years, the second at 6.5 years, and the third at 1 year after the original test. This may be due to the transition of these horses into an asymptomatic stage of infection, which has no circulating virus or antibodies, at the moment of testing. If retested at a later time, these horses may present positive EIAV reactions regardless of the test used [5].

We performed ELISAs in this study owing to the aforementioned advantages of this technique. In conclusion, none of the 217 blood samples from horses from all over the island resulted in a positive EIAV reaction. In 20 years, we have not had a positive result in Taiwan, which can be seen as a huge success of our EIA elimination strategies.

REFERENCES

1. 張詠樂. 台灣地區馬病毒性疾病之血清學檢測. 台灣大學獸醫學研究所論文 1991*.
2. 楊揚輝, 林文華, and 邱仕炎. 台灣地區馬傳染性貧血症的抗體調查. 中華獸醫誌 18: 33-34, 1992.
3. Manual of diagnostic tests and vaccines for terrestrial animals : mammals, birds, and bees. World Organisation for Animal Health, Paris, 2008.
4. Body M, Al-Rawahi A, Hussain MH, Al-Lamki K, Al-Habsy S, Almaawali M, and Alrawahi QA. Sero-Survey of Equine Infectious Anemia in the Sultanate of Oman during 2007-2009. Pak Vet J 31: 235-238, 2011.
5. Bolfa P. MS, C. Catoi, M. Taulescu, A. Gal, V.I. Rus, Niculae Mihaela. The relation between coggins test and ELISA in the diagnosis of equine infectious anemia. Buletin USAMV Veterinary Medicine 65: 2008.
6. Charman HP, Bladen S, Gilden RV, and Coggins L. Equine infectious anemia virus: evidence favoring classification as a retravirus. Journal of virology 19: 1073-1079, 1976.
7. Cheevers WP, and McGuire TC. Equine infectious anemia virus: immunopathogenesis and persistence. Reviews of infectious diseases 7: 83-88, 1985.
8. Coggins L, and Auchnie JA. Control of equine infectious anemia in horses in Hong Kong. Journal of the American Veterinary Medical Association 170: 1299-1301, 1977.
9. Coggins L, Norcross NL, and Nusbaum SR. Diagnosis of equine infectious anemia by immunodiffusion test. American journal of veterinary research 33: 11-18, 1972.
10. Cook SJ, Cook RF, Montelaro RC, and Issel CJ. Differential responses of Equus caballus and Equus asinus to infection with two pathogenic strains of equine infectious anemia virus. Veterinary microbiology 79: 93-109, 2001.
11. Craigo JK, and Montelaro RC. Equine Infectious Anemia Virus Infection and Immunity: Lessons for Aids Vaccine Development. Future virology 6: 139-142, 2011.
12. Crawford TB, Mcguire TC, Cheevers WP, and Anderson PK. Equine Infectious-Anemia - Persistent Retravirus of Horses. J Supramol Str Cell 239-239, 1978.
13. Cullinane A, Quinlivan M, Nelly M, Patterson H, Kenna R, Garvey M, Gildea S, Lyons P, Flynn M, Galvin P, Neylon M, and Jankowska K. Diagnosis of equine infectious anaemia during the 2006 outbreak in Ireland. The Veterinary record 161: 647-652, 2007.
14. Davila AM, Souza SS, Campos C, and Silva RA. The seroprevalence of equine trypanosomosis in the pantanal. Memorias do Instituto Oswaldo Cruz 94: 199-202, 1999.
15. dos Reis JK, Melo LM, Rezende MR, and Leite RC. Use of an ELISA test in the eradication of an equine infectious anaemia focus. Tropical animal health and production 26: 65-68, 1994.
16. Foil LD, Adams WV, McManus JM, and Issel CJ. Bloodmeal residues on mouthparts of Tabanus fuscicostatus (Diptera: Tabanidae) and the potential for mechanical transmission of pathogens. Journal of medical entomology 24: 613-616, 1987.

17. Hans A, Poncon N, and Zientara S. Epidemiology of Equine Infectious Anemia in France and in Europe from 1994 to 2011. B Acad Vet France 165: 27-34, 2012.
18. Issel CJ, and Coggins L. Equine infectious anemia: current knowledge. Journal of the American Veterinary Medical Association 174: 727-733, 1979.
19. Issel CJ, and Cook RF. A review of techniques for the serologic diagnosis of equine infectious anemia. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 5: 137-141, 1993.
20. Issel CJ, and Foil LD. Studies on equine infectious anemia virus transmission by insects. Journal of the American Veterinary Medical Association 184: 293-297, 1984.
21. Issel CJ, Scicluna MT, Cook SJ, Cook RF, Caprioli A, Ricci I, Rosone F, Craigo JK, Montelaro RC, and Autorino GL. Challenges and proposed solutions for more accurate serological diagnosis of equine infectious anaemia. The Veterinary record 172: 210, 2013.
22. Kemeny LJ, Mott LO, and Pearson JE. Titration of equine infectious anemia virus. Effect of dosage on incubation time and clinical signs. The Cornell veterinarian 61: 687-695, 1971.
23. Lavoie J-P, Hinchcliff KW, and Brown CM. Blackwell's five-minute veterinary consult : equine. Wiley-Blackwell, Ames, Iowa, xxxvii, 874 p., 2008.
24. Leroux C, Cadore JL, and Montelaro RC. Equine Infectious Anemia Virus (EIAV): what has HIV's country cousin got to tell us? Veterinary research 35: 485-512, 2004.
25. Lew AM, Thomas LM, and Huntington PJ. A comparison of ELISA, FAST-ELISA and gel diffusion tests for detecting antibody to equine infectious anaemia virus. Veterinary microbiology 34: 1-5, 1993.
26. Mahy BWJ, and Van Regenmortel MHV. Desk encyclopedia of animal and bacterial virology. Academic, Oxford, 2010.
27. Malmquist WA, Barnett D, and Becvar CS. Production of equine infectious anemia antigen in a persistently infected cell line. Archiv fur die gesamte Virusforschung 42: 361-370, 1973.
28. McClure JJ, Lindsay WA, Taylor W, Ochoa R, Issel CJ, and Coulter SJ. Ataxia in four horses with equine infectious anemia. Journal of the American Veterinary Medical Association 180: 279-283, 1982.
29. Newman MJ, Issel CJ, Truax RE, Powell MD, Horohov DW, and Montelaro RC. Transient suppression of equine immune responses by equine infectious anemia virus (EIAV). Virology 184: 55-66, 1991.
30. Noiman S, Yaniv A, Sherman L, Tronick SR, and Gazit A. Pattern of transcription of the genome of equine infectious anemia virus. Journal of virology 64: 1839-1843, 1990.
31. Pearson JE, and Gipson CA. Standardization of Equine Infectious-Anemia Immunodiffusion and Celisa Tests and Their Application to Control of the Disease in the United-States. J Equine Vet Sci 8: 60-61, 1988.
32. Sellon DC, and Long MT. Equine infectious diseases. Saunders/Elsevier, St. Louis, Mo., xvii, 653 p., 2007.
33. Steagall WK, Robek MD, Perry ST, Fuller FJ, and Payne SL. Incorporation of uracil into viral DNA correlates with reduced replication of EIAV in macrophages. Virology 210: 302-313, 1995.
34. Telesnitsky A. Retroviruses: Molecular Biology, Genomics and Pathogenesis.

Future virology 5: 539-543, 2010.
35. Tumas DB, Hines MT, Perryman LE, Davis WC, and McGuire TC. Corticosteroid immunosuppression and monoclonal antibody-mediated CD5+ T lymphocyte depletion in normal and equine infectious anaemia virus-carrier horses. The Journal of general virology 75 (Pt 5): 959-968, 1994.
36. van der Kolk JH. Reintroducing Veterinary Quarterly. The Veterinary quarterly 31: 1, 2011.
37. van Regenmortel MH, Mayo MA, Fauquet CM, and Maniloff J. Virus nomenclature: consensus versus chaos. Archives of virology 145: 2227-2232, 2000.
38. Zhang B, Jin S, Jin J, Li F, and Montelaro RC. A tumor necrosis factor receptor family protein serves as a cellular receptor for the macrophage-tropic equine lentivirus. Proceedings of the National Academy of Sciences of the United States of America 102: 9918-9923, 2005.

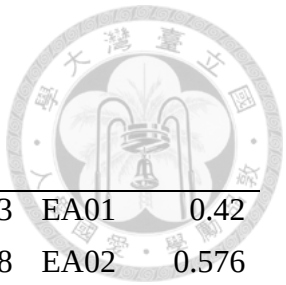
APPENDIX

The O.D. at a wavelength of 650 nm of samples

NA01	0.336	NB02	0.51	NE07	0.389	NG08	0.364	NH18	0.385
NA02	0.337	NB03	0.468	NE08	0.482	NG09	0.352	NH19	0.402
NA03	0.432	NB04	0.636	NE09	0.416	NG10	0.384	NH20	0.375
NA04	0.314	NB05	0.625	NE10	0.388	NG11	0.425	NH21	0.384
NA05	0.308	NC01	0.432	NE11	0.408	NH01	0.476	NH22	0.341
NA06	0.409	NC02	0.383	NE12	0.39	NH02	0.407	NI01	0.403
NA07	0.414	NC03	0.323	NE13	0.423	NH03	0.403	NI02	0.431
NA08	0.466	NC04	0.338	NE14	0.282	NH04	0.36	NI03	0.377
NA09	0.484	ND01	0.54	NE15	0.396	NH05	0.361	NI04	0.364
NA10	0.405	ND02	0.589	NF01	0.371	NH06	0.356	NI05	0.393
NA11	0.47	ND03	0.494	NF02	0.38	NH07	0.402	NI06	0.388
NA12	0.403	ND04	0.597	NF03	0.432	NH08	0.296	NI07	0.43
NA13	0.385	ND05	0.476	NF04	0.374	NH09	0.386	NI08	0.376
NA14	0.381	ND06	0.568	NF05	0.372	NH10	0.354	NI09	0.294
NA15	0.356	ND07	0.496	NG01	0.391	NH11	0.349	NI10	0.395
NA16	0.327	NE01	0.351	NG02	0.373	NH12	0.364	NI11	0.321
NA17	0.373	NE02	0.281	NG03	0.343	NH13	0.325	NJ01	0.509
NA18	0.338	NE03	0.237	NG04	0.407	NH14	0.343	NJ02	0.575
NA19	0.396	NE04	0.265	NG05	0.322	NH15	0.387	NJ03	0.607
NA20	0.39	NE05	0.391	NG06	0.397	NH16	0.671	NJ04	0.626
NB01	0.59	NE06	0.379	NG07	0.34	NH17	0.447	NJ05	0.629

positive controls were 0.153 & 0.193

negative controls were 0.442 & 0.447



The O.D. at a wavelength of 650 nm of samples

NK01	0.597	MB04	0.473	ME01	0.513	SC01	0.603	EA01	0.42
NK02	0.574	MB05	0.591	ME02	0.533	SC02	0.528	EA02	0.576
NK03	0.53	MB06	0.558	ME03	0.617	SC03	0.575	EA03	0.605
NK04	0.546	MB07	0.535	ME04	0.593	SC04	0.579	EA04	0.64
NK05	0.457	MB08	0.498	ME05	0.519	SC05	0.624	EA05	0.388
MA01	0.548	MB09	0.68	ME06	0.637	SC06	0.681	EA06	0.409
MA02	0.647	MB10	0.635	ME07	0.659	SC07	0.69	EA07	0.464
MA03	0.65	MB11	0.591	ME08	0.579	SC08	0.545	EA08	0.467
MA04	0.591	MC01	0.576	ME09	0.567	SC09	0.686	EA09	0.5
MA05	0.63	MC02	0.649	ME10	0.663	SC10	0.703	EA10	0.533
MA06	0.432	MC03	0.524	ME11	0.624	SC11	0.501	EA11	0.52
MA07	0.609	MC04	0.517	SA01	0.487	SC12	0.652	EB01	0.603
MA08	0.526	MC05	0.36	SA02	0.475	SC13	0.493	EB02	0.535
MA09	0.611	MD01	0.487	SA03	0.662	SC14	0.666	EB03	0.409
MA10	0.593	MD02	0.569	SA04	0.441	SD01	0.36	EC01	0.376
MA11	0.632	MD03	0.533	SA05	0.647	SD02	0.493	EC02	0.786
MA12	0.585	MD04	0.558	SA06	0.497	SD03	0.739	EC03	0.409
MA13	0.552	MD05	0.673	SA07	0.643	SD04	0.509	EC04	0.562
MA14	0.514	MD06	0.63	SA08	0.781	SD05	0.58	EC05	0.392
MA15	0.538	MD07	0.597	SB01	0.576	SD06	0.645	EC06	0.576
MB01	0.434	MD08	0.656	SB02	0.441	SD07	0.657		
MB02	0.563	MD09	0.536	SB03	0.608	SD08	0.353		
MB03	0.525	MD10	0.573	SB04	0.488	SD09	0.467		

positive controls were 0.17 & 0.197

negative controls were 0.402 & 0.422

The information of the sampling horses

馬場編號 NA

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NA01	8	Belgium	No	Neutered	22
NA02	3	Holland	No	Female	13
NA03	3	USA	No	Neutered	14
NA04	10	Holland	No	Neutered	19
NA05	4	New Zealand	No	Neutered	20
NA06	3	Holland	No	Neutered	11
NA07	5	Belgium	No	Neutered	20
NA08	7	New Zealand	No	Neutered	15
NA09	5	Australia	No	Neutered	9
NA10	6	Australia	No	Neutered	19
NA11	2	Belgium	No	Neutered	14
NA12	8	New Zealand	No	Neutered	14
NA13	10	Germany	No	Neutered	19
NA14	9	Taiwan	No	Neutered	10

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NA15	4	New Zealand	No	Female	9
NA16	12	Japan	No	Neutered	22
NA17	8	Taiwan	No	Neutered	9
NA18	4	Australia	No	Neutered	17
NA19	5	Germany	No	Female	9
NA20	12	Taiwan	No	Female	13

The information of the sampling horses

馬場編號 NB

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NB01	3	New Zealand	No	Female	21
NB02	4	Belgium	No	Neutered	20
NB03	13	Germany	No	Neutered	19
NB04	6	Germany	No	Neutered	10
NB05	6	Germany	No	Neutered	15

The information of the sampling horses

馬場編號 NC

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NC01	4	Australia	No	Neutered	23
NC02	4	USA	No	Female	23
NC03	6	Taiwan	No	Neutered	13
NC04	8	Australia	No	Neutered	23

The information of the sampling horses

馬場編號 ND

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
ND01	11	Germany	No	Neutered	27
ND02	3	Germany	No	Neutered	17
ND03	4	Belgium	No	Neutered	17
ND04	3	Australia	No	Neutered	20
ND05	8	Germany	No	Neutered	17
ND06	3	Germany	No	Neutered	11
ND07	3	Germany	No	Neutered	19

The information of the sampling horses

馬場編號 NE

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NE01	12	Germany	No	Neutered	22
NE02	5	Holland	No	Neutered	7
NE03	5	Germany	No	Neutered	17
NE04	4	Holland	No	Neutered	Unknown
NE05	5	Germany	No	Neutered	17
NE06	7	Germany	No	Neutered	Unknown
NE07	2	Unknown	No	Male	Unknown
NE08	5	Germany	No	Neutered	20
NE09	5	Germany	No	Neutered	9
NE10	6	Germany	No	Neutered	Unknown
NE11	5	Unknown	No	Neutered	13
NE12	8	Holland	No	Female	Unknown
NE13	4	Holland	No	Neutered	12
NE14	4	Holland	No	Male	13

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NE15	5	Unknown	No	Neutered	24

The information of the sampling horses

馬場編號 NF

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NF01	10	Belgium	No	Neutered	17
NF02	4	Germany	No	Neutered	16
NF03	5	Germany	No	Neutered	12
NF04	4	Germany	No	Female	12
NF05	6	France	No	Neutered	12

The information of the sampling horses

馬場編號 NG

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NG01	3	Germany	No	Neutered	15
NG02	26	USA	No	Neutered	34
NG03	3	USA	No	Neutered	8
NG04	6	France	No	Neutered	18
NG05	7	Germany	No	Neutered	19
NG06	2	Germany	No	Female	16
NG07	6	Germany	No	Neutered	18
NG08	5	Germany	No	Neutered	18
NG09	6	Germany	No	Neutered	20
NG10	4	Germany	No	Female	17
NG11	3	Germany	No	Neutered	15

The information of the sampling horses

馬場編號 NH

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NH01	10	New Zealand	No	Neutered	26
NH02	20	Australia	No	Female	29
NH03	17	Taiwan	No	Neutered	18
NH04	2	New Zealand	No	Female	12
NH05	3	Australia	No	Neutered	15
NH06	8	Australia	No	Neutered	19
NH07	10	France	No	Neutered	23
NH08	10	Belgium	No	Neutered	23
NH09	7	Germany	No	Neutered	18
NH10	4	USA	No	Neutered	12
NH11	11	USA	No	Neutered	16
NH12	4	France	No	Neutered	15
NH13	11	Holland	No	Neutered	17
NH14	12	New Zealand	No	Neutered	16

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NH15	8	Australia	No	Neutered	18
NH16	7	Taiwan	No	Neutered	7
NH17	8	Belgium	No	Neutered	23
NH18	10	Australia	No	Neutered	19
NH19	11	USA	No	Neutered	17
NH20	10	Australia	No	Neutered	22
NH21	10	Germany	No	Neutered	21
NH22	3	Australia	No	Neutered	18

The information of the sampling horses

馬場編號 NI

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NI01	15	Germany	No	Neutered	26
NI02	15	USA	No	Neutered	21
NI03	21	Taiwan	No	Neutered	21
NI04	14	Taiwan	No	Female	14
NI05	8	Unknown	No	Neutered	21
NI06	Unknown	Unknown	No	Female	21
NI07	20	USA	No	Female	21
NI08	15	USA	No	Neutered	21
NI09	5	USA	No	Female	13
NI10	Unknown	Unknown	No	Neutered	Unknown
NI11	20	USA	No	Neutered	26

The information of the sampling horses

馬場編號 NJ

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NJ01	4	Australia	No	Neutered	20
NJ02	6	Holland	No	Neutered	21
NJ03	6	Holland	No	Neutered	21
NJ04	1	New Zealand	No	Neutered	10
NJ05	1	New Zealand	No	Neutered	5

The information of the sampling horses

馬場編號 NK

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
NK01	Unknown	Taiwan	No	Neutered	Unknown
NK02	Unknown	Taiwan	No	Neutered	Unknown
NK03	Unknown	Taiwan	No	Male	Unknown
NK04	Unknown	Taiwan	No	Neutered	Unknown
NK05	Unknown	Taiwan	No	Female	Unknown

The information of the sampling horses

馬場編號 MA

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
MA01	2	Holland	No	Neutered	7
MA02	5	Holland	No	Female	11
MA03	7	Taiwan	No	Male	7
MA04	8	Holland	No	Neutered	18
MA05	6	USA	No	Neutered	13
MA06	13	Taiwan	No	Male	13
MA07	4	Holland	No	Female	13
MA08	15	Finland	No	Neutered	19
MA09	4	USA	No	Female	10
MA10	8	Holland	No	Neutered	15
MA11	6	USA	No	Neutered	11
MA12	15	USA	No	Neutered	31
MA13	6	Holland	No	Neutered	15
MA14	3	Holland	No	Neutered	15

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
MA15	8	Holland	No	Neutered	19

The information of the sampling horses

馬場編號 MB

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
MB01	5	Germany	No	Neutered	17
MB02	5	Belgium	No	Neutered	21
MB03	21	Taiwan	No	Neutered	21
MB04	5	Unknown	No	Neutered	12
MB05	15	Taiwan	No	Female	15
MB06	6	USA	No	Neutered	21
MB07	4	Germany	No	Neutered	11
MB08	5	Taiwan	No	Neutered	5
MB09	2	Holland	No	Neutered	21
MB10	10	Taiwan	No	Neutered	10
MB11	2	Germany	No	Neutered	19

The information of the sampling horses

馬場編號 MC

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
MC01	2	Unknown	No	Male	11
MC02	3	Belgium	No	Neutered	16
MC03	1	Unknown	No	Neutered	5
MC04	1	Australia	No	Male	3
MC05	1	Unknown	No	Female	3

The information of the sampling horses

馬場編號 MD

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
MD01	7	Taiwan	No	Female	7
MD02	3	USA	No	Neutered	15
MD03	2	Germany	No	Neutered	14
MD04	3	Holland	No	Neutered	12
MD05	7	Holland	No	Female	16
MD06	4	Taiwan	No	Neutered	4
MD07	5	Germany	No	Female	8
MD08	1	Germany	No	Neutered	14
MD09	12	Taiwan	No	Neutered	12
MD10	11	Taiwan	No	Female	11

The information of the sampling horses

馬場編號 ME

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
ME01	4	Belgium	No	Neutered	17
ME02	Unknown	New Zealand	No	Neutered	14
ME03	Unknown	New Zealand	No	Neutered	14
ME04	Unknown	New Zealand	No	Neutered	19
ME05	6	Taiwan	No	Male	6
ME06	3	Unknown	No	Neutered	21
ME07	Unknown	Holland	No	Neutered	21
ME08	5	New Zealand	No	Neutered	17
ME09	Unknown	Unknown	No	Neutered	6
ME10	4	New Zealand	No	Neutered	13
ME11	Unknown	New Zealand	No	Female	16

The information of the sampling horses

馬場編號 SA

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
SA01	2	Germany	No	Neutered	9
SA02	1	Holland	No	Neutered	14
SA03	1	USA	No	Neutered	11
SA04	6	USA	No	Neutered	13
SA05	1	Germany	No	Neutered	14
SA06	3	Holland	No	Neutered	15
SA07	2	Holland	No	Neutered	8
SA08	13	Taiwan	No	Neutered	13

The information of the sampling horses

馬場編號 SB

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
SB01	12	Taiwan	No	Female	12
SB02	20	Australia	No	Neutered	29
SB03	2	USA	No	Neutered	14
SB04	8	Taiwan	No	Neutered	8

The information of the sampling horses

馬場編號 SC

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
SC01	＞1	USA	No	Female	10
SC02	＞1	Unknown	No	Neutered	16
SC03	＞1	USA	No	Female	11
SC04	＞1	Unknown	No	Neutered	14
SC05	＞1	France	No	Neutered	13
SC06	＞1	Unknown	No	Neutered	12
SC07	＞1	Taiwan	No	Neutered	8
SC08	＞1	USA	No	Neutered	11
SC09	＞1	USA	No	Neutered	11
SC10	＞1	Germany	No	Neutered	11
SC11	＞1	Belgium	No	Neutered	16
SC12	＞1	USA	No	Neutered	11
SC13	＞1	USA	No	Neutered	11
SC14	＞1	USA	No	Neutered	11

The information of the sampling horses

馬場編號 SD

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
SD01	3	England	No	Female	10
SD02	2	Unknown	No	Female	7
SD03	3	England	No	Female	10
SD04	4	England	No	Neutered	11
SD05	9	Taiwan	No	Neutered	9
SD06	8	Taiwan	No	Neutered	8
SD07	3	New Zealand	No	Neutered	12
SD08	4	USA	No	Female	10
SD09	4	USA	No	Female	10

The information of the sampling horses

馬場編號 EA

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
EA01	10	Holland	No	Neutered	23
EA02	9	Taiwan	No	Female	9
EA03	6	Taiwan	No	Female	6
EA04	16	Taiwan	No	Neutered	16
EA05	13	Taiwan	No	Female	13
EA06	5	Taiwan	No	Female	5
EA07	5	USA	No	Neutered	26
EA08	7	USA	No	Neutered	16
EA09	8	Taiwan	No	Neutered	8
EA10	20	Taiwan	No	Neutered	20
EA11	6	USA	No	Neutered	14

The information of the sampling horses

馬場編號 EB

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
EB01	＞1	Germany	No	Neutered	12
EB02	14	Taiwan	No	Female	14
EB03	＞1	Unknown	No	Male	16

The information of the sampling horses

馬場編號 EC

Horse Code	Years in Taiwan	Birth Place	EIA Vaccination	Gender	Age
EC01	11	Taiwan	No	Male	11
EC02	12	Australia	No	Neutered	22
EC03	18	Taiwan	No	Male	18
EC04	12	Australia	No	Neutered	19
EC05	12	Australia	No	Neutered	21
EC06	15	Taiwan	No	Female	15