Epizootiological investigation of the most important infectious equine diseases in Greece

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Summary

During the period 2001 to 2008, a total of 7,872 equine sera were tested at the Athens Centre of Veterinary Institutes. Antibodies against seven infectious diseases of equids were determined: equine infectious anaemia (EIA), African horse sickness (AHS), equine viral arteritis (EVA), West Nile encephalitis (WNE), glanders, piroplasmosis and dourine. Tests for the four viral diseases found 4.5% seropositivity for EIA, 0% for AHS, 3.3% for EVA and 4% for WNE. All sera tested for glanders antibodies were negative. Tests for piroplasmosis detected antibodies against *T. equi* and *B. caballi* in 12.9% and 1.3% of the sera, respectively. No sample tested positive for dourine. The results of this epidemiological survey provide strong evidence that Greece is free from the diseases of AHS, glanders and dourine.

Keywords

Bacterial disease – Epizootiological investigation – Equid – Horse – Parasitic disease – Viral disease.

Introduction

The Centre of Veterinary Institutes of Athens routinely receives horse serum samples originating from all over Greece. In addition, an official epidemiological investigation programme for the most important equine infectious diseases was organised for the period from 2001 to 2002. The purpose of the programme was to evaluate the present situation by collecting and examining a large number of serum samples from equids and to plan responses in case of possible nosological problems. The programme was carried out in cooperation with the Animal Health Directorate Veterinary Authorities at local (prefecture) level, the Hellenic Equestrian Federation and the Jockey Club of Greece, and involved the registration of national equid holdings.

All testing on routine samples and samples collected within the framework of the programme took place at laboratories of the Institute of Infectious and Parasitic Diseases within the Athens Centre of Veterinary Institutes; namely, the laboratories of Virology, Microbiology (glanders) and Parasitology (piroplasmosis and dourine), during the period 2001 to 2008. All the methods used are considered reference methods for the European Union and were conducted in accordance with the protocols laid down in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) of the World Organisation for Animal Health (OIE) (1, 2, 3).

Equine infectious anaemia

Equine infectious anaemia (EIA) is a viral disease found on all continents, caused by an RNA virus of the genus *Lentivirus* of the family *Retroviridae* (4, 5). The disease can be transmitted vertically or horizontally: by the intrauterine route and by suckling, through the use of infected surgical equipment, mechanically by the bite of blood-

feeding insects, and from the nasal mucosa or from skin lesions (traumatic ruptures of the skin). The virus is found in all tissues and secretions of the animals, with higher concentrations in the spleen and lymph nodes.

Clinical signs include weakness, loss of appetite, depression, jaundice, anaemia, oedema, transient fever and mucosal petechiae. The morbidity of the disease is variable but can reach 100% where there is a very high concentration of animals (6). Those animals that survive the infection become life-long carriers of the virus. The disease has been notifiable in Greece since 1960 (6).

African horse sickness

African horse sickness (AHS) is caused by an RNA virus of the genus *Orbivirus* of the family *Reoviridae* (1, 5, 7). There are nine serotypes of the virus, which is transmitted via biting midges of the genus *Culicoides*. Infection is characterised by high fever, dyspnoea, circumocular and inframandibular oedema, swelling in the chest and neck, and ultimately death.

Haemorrhages are noticeable at necropsy, together with pulmonary oedema and exudate. Petechiae are found in the small and large intestine, and bleeding in the heart and lungs. The virus can be isolated from whole blood samples collected with anticoagulant and from the spleen and lymph nodes.

The disease is enzootic in Africa, south of the Sahara desert (1, 8), and appeared in North Africa in 1928, 1943 and 1944, in the Middle East and Cyprus in 1959 and 1960, and in Spain between 1987 and 1990. It has not been found in Greece, even though it belongs to the same group as bluetongue and is transferred by the same vector, *Culicoides imicola*, which has been found in both mainland Greece and the Aegean Islands. African horse sickness is notifiable.

Equine viral arteritis

Equine viral arteritis (EVA) is a disease caused by an RNA virus of the genus *Arterivirus* of the family *Arteriviridae* (5, 9, 10). The primary routes of transmission of this virus are respiratory and genital. Stallions become carriers of the disease. Infection with the virus results in necrosis of the muscle layer in the walls of small vessels and affects the respiratory and genital systems. The virus can be isolated from white blood cells and nasal, pharyngeal and tracheal secretions, as well as from semen and urine.

Affected horses may have elevated body temperature, leucopaenia, decreased appetite, depression, conjunctivitis, nasal discharge, oedemas, diarrhoea, jaundice, muscular pain, inflammation of the male genital organs, and abortions.

The spread of the virus is global but clinical cases are few. The financial consequences of the disease are of considerable importance (10). The present survey for EVA is the first in Greece.

West Nile encephalitis

West Nile encephalitis (WNE), a zoonosis, is caused by an RNA virus of the genus *Flavivirus* of the family *Flaviviridae* (11, 12). The virus is transmitted by ornithophilous mosquitoes of the genus *Culex*. Birds develop a high titre of the virus in their bloodstream and play an important role in the epidemiology of the disease. It is believed that the life cycle of the virus is between wild birds and mosquitoes. Mammals are occasional hosts, with clinical signs observed only in equids (mostly horses) and humans.

The disease in mammals is usually latent and subclinical, with few cases developing clinical disease. Fatalities usually occur when the virus appears in a region for the first time. Horses and humans affected with clinical disease present with encephalitis, the main signs of which are a two-phase fever and neurological problems such as paresis, ataxia, and disorders in hearing and vision. In endemic

regions the disease is usually latent. Seropositive horses without clinical signs are not uncommon.

The disease was first described in Egypt in 1963. Until 1999, it was endemic in Africa, India, the Middle East and western and central Asia, with sporadic epizootics in Europe. Since 1999, the disease has appeared in the United States (USA), Canada, Mexico and the Caribbean islands, with encephalitis in animals and humans.

Glanders

Glanders is caused by the bacterium *Burkholderia mallei* (13) and horses, donkeys and mules are susceptible to the infection. Bovines, sheep, goats and pigs are considered resistant. Canines can be infected through eating infected meat, whereas felines appear to be more resistant.

The bacterium is transmitted through secretions of the digestive and respiratory system, as well as through the skin. Humans can be infected by direct contact with a sick animal or infectious materials. The incubation period lasts from a few days to months. The acute form of glanders appears mostly in donkeys, with high fever, respiratory signs (dyspnoea, pneumonia) and death after a few days. Horses demonstrate two chronic forms:

a) the respiratory form (glanders), with nodules and ulcers in the nasal cavities and lungs, thick nasal secretions, hyperplasia of the inframandibular lymph nodes, cough, dyspnoea, fever and depression

b) the cutaneous form (farcy), with nodules and ulcers in the lymphatic vessels of the limbs and body.

A subclinical form of glanders without any characteristic signs, apart from nasal excretion and fatigue, is also common in horses and is considered responsible for maintaining the pathogen in the environment and the occurrence of epidemics (13). Mules usually present with the acute or subacute forms, although there have been reports of some chronic and subclinical forms (14).

The disease is found in the countries of the Middle East and Asia (Iran, Iraq, India, Pakistan, Mongolia, Turkey, China and the United Arab Emirates), Africa and sporadically in South America. According to data from the OIE, there were reports of glanders in Western Europe up until 1965, and in Turkey, which borders Greece, in 1988.

Glanders is considered a very serious zoonosis and a potential biological weapon (15). Strict controls in the transportation of equines, together with the use of the mallein test (16) and complement fixation testing (17), have led to the control and eradication of the disease in Europe, America and Australia. Glanders is a notifiable disease in Greece.

Equine piroplasmosis

Equine piroplasmosis is an infection of horses, donkeys, mules and zebras and results from infection with two protozoa, *T. equi* (formerly *Babesia equi*) and *B. caballi*. These protozoa are intracellular parasites of blood cells and follow an obligatory life cycle involving equids and ticks of the genera *Rhipicephalus*, *Dermacentor* and *Hyalomma*. There have been cases of transmission via contaminated needles and syringes. Infected animals become carriers for long periods of time and act as reservoirs of the disease for ticks (18, 19).

The incubation period for *T. equi* infection is 12 to 19 days and, for *B. caballi*, 10 to 30 days. The clinical signs of piroplasmosis vary. In rare peracute forms the animals are found dead. More often, the disease is acute, with fever (>41°C), loss of appetite, laboured breathing, congestion of the mucous membranes, infrequent dry faeces, anaemia, jaundice, haemoglobinuria (rare in *B. caballi* infection), sweating, petechial haemorrhages on the conjunctiva, swollen abdomen, and posterior weakness or swaying. Subacute cases of piroplasmosis may demonstrate fever (sometimes intermittent), loss of appetite, loss of weight, signs of mild colic, and mild oedema of the distal limbs. The mucous membranes can be pink or yellow, and may have petechiae or ecchymoses. In chronic forms, common signs include mild loss of appetite, poor tolerance of exercise, loss of weight, transient fevers and an enlarged spleen (palpable on rectal

examination). Foals infected *in utero* are usually weak at birth and rapidly develop anaemia and severe jaundice.

Immunity lasts for more than a year. Thus, equines in endemic areas are resistant, whereas the mortality rate can be up to 20% in animals previously unexposed to the parasite. Treatment is usually more effective for *B. caballi* than for *T. equi* (18, 19).

Equine piroplasmosis is encountered worldwide. In Greece, sporozoites of *B. caballi* have been detected in the tick *Rhipicephalus sanguineus* and *T. equi* in the tick *Hyalomma plumbeum* (19).

Dourine

Dourine is a venereal disease of horses and donkeys and is caused by the haemoprotozoon *Trypanosoma brucei equiperdum* of the family *Trypanosomatidae*, Doflein 1901. The infection is transmitted during copulation via minor wounds of the mucosa. There are also limited reports of transmission through fly bites (18). *Trypanosoma equiperdum* (18 µm to 28 µm) demonstrates a direct biological cycle.

The signs of dourine appear weeks or months after infection. The first signs include discharge of mucus and purulent material from the urethra or vagina of the horse; this is followed by swelling of the genitalia. Later, pathognomonic round urticarial plaques of 5 cm to 8 cm in diameter and 1 cm thick appear on the skin of the ventral area (20). The signs of chronic forms of dourine include emaciation, transient fever and paralysis, followed by death. In donkeys, the disease produces no clinical signs and there is no swelling of the genitalia; urticarial plaques are observed in fewer than 10% of infected animals. Donkeys are considered a permanent reservoir of the protozoon because the sperm and the vaginal discharge are very infectious (18).

Dourine is present in Arabia, the Middle East, South Africa, Russia, the Mediterranean countries, South-East Asia and South America. In Greece, single infected horses were found in the area of Trikala-Karditsa in 1935 by Temponeras and in 1956 by Papadakis (21).

Materials and methods

The study involved all 51 prefectures of Greece and was implemented:

a) on every stud farm in Greece

b) at all border inspection posts, for 10% of all imported batches of equines, regardless of their time of stay in Greece or the accompanying documentation from their country of origin

c) in all places where equines are concentrated, such as markets, traditional trade fairs and equestrian clubs.

The local Veterinary Services at the prefecture level collected blood samples and sent them to the laboratories of the Athens Centre of Veterinary Institutes, in accordance with Ministerial Decisions 333468/29-01-2001 and 228868/12.02.2002 (of the Ministry of Agriculture, now the Ministry of Rural Development and Food). The Hellenic Equestrian Federation and the Jockey Club of Greece collected blood samples from every relevant stud farm (at least two samples from farms with fewer than 20 animals). The scheme aimed at sampling a minimum 5% of the total equine population over 12 months of age.

[Table I, Figure 1]

Equine infectious anaemia

Serum samples were tested for EIA antibody using agar gel immunodiffusion, also known as Coggins' test (22), with reagents from IDDEX Laboratories, Inc. (Hoofddorp, the Netherlands) and VMRD, Inc. (Pullman, WA, USA). Positive results were confirmed with an enzyme-linked immunosorbent assay (ELISA) from IDDEX Laboratories (23).

African horse sickness

Samples were tested for antibody in competitive ELISAs with reagents from the Pirbright Institute (United Kingdom) (24) or from Ingenasa (Madrid, Spain). Where results were inconclusive, the test

was repeated with reagents from both sources. The ELISA was followed by clinical examination of all animals at the holding of origin. Local veterinarians collected blood samples with and without anticoagulant for virus isolation in embryonated hen eggs, lactating mice (intracerebral inoculation) and cell culture. Viral antigen was detected by ELISA (25), using Ingenasa reagents.

Equine viral arteritis

Serum samples were tested for EVA antibody in a virus neutralisation test in rabbit kidney (RK_{13}) cells with the addition of guinea-pig complement. Many samples were also tested in an ELISA with Ingenasa reagents.

West Nile encephalitis

Samples were tested in a serum neutralisation assay in cultures of Vero E6 cells.

Glanders

Sera were tested for glanders (*B. mallei*) using a complement fixation assay with reagents from the National Veterinary Services Laboratories USA (NVSL-USA), and the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS). Where results were inconclusive, local veterinarians collected further blood samples and sent them to the microbiology laboratory for repeat testing; this was followed by clinical examination of all equids at the holding of origin.

Equine piroplasmosis

Samples were tested for piroplasmosis (*T. equi*, *B. caballi*) at the parasitology laboratory; however, because of a shortage of commercially available reagents, not all sera were examined. Nevertheless, a total of 2,685 sera were tested (35% of the total number of samples) from different regions of the country to ensure a balanced geographical representation. An indirect immunofluorescence assay (IFA) (3) was used with reagents from

Sigma-Aldrich (Saint Louis, MO, USA), the Hanover School of Veterinary Medicine (Germany), and NVSL-USA and USDA-APHIS.

Dourine

Sera were tested for infection with *T. equiperdum* using an IFA (3), with reagents from Sigma-Aldrich and the Instituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (Teramo, Italy). A shortage of reagents for dourine testing meant that only 1,091 sera (approximately 14% of the total samples) could be examined.

Tests on horses imported into Greece

Samples from imported animals originating from Bulgaria or the Former Yugoslav Republic of Macedonia were tested for antibodies to five of the diseases under investigation: EIA, AHS, WNE, EVA and glanders.

Results

Equine infectious anaemia

A total of 7,872 equine sera from animals in 49 prefectures of Greece were tested for EIA, of which 353 (4.5%) were seropositive (Table II).

African horse sickness

A total of 7,661 sera from 49 prefectures were tested and one sample was found to have borderline ELISA values for AHS antibody (Table II).

[Table II]

Equine viral arteritis

A total of 7,579 equine sera from 49 prefectures were tested for EVA. Of these, 249 (3.3%) were positive (Table III).

West Nile encephalitis

A total of 7,549 equine sera from 49 prefectures were tested for WNE antibody, among which 302 (4.0%) were positive and 1,122 were toxic (Table III).

[Table III]

Glanders

A total of 7,697 equine sera from 49 prefectures were tested for glanders. Antibodies were initially detected in one horse from the region of Rodopi (Table IV). The suspect case was retested but with negative results. Supplementary sampling of the associating animals gave negative results for all sera.

[Table IV]

Equine piroplasmosis

A total of 2,685 equine sera from 41 prefectures were tested for piroplasmosis. Antibodies to *T. equi* (*B. equi*) were detected in 347 sera (12.9%); antibodies to *B. caballi* were detected in 35 sera (1.3%) (Table V).

[Table V]

Dourine

A total of 1,091 equine sera from animals in 29 prefectures were tested for dourine antibodies, all with negative results (Table VI).

[Table VI]

Tests on horses imported into Greece

Among 654 samples from imported animals, one tested positive for EIA, two for WNE and 48 for EVA. There were no positive results for AHS or glanders (Table VII).

[Table VII]

Discussion

Equine infectious anaemia

Test results revealed EIA-seropositive animals in 23 of the 49 prefectures that contributed serum samples, with the highest rate found in northern and central Greece. Regions with a high percentage of seropositive animals were those that had experienced clinical disease in the past. No EIA seropositivity was found in samples from Crete or the islands of the eastern Aegean and Ionian Sea. No cases of clinical disease were observed. In one case, an animal from Bulgaria was found to be seropositive and was denied entry into Greece.

Greek policy on animals that test seropositive for EIA requires that all animals in the same stable should undergo clinical and serological examination, and that restriction measures and movement control measures should be kept in place for as long as there are still seropositive animals present in the holding.

African horse sickness

Greece is considered a country free of AHS. Antibodies were detected in only one animal, an old horse in Pieria that had been bought in Karditsa but had originally come from Spain, where all equines received preventive vaccination during the AHS epidemic there (26, 27). An attempt to isolate the virus was negative and it was concluded that the detected antibodies were the result of the earlier vaccination. All the animals in the same stable were tested and all were found negative for AHS.

Equine viral arteritis

Tests for EVA identified seropositive animals in 27 of the 49 prefectures that contributed samples. All attempts to isolate the virus (three sequential passages of pathological materials from ill equines in sensitive cell cultures) gave negative results. Present techniques do not allow distinction between natural antibodies and vaccine antibodies, so the detection of EVA-seropositive animals cannot be definitively attributed to natural infection. A killed EVA vaccine has been

available in the United Kingdom and Ireland since 1993; an attenuated vaccine that can cause disease is used in the USA. Further efforts are being made to produce a safe commercial vaccine. Strict enforcement of hygiene regulations on animal movements can detect the transfer of disease carriers.

The authors found significant variation between the results of the serum neutralisation test and those of the ELISA. The rate of EVA seropositivity found when using the ELISA was extremely high, and a comparative study is needed to evaluate the diagnostic ability of ELISA kits. Equine transportation and the world trade in horses make a fast and reliable method of antibody detection a necessity.

West Nile encephalitis

Tests for WNE detected antibodies in 36 of the 49 contributing prefectures. Earlier studies in Greece (28, 29) showed the presence of antibodies against the virus in humans, as well as in many animal species. As known from studies in previous years, seropositive animals have been found in the Balkan countries and Turkey (30), and in some areas even clinical disease has been identified. The virus is endemic in the Camargue, southern France, causing encephalitis in horses and humans (31). No clinical disease has yet been detected in animals or humans in Greece, but the intermediate vectors of the virus are present in the Balkan region and there is an urgent need to further investigate WNE. In the present study, an additional 334 sera from sheep, fowl, dogs, cats and people working in the laboratory were tested for WNE but with negative results. Attempts to isolate the virus from the brains of birds with an unknown cause of death and from the cerebrospinal fluid of a patient with encephalomyelitis were negative.

Glanders

The authors' results show that glanders does not exist in Greece; antibodies were detected in only a single asymptomatic animal in the area of Rodopi. When evaluating that case, three possible explanations were considered:

a) a false-positive result: this is a problem with the complement fixation test because of the nature of the non-purified antigen (13, 32, 33) and the quality of the serum (immune complexes)

b) a cross-reaction with bacteria of the genera *Pseudomonas* and *Burkholderia* (34)

c) chronic subclinical disease: an unlikely explanation because of the young age of the animal and the absence of other seropositive animals in the same stable.

Equine piroplasmosis

Antibodies against the organisms causing piroplasmosis were detected in samples from 39 of the 41 submitting prefectures, suggesting that the disease is widely spread through many regions of Greece. There were very high rates of seropositivity for *T. equi*, which is considered more infectious than *B. caballi* (19, 34, 35). In general, piroplasmosis is a disease of great importance in the warmer regions of the world, mostly in Africa (18). The disease can be controlled by decreasing the number of ticks in the environment and through the use of effective insecticides in animals. It is also important to provide extra care for animals entering an endemic area if they have not previously been exposed to the parasites. The introduction of disease carriers into areas where the vectors are present could lead to epidemics.

Dourine

Tests on equine sera from 29 prefectures indicated that dourine is not present in Greece. International regulations state that all animals testing seropositive for dourine must be euthanised, because the disease is considered untreatable. This policy, together with the use of artificial insemination, has led to the eradication of dourine from Greece and most other parts of the world. According to the OIE, the last reports of dourine came mainly from Botswana, Ethiopia, Germany, Kyrgyzstan, Namibia, Pakistan, Russia, South Africa and Uzbekistan (20). Further investigation of the disease in all prefectures of Greece would be informative.

International trading of equines

Many of the animals that are transported through Greece have antibodies for EVA and WNE (Table VII). According to the legislation, stallions used for breeding are not allowed to enter the country if found to be seropositive for EVA. Strict adherence to the veterinary hygiene regulations governing the movement of equines should be sufficient to prevent problems now and in the future.

Conclusions

This epizootiological survey of seven important diseases of horses identified antibodies to four of these diseases in serum samples collected from many areas of Greece. The authors conclude that the country is free from glanders, dourine and AHS. In the case of dourine, however, a larger number of samples should be examined to gain a more thorough understanding of the situation of this disease in Greece.

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Fig. 1
Greek prefectures

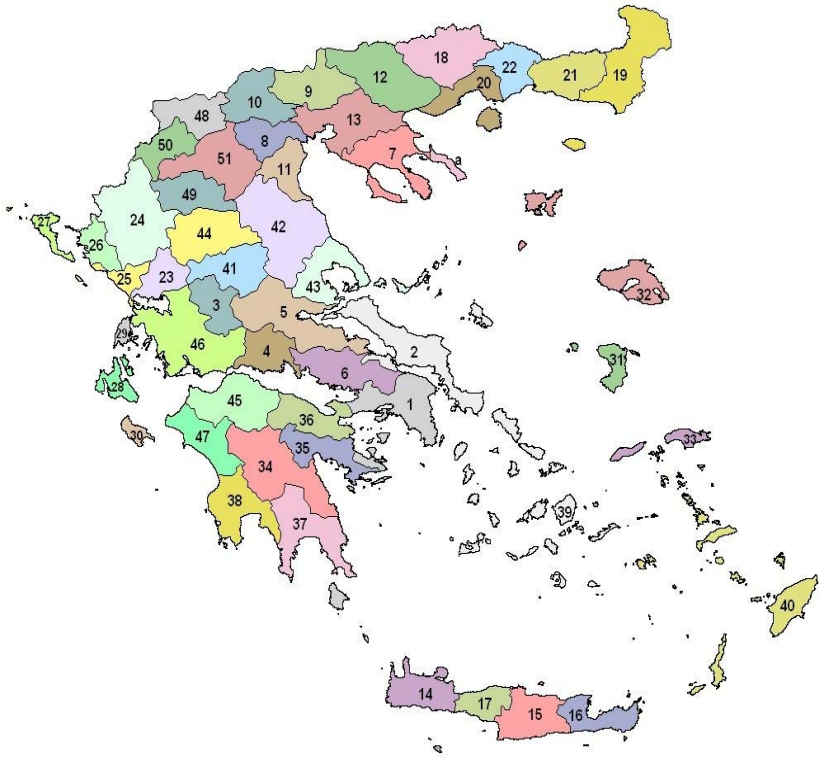

Table I
Greek prefectures

No. on map	Prefecture	No. on map	Prefecture
1	Attici	27	Corfu
2	Evia	28	Kefallonia
3	Evritania	29	Lefkada
4	Fokida	30	Zakynthos
5	Phthiotida	31	Chios
6	Viotia	32	Lesvos
7	Chalkidiki	33	Samos
8	Imathia	34	Arcadia
9	Kilkis	35	Argolida
10	Pella	36	Korinthia
11	Pieria	37	Laconia
12	Serres	38	Messinia
13	Thessaloniki	39	Cyclades
14	Chania	40	Dodekanisa
15	Heraklion	41	Karditsa
16	Lasithi	42	Larisa
17	Rethymno	43	Magnesia
18	Drama	44	Trikala
19	Evros	45	Achaia
20	Kavala	46	Aetoloakarnania
21	Rhodopi	47	Ilia
22	Xanthi	48	Florina
23	Arta	49	Grevena
24	Ioannina	50	Kastoria
25	Preveza	51	Kozani
26	Thesprotia	a	Mount Athos

Table II

Serological results for equine infectious anaemia and African horse sickness in equine serum samples in Greece, tested between 2001 and 2008, by prefecture (samples were taken from 49 of the 51 prefectures)

Prefecture	Equine infectious anaemia		African horse sickness	
	Samples tested	Positive (%)	Samples tested	Positive
Argolidα	6	0 (0.0)	6	0
Arkadia	872	10 (1.1)	872	0
Arta	25	0 (0.0)	25	0
Attici	937	0 (0.0)	843	0
Achaia	120	0 (0.0)	120	0
Viotia	20	0 (0.0)	20	0
Grevena	48	10 (20.8)	48	0
Drama	85	25 (29.4)	85	0
Dodekanisa	46	0 (0.0)	46	0
Evros	84	9 (10.7)	84	0
Evia	118	5 (4.2)	116	0
Evrytania	74	0 (0.0)	74	0
Zakynthos	14	0 (0.0)	14	0
Ilia	150	0 (0.0)	150	0
Imathia	102	1 (1.0)	102	0
Heraklion	238	0 (0.0)	238	0
Thessaloniki	431	28 (6.5)	420	0
Thesprotia	19	0 (0.0)	19	0
Ioannina	241	5 (2.1)	241	0
Kavala	62	10 (16.1)	62	0
Karditsa	82	3 (3.7)	82	0
Kastoria	22	3 (13.6)	22	0
Corfu	9	0 (0.0)	9	0
Kefallinia	12	0 (0.0)	12	0
Kilkis	31	1 (3.2)	31	0
Kozani	162	2 (1.2)	162	0
Korinthia	292	0 (0.0)	292	0
Cyclades	97	1 (1.0)	97	0
Lakonia	140	0 (0.0)	140	0
Larissa	221	89 (40.3)	221	0
Lasithi	139	0 (0.0)	139	0
Lesvos	37	0 (0.0)	37	0
Magnesia	291	25 (8.6)	291	0

Messinia	581	0 (0.0)	475	0
Xanthi	115	11 (9.6)	115	0
Pella	6	0 (0.0)	6	0
Pieria	242	54 (22.3)	244	1
Preveza	21	0 (0.0)	21	0
Rethymno	180	0 (0.0)	180	0
Rodopi	150	1 (0.7)	150	0
Samos	120	0 (0.0)	120	0
Serres	792	16 (2.0)	792	0
Trikala	20	0 (0.0)	20	0
Fthiotida	139	2 (0.7)	139	0
Florina	12	4 (33.3)	12	0
Fokida	18	0 (0.0)	18	0
Chalkidiki	176	39 (22.2)	176	0
Chania	30	0 (0.0)	30	0
Chios	49	0 (0.0)	49	0
Total	7,872	353 (4.5)	7,661	1

Table III

Serological results for equine viral arteritis and West Nile encephalitis in equine serum samples in Greece, tested between 2001 and 2008, by prefecture (samples were taken from 49 of the 51 prefectures)

Prefecture	Equine viral arteritis		West Nile encephalitis		
	Samples tested	Positive (%)	Samples tested	Positive (%)	Toxic for Vero cells
Argolida	6	0 (0.0)	6	0 (0.0)	0
Arkadia	872	4 (0.5)	872	32 (3.7)	36
Arta	25	0 (0.0)	25	0 (0.0)	5
Attici	901	63 (7.0)	892	28 (3.1)	226
Achaia	120	32 (26.7)	120	14 (11.7)	45
Viotia	20	0 (0.0)	20	4 (20.0)	3
Grevena	48	0 (0.0)	48	2 (4.2)	11
Drama	85	6 (7.1)	85	5 (5.9)	9
Dodekanisa	46	1 (2.2)	46	0 (0.0)	4
Evros	84	7 (8.3)	82	3 (3.7)	24
Evia	119	4 (3.4)	116	2 (1.7)	13
Evrytania	74	0 (0.0)	74	0 (0.0)	6
Zakynthos	14	0 (0.0)	14	0 (0.0)	3
Ilia	143	0 (0.0)	150	7 (4.7)	27
Imathia	102	2 (2.0)	92	9 (9.8)	2
Heraklion	238	16 (6.7)	238	4 (1.7)	26
Thessaloniki	419	18 (4.3)	410	25 (6.1)	56
Thesprotia	19	0 (0.0)	19	1 (5.3)	2
Ioannina	220	2 (0.9)	241	6 (2.5)	30
Kavala	62	3 (4.8)	62	5 (8.1)	4
Karditsa	82	3 (3.7)	82	6 (7.3)	3
Kastoria	22	0 (0.0)	22	0 (0.0)	2

Corfu	9	0 (0.0)	9	0 (0.0)	4
Kefallinia	12	0 (0.0)	12	0 (0.0)	7
Kilkis	31	1 (3.2)	31	2 (6.5)	4
Kozani	162	7 (4.3)	162	6 (3.7)	33
Korinthia	295	10 (3.4)	292	8 (2.7)	40
Cyclades	97	0 (0.0)	97	1 (1.0)	18
Lakonia	121	1 (0.8)	112	3 (2.7)	27
Larissa	176	1 (0.6)	190	4 (2.1)	16
Lasithi	139	0 (0.0)	139	5 (3.6)	32
Lesvos	37	0 (0.0)	37	0 (0.0)	2
Magnesia	288	2 (0.7)	291	2 (0.7)	29
Messinia	466	7 (1.5)	466	54 (11.6)	71
Xanthi	115	1 (0.9)	115	3 (2.6)	2
Pella	6	0 (0.0)	6	0 (0.0)	0
Pieria	244	2 (0.8)	244	10 (4.1)	26
Preveza	21	0 (0.0)	21	2 (9.5)	3
Rethymno	179	0 (0.0)	177	9 (5.1)	47
Rodopi	150	0 (0.0)	150	4 (2.7)	32
Samos	120	2 (1.7)	120	3 (2.5)	27
Serres	753	48 (6.4)	728	19 (2.6)	92
Trikala	20	0 (0.0)	20	0 (0.0)	1
Fthiotida	139	1 (0.7)	139	9 (6.5)	16
Florina	12	0 (0.0)	12	0 (0.0)	9
Fokida	18	0 (0.0)	18	2 (11.1)	8
Chalkidiki	176	4 (2.3)	172	2 (1.2)	24
Chania	29	0 (0.0)	30	1 (3.3)	13
Chios	49	1 (2.0)	49	0 (0.0)	2
Total	7,579	249	7,549	302 (4.0)	1,122

Table IV

Serological results for glanders in equine serum samples in Greece, tested between 2001 and 2008, by prefecture (samples were taken from 49 of the 51 prefectures)

Prefecture	Glanders		
	Samples tested	Positive	Suspected
Argolidα	6	0	0
Arkadia	872	0	0
Arta	25	0	0
Attici	750	0	0
Achaia	120	0	0
Viotia	20	0	0
Grevena	48	0	0
Drama	85	0	0
Dodekanisa	46	0	0
Evros	84	0	0
Evia	118	0	1
Evrytania	74	0	0
Zakynthos	14	0	0
Ilia	150	0	0
Imathia	108	0	0
Heraklion	238	0	0
Thessaloniki	431	0	0
Thesprotia	19	0	0
Ioannina	241	0	0
Kavala	62	0	0
Karditsa	82	0	0
Kastoria	22	0	0
Corfu	9	0	0
Kefallinia	12	0	0
Kilkis	31	0	0
Kozani	162	0	2
Korinthia	292	0	0
Cyclades	97	0	0
Lakonia	140	0	0
Larissa	221	0	0
Lasithi	139	0	0
Lesvos	37	0	0
Magnesia	291	0	2
Messinia	581	0	0
Xanthi	115	0	0

Pella	6	0	0
Pieria	242	0	3
Preveza	21	0	0
Rethymno	180	0	0
Rodopi	150	0	1
Samos	120	0	0
Serres	792	0	0
Trikala	20	0	0
Fthiotida	139	0	0
Florina	12	0	0
Fokida	18	0	0
Chalkidiki	176	0	0
Chania	30	0	0
Chios	49	0	0
Total	7,697	0	9

Table V

Serological results for equine piroplasmosis in equine serum samples in Greece, tested between 2001 and 2008, by prefecture (samples were taken from 41 of the 51 prefectures)

Prefecture	Piroplasmosis *Theileria equi*		Piroplasmosis *Babesia caballi*	
	Samples tested	Positive (%)	Samples tested	Positive (%)
Argolidα	6	2 (33.3)	6	0 (0.0)
Arkadia	271	21 (7.7)	271	0 (0.0)
Arta	17	0 (0.0)	17	0 (0.0)
Attici	359	35 (9.7)	359	8 (2.2)
Viotia	13	0 (0.0)	13	0 (0.0)
Grevena	44	10 (22.7)	44	0 (0.0)
Dodekanisa	46	2 (4.3)	46	0 (0.0)
Evros	24	6 (25.0)	24	0 (0.0)
Evia	53	7 (13.2)	53	0 (0.0)
Evrytania	43	2 (4.7)	43	0 (0.0)
Zakynthos	3	1 (33.3)	3	0 (0.0)
Ilia	86	15 (17.4)	86	1 (1.2)
Imathia	58	5 (8.6)	58	0 (0.0)
Heraklion	82	2 (2.4)	82	0 (0.0)
Thessaloniki	101	19 (18.8)	101	0 (0.0)
Thesprotia	11	2 (18.2)	11	0 (0.0)
Ioannina	97	13 (13.4)	97	1 (1.0)
Kavala	20	2 (10.0)	20	0 (0.0)
Karditsa	66	10 (15.2)	66	1 (1.5)
Kastoria	8	1 (12.5)	8	0 (0.0)
Corfu	6	4 (66.7)	6	0 (0.0)
Kefallinia	12	1 (8.3)	12	0 (0.0)
Kilkis	13	1 (7.7)	13	0 (0.0)
Korinthia	153	13 (8.5)	153	2 (1.3)
Cyclades	71	6 (8.5)	71	0 (0.0)
Lakonia	59	3 (5.1)	59	0 (0.0)
Larissa	94	16 (17.0)	94	2 (2.1)
Lesvos	20	5 (25.0)	20	1 (5.0)
Magnesia	124	39 (31.5)	124	2 (1.6)
Messinia	124	8 (6.5)	124	0 (0.0)
Xanthi	115	13 (11.3)	115	1 (0.9)
Pella	10	2 (20.0)	10	0 (0.0)
Preveza	21	7 (33.3)	21	2 (9.5)
Rethymno	76	1 (1.3)	76	1 (1.3)
Samos	29	5 (17.2)	29	0 (0.0)

Serres	103	27 (26.2)	103	4 (3.9)
Fthiotida	99	14 (14.1)	99	0 (0.0)
Florina	12	1 (8.3)	12	1 (8.3)
Fokida	18	3 (16.7)	18	0 (0.0)
Chalkidiki	83	19 (22.9)	83	5 (6.0)
Chios	35	4 (11.4)	35	3 (8.6)
Total	2,685	347 (12.9)	2,685	35 (1.3)

Table VI

Serological results for dourine (*Trypanosome equiperdum)* in equine serum samples in Greece, tested between 2001 and 2008, by prefecture (samples were taken from 29 of the 51 prefectures)

Prefecture	Samples tested	Positive
Argolidα	6	0
Attici	318	0
Grevena	21	0
Evros	16	0
Evia	22	0
Evrytania	25	0
Ilia	3	0
Imathia	44	0
Heraklion	51	0
Thessaloniki	103	0
Thesprotia	11	0
Kavala	2	0
Karditsa	3	0
Kastoria	8	0
Corfu	6	0
Kilkis	13	0
Korinthia	9	0
Cyclades	32	0
Lakonia	10	0
Larissa	94	0
Lesvos	20	0
Magnesia	30	0
Messinia	124	0
Pella	10	0
Rethymno	30	0
Serres	18	0
Fokida	10	0
Chalkidiki	23	0
Chios	29	0
Total	1,091	0

Table VII

Tests on serum samples from horses imported into Greece at two veterinary health inspection stations, between 2001 and 2008

Disease	Evzoni[a]		Promachonas[a]		Total samples	
	Tested	Positive	Tested	Positive	Tested	Positive
EIA	14	0	640	1	654	1
AHS	14	0	640	0	654	0
WNE	14	1	576	1 (104 toxic)[b]	590	2 (104 toxic [b]
EVA	14	1	601	47	615	48
Glanders	14	0	640	0	654	0

a) veterinary health inspection station

b) toxic for Vero cells

EIA: equine infectious anaemia

AHS: African horse sickness

WNE: West Nile encephalitis

EVA: equine viral arteritis