

SEROLOGICAL RESULTS OF EQUINE INFECTIOUS ANEMIA IN ALBANIA AND ECOLOGICAL RISK FACTORS

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Received May 2025; Accepted May 2025; Published July 2025;

DOI: <https://doi.org/10.31407/ijeess15.307>

ABSTRACT

Equine infectious anemia (EIA), a persistent lentiviral infection of equids, causes significant health and economic challenges due to its range of clinical manifestations, from subclinical disease to fatal anemia, and trade restrictions as a WOAHP-notifiable disease. Despite its importance, no comprehensive nationwide studies on EIA prevalence had previously been conducted in Albania. This study aimed to assess the seroprevalence of EIA in a total of 486 equids from southeastern Albania, sampled during the period 2023–2024, from 10 Albanian districts: Tirana, Elbasan, Durrës, Peqin, Mat, Librazhd, Berat, Kolonjë, Pogradec, and Devoll. Samples were tested using a parallel approach with the agar gel immunodiffusion test and the fluorescence polarization assay. Animals were categorized based on age groups (0–5, 6–20, and 21–35 years), sex (65 males and 85 females), and additional management practice information was documented. Serum samples were analyzed using a dual-test protocol: screening with either the IDvet EIAV Double Antigen ELISA or the Fluorescence Polarization Assay (FPA), followed by confirmation with the Agar Gel Immunodiffusion Test (AGID). These findings suggest an absence of EIAV circulation in Albania to date. However, considering ecological risk factors such as vector density, climatic variability, and potential cross-border equine movements, continued surveillance is crucial. This study provides important baseline data for future epizootiological monitoring and risk assessment efforts concerning EIA in Albania.

Key words: Equine infectious anemia, equids, seroprevalence, ELISA, Fluorescence Polarization Assay, Agar Gel Immunodiffusion, Albania.

INTRODUCTION

Equine infectious anemia (EIA) represents a viral disease which is lifelong infection in horse, donkey and mules at all ages. The virus targets macrophages, with clinical outcomes ranging from asymptomatic forms to acute and chronic disease (Leroux et al., 2004). Acute manifestations include intermittent fever, thrombocytopenia, hemolytic anemia, mucosal hemorrhages, edema, fatigue, and in some cases, death (Wang et al., 2018). Chronic cases enable

silent viral persistence in reservoirs (Leroux et al., 2004). The disease is not treatable and it has been shown that vaccines against the virus are not effective. EIAV's high genetic variability (due to error-prone reverse transcriptase) and rapid antigenic drift complicate vaccine development and diagnostic sensitivity. The pathogen is a Lentivirus of the Retroviridae family (Cook et al., 2013). The virus causing EIA is usually very sensitive to different factors such as temperature, detergents and common commercial disinfectants. EIA is mechanically transmitted from horse flies (*Tabanus spp.* and *Hybomitra spp.*), deer flies (*Chrysops spp.*) and stable flies (*Stomoxys calcitrans*) (Lupulovic et al., 2021). Moreover, iatrogenic transmission occurs through using contaminated plasma from affected horses or surgical instruments or needles (Brangan et al., 2008; More et al., 2008). The virus remains viable in needles for 96 hours and resists environmental degradation in organic matter (Williams et al., 1981; Lupulovic et al., 2021).

The EIA virus persists within white blood cells for life, and also it is present in plasma during febrile intervals. The seasonality of EIA outbreaks is related with high activity of the vectors involved. In addition to the major route of transmission, several minor routes have been identified, such as milk, semen, aerosol, imported cloned horse embryos (Asseged et al., 2012). Vertical transmission occurs in cases where the virus is in its acute phase (Quinlivan et al., 2013). Equine infectious anemia has a worldwide spread but North and Central America are considered to be the natural sites of the infection (Jara et al., 2020). Japan and tropical Australia have a high impact on the EIA distribution. However, the disease has been reported on all continents (Capomaccio et al., 2012).

Epidemiology in Europe exhibits heterogeneous EIA distribution, with endemicity in Italy and Romania, where national surveillance programs have documented seroprevalence rates of 0.18% (2007–2012) and recurrent outbreaks linked to equine movement (Carvelli et al., 2024). Notably, Italy's risk-based surveillance identified mules as high-risk carriers (IRR=48.9 vs. horses), emphasizing their role in maintaining viral circulation (Carvelli et al., 2024). Some European countries such as France, Ireland, Hungary, Poland, North Macedonia and Serbia have reported disease outbreaks contributing in an update of the disease spread in Europe comparing with previous reports (Bolfà et al., 2016; Gaudaire et al., 2018; Quinlivan et al., 2013). Sporadic outbreaks in historically EIA-free regions such as Ireland (2006), Germany, and Belgium—have been traced to imported equine plasma or subclinically infected animals (Bolfà et al., 2016). Greece, bordering Albania, reports 4.5% seroprevalence, with hotspots in Thessaloniki (28% positivity) and Ioannina (5%) (Mangana-Vougiouka et al., 2013), underscoring cross-border transmission risks. Despite Albania's proximity to endemic regions and unregulated equine trade with Greece (Mangana-Vougiouka et al., 2013), no systematic EIA surveillance exists. Equine infectious anemia is a WOA listed disease and all control strategies are provided by the terrestrial animal health code. The EIA, despite it not being a zoonotic disease, has an important economic impact, particularly for the sport horse industry. Severe disease occurs when the disease is introduced to the native horse population. Active surveillance must be applied in all cases, as it can help in the early detection of affected farms and individual animals and effectively control and prevent the disease.

The veterinary legislation is very strict, and horses, donkeys, and mule's movements require negative AGID results before animal movements. While AGID (Coggins test) remains the OIE gold standard, its limited sensitivity ($\leq 80\%$) may underestimate prevalence, as demonstrated in Italy's false-negative cases (Carvelli et al., 2024). Modern protocols combine ELISA (screening) with AGID (confirmation) to optimize detection (Carvelli et al., 2024). OIE recommends different tests such as AGID, ELISA, and immunoblot for surveillance in order to allow the free movement of the animals. Fluorescent polarization assay has been proposed to detect the EIA and has been described as compatible with the other methods mentioned above (Tencza et al., 2000; Espasandin et al., 2021). The Albanian ecological profile temperate climate, wooded areas, and seasonal vector activity mirrors risk factors identified in Italian clusters (Carvelli et al., 2024). Donkeys and mules, which dominate Albania's working equid population, are understudied but may act as cryptic reservoirs, as evidenced by Romania's 8.3% seroprevalence in donkeys (Bolfà et al., 2016).

To the best of the authors' knowledge, no studies have been conducted to assess the epidemiological status of EIA in Albania. This study aims to describe the epidemiological situation of EIA across the country during the period 2023–2024, using a dual serological testing approach Fluorescence Polarisation Assay (FPA) and Agar Gel Immunodiffusion Assay (AGID) in one group, and ELISA and AGID in another, with AGID applied as a confirmatory test in both testing protocols.

MATERIALS AND METHODS

Study area, sampling and serological analyses

Domestic equine animals with a healthy status and without a history of clinical diseases were eligible to be involved in this study. Different areas of Albania were selected for sample collection. Regions of Elbasan, Korça, Berat, Tirana, and Mat were chosen for sample collection. In total, 486 equids (295 horses, 56 mules, and 135 donkeys) aged 0–35 years were sampled across various villages. Sex distribution, body condition scores, management practices, and geographical coordinates were documented for each animal. Blood samples (5 mL) were aseptically collected from the jugular vein using plain vacutainer tubes and sent to the infectious disease laboratory at the Faculty of Veterinary Medicine in Tirana. After an overnight period, the horse sera were separated by centrifugation at 3000 rpm for 5 minutes and stored at -20°C until testing. The serum was then aliquoted and kept at -20°C until analysis, in accordance with protocols validated by the OIE Terrestrial Manual (WOAH, 2023). 336 samples were tested in parallel using Fluorescence Polarisation Assay (FPA) and Agar Gel Immunodiffusion (AGID) at the Faculty of Veterinary Medicine in Tirana, Albania. Additionally, the 150 samples from the Korça region were tested with the ELISA (IDvet D Screen® EIA Double Antigen ELISA) as a screening test at the Faculty of Veterinary Medicine in Tirana and confirmed with the Coggins (AGID) test at the Faculty of Veterinary Medicine in Skopje, North Macedonia, where the ELISA was also performed as part of the testing process (Winston et al., 1987). The FPA results were expressed in Δmp , calculated by subtracting the mean negative control values of mp (milipolarisation) from the mp sample values. The criterion for determining animal health status was based on the FPA titer value (Table 1).

Table 1. Criteria used to determine animal status based on Fluorescence Polarisation Assay.

Titer unit Δmp Value	Animal Status		
	Negative	Suspected	Positive
	≤ 10	10 – 20	> 20

The calculating formula is as follows: $\Delta mp = (\text{analyzed sample } mP - \text{Average of negative control } mP)$.

RESULTS

In total, 486 equids (107 donkeys, 56 mules, and 323 horses) were sampled from various regions of Albania. 336 samples were negative on both AGID and FPA in both the Faculty of Veterinary Medicine in Tirana and Skopje, as presented in Table 2.

Table 2. Serological Results According to AGID and FPA Test.

District	Mules	AGID Negative	FPA Negative	Donkeys	AGID Negative	FPA Negative	Horses	AGID Negative	FPA Negative
Tirana	7	7	7	2	2	2	23	23	23
Elbasan	5	5	5	28	28	28	2	2	2
Durrës				5	5	5	1	1	1
Peqin				6	6	6	5	5	5
Mat	5	5	5	5	5	5	2	2	2
Librazhd				2	2	2	13	13	13
Berat	2	2	2	36	36	36	24	24	24
Korça	5	5	5	9	9	9	67	67	67
Devoll	3	3	3				27	27	27
Pogradec	10	10	10	8	8	8	9	9	9
Kolonja	3	3	3	6	6	6	16	16	16
Total	40	40	40	107	107	107	189	189	189

Initial screening of 150 equids (106 horses, 28 donkeys, 16 mules) from southeastern Albania's Korçë region identified three ELISA-reactive cases (2.0% apparent prevalence), comprising two horses (1.9%) and one donkey (3.6%), as presented in Table 3. The reactive samples demonstrated optical densities ranging from 0.629 to 1.951, with S/P values between 41.2% and 122.0%. Notably, the highest reactivity occurred in an 11-year-old male horse (S/P=122.0%, OD=1.951), followed by a 25-year-old female horse (S/P=45.1%, OD=0.632) and a 10-year-old male donkey (S/P=41.2%, OD=0.629). Among the 336 equids tested by AGID and FPA, no seropositive cases were detected. This results in an apparent prevalence of 0%. Using the exact binomial method, the 95% confidence interval (CI) for disease freedom is: 0.0% to 1.09%. The seroprevalence of equine infectious anemia (EIA) as determined by ELISA was 1.89% in horses (2/106), 3.57% in donkeys (1/28), and 0% in mules (0/16). However, none of the ELISA-positive animals were confirmed by AGID, which is regarded as the gold standard diagnostic method for EIA. These ELISA-positive cases may reflect false positives, early stages of seroconversion, or non-specific cross-reactivity.

Table 3. Serological Results According to AGID and ELISA Test.

Species	Tested	ELISA+	Proportions (95%, CI)	AGID+	AGID-
Horses	106	2	1.89 (0.52 -6.62)	0	106
Donkeys	28	1	3.57 (0.63 -17.71)	0	28
Mules	16	0	0 (0.0 -19.36)	0	16

Confirmatory testing via agar gel immunodiffusion (AGID) yielded negative results for all ELISA-reactive specimens, consistent with World Organization for Animal Health (WOAH) diagnostic standards. No seropositivity was detected among mules. Demographic analysis revealed all reactive animals were mature working equids (10-25 years) without apparent sex predilection.

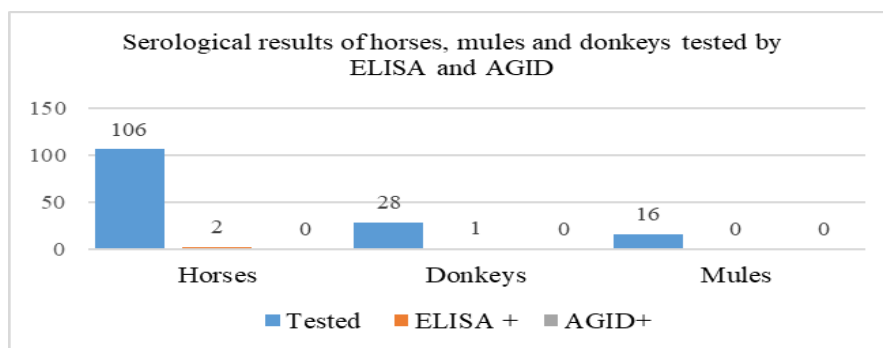


Figure 1. Seroprevalence of EIAV antibodies in equids from southeastern Albania by species, as detected by ELISA screening and AGID confirmation tests.

All laboratory test results from the Faculty of Veterinary Medicine in Tirana were identical to those of the reference laboratory in Skopje.

Discussion

Equine Infectious Anemia (EIA) is a significant disease affecting equine species worldwide and has been listed as one of the 11 equine-specific diseases by the World Organization for Animal Health (WOAH, 2023; More et al., 2008). Despite being recognized in many European countries between 2007 and 2014, including Belgium, Bosnia and Herzegovina, Croatia, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Romania, Serbia, Slovenia, and the United Kingdom, it remains endemic in certain regions such as Italy and Romania. This indicates the ongoing circulation of the virus within the European Union, despite active surveillance systems, including systematic yearly testing of equine animals in these countries.

EIA prevalence in Europe remains variable. In Italy, during 2007–2012, a seroprevalence of 0.18% was detected in over 1.3 million samples, with higher rates in mules than horses (Carvelli et al., 2024).

The seroprevalence of EIA showed a significant decline across all species and hybrids over the course of the study. Mules had a higher occurrence of EIA (IRR=48.90), while donkeys had a lower occurrence (IRR=0.56). Similarly, a long-term study in Serbia reported a 0.17% prevalence across 11,972 horses tested between 1994 and 2013, indicating low-level endemicity possibly controlled through systematic surveillance program implemented by Serbian authority since 1981 (Lupulovic et al., 2021). The results indicate that 21 horses tested positive for EIA in the southern regions of Backa and Srem. In Hungary, targeted surveillance in 2019 detected 6 seropositive cases from a national equine population of ~60,000, reaffirming the importance of continuous monitoring even when national prevalence is low. Data from the Western Balkans (2021–2023) suggest localized clusters of infection, particularly in regions with limited veterinary control (OIE, 2024).

In our country the disease has not been reported previously and the results of our study support these results. The present study provides the first serological evidence regarding the absence of Equine Infectious Anemia (EIA) in Albania. We emphasise the fact that equine species in Albania are bred extensively and the majority of the species is found in rural areas and in low numbers per farm/animal keeper. Moreover the contact from farm to farm is rare. Despite the negative findings, EIA remains a notifiable disease under OIE regulations and a concern due to its epidemiological implications. The presence of cases in neighboring countries such as Greece, North Macedonia, and Serbia, where sporadic outbreaks have occurred in the last decades, underscores the risk of transboundary spread, particularly in regions with equine movements or inadequate surveillance (Koptopoulos, 1999). In 2016 and 2018, two cases of EIA were identified in Kastoria, Greece, while six cases were reported in Edessa, Greece, in 2019, 2023, and 2024.

These regions are geographically close to southeastern Albania, an area with active animal movement across the border. In North Macedonia, for example, sporadic seropositive cases were identified between 2017–2021 in regions bordering Albania, though not always confirmed with molecular techniques (OIE, 2024). Although the clinical transmission risk is relatively low especially during the in apparent carrier stage of infection the infected animals remain reservoirs of the virus. Immunosuppression or stress can reactivate viral replication, posing a risk for horizontal transmission via hematophagous insects (Bolfa et al., 2016). Such dynamics demand vigilance even in apparently disease-free zones, as highlighted by past Romanian experiences where undetected carriers remained infectious despite long periods of stability (Bolfa et al., 2016).

Thus structured epidemiological studies would be of great interest in order to accurately determine the spread of the disease in the Balkan's region and beyond. The FPA test is a relatively simple and rapid test with high sensitivity and specificity (Espasandin et al., 2021). It is compatible with AGID and provides the results within 10 minutes compare to 2-3 days of Coggins test. Despite we did not found any positive cases to validate the FPA verse AGID test, the positive control sample provided generously by EIA reference laboratory were strong positive based on delta mP value. Among the 150 equine serum samples tested, 2% (95% CI 0.7 to 5.7) were positive in the ELISA test, but none were confirmed by the AGID, the confirmatory test for EIA diagnosis (Issel et al., 2013). This suggests the absence of active infection and highlights the importance of confirmatory testing to rule out false-positive ELISA results, a phenomenon well-documented in international studies due to cross-reactivity with p26 antigens (Cook et al., 2013).

However, Albania faces specific vulnerabilities that merit attention. Although the study area did not confirm any active infections, the broader national picture is still unknown. Albania lacks a systematic surveillance program for EIA, and no national sero-monitoring scheme is currently implemented. This absence of routine screening is partly due to limited financial resources, institutional capacity, and the historically low perceived risk for the disease. Such assumptions, however, risk underestimating the true level of exposure, particularly given the informal and often unregulated movement of equids across borders, often without veterinary certification.

The country's equine sector is characterized by smallholder ownership, lack of identification and registration systems, and limited veterinary oversight in rural and mountainous areas. These structural limitations amplify the risk for silent introduction and undetected spread of EIA. Informal trade, shared pastures, and lack of biosecurity awareness contribute further to the vulnerability. In this context, even a single in apparent carrier could pose a threat, especially where vector control is minimal and veterinary infrastructure is weak. No active cases of Equine Infectious Anemia (EIA) were confirmed in this study; however, several ecological and structural risk factors indicate that Albania remains potentially vulnerable to the introduction and silent spread of the disease.

The country's equine population is largely kept under extensive management systems, particularly in rural and mountainous areas, where horses and mules are used for transport and agricultural work. These animals often share pastures, lack official identification, and move across unmonitored borders, especially in southeastern and northeastern regions adjacent to Greece and North Macedonia, where sporadic EIA cases have been reported in recent years. The presence of hematophagous insects such as *Tabanus* spp. and *Stomoxys* spp. in these ecological zones further increases the risk of vector-borne transmission. Combined with the lack of a national surveillance program, weak veterinary infrastructure, and minimal biosecurity practices, these environmental and socioeconomic conditions constitute key ecological risk factors for EIA introduction and persistence in Albania.

The ELISA screening revealed a limited number of seropositive equids; however, none of these cases were confirmed by AGID, indicating either false positives or animals in the early stages of seroconversion.

This ELISA+/AGID- trend is not uncommon in low-prevalence areas or where nonspecific reactivity may occur. The absence of AGID-confirmed positives suggests that there were no ongoing or confirmed cases of infection in the study population at the time of testing. Because of the small number of positives, statistical comparisons between species (for example, horses vs. mules) produced undefined odds ratios and relative risks. After applying a continuity adjustment, the estimated odds ratio for ELISA positivity in horses versus mules was 0.79, however this finding is not statistically significant due to the small sample sizes.

These data support the conclusion that the examined equid population is now free of confirmed infection; however, ongoing surveillance is critical for early diagnosis and disease prevention. Given Albania's fragmented equine husbandry, widespread screening is challenging due to financial and logistical constraints.

A cost-effective approach would focus on targeted surveillance in high-risk areas, using mobile diagnostic units, local veterinarians, and pooled sample testing. Raising awareness and integrating EIA testing in routine visits could help build a national risk map. Discussions suggest shifting from general serological screening to more strategic, molecular approaches in resource-limited settings like Albania (Issel et al., 2013).

CONCLUSIONS

This is a first approach that aims to evaluate the presence of Equine Infectious Anemia in Albania. The results from the seroepidemiological study indicate the absence of specific immunoglobulins against the p26 protein of the EIA virus, as tested by the Agar Gel Immunodiffusion Assay (AGID) and Fluorescence Polarisation Assay (FPA). EIA appears to be absent or present at a very low prevalence in the tested equine populations. The absence of AGID-confirmed positives suggests that no active or confirmed infections were circulating at the time of sampling. Nonetheless, continued surveillance is warranted especially in light of the ELISA findings to monitor for potential early infections or emerging risk in specific areas or species. Despite a small percentage of samples testing positive in the initial ELISA screening, none were confirmed positive by AGID, further supporting the conclusion that the country is free from EIA. Clinical signs observed in some animals were not associated with EIAV infection. The consistency between local and reference laboratory results reinforces the reliability of findings. While the results strongly suggest Albania's EIA-free status, the proximity of the disease in neighboring countries such as Greece and North Macedonia, combined with Albania's limited surveillance capacity, underlines the need for continuous monitoring. The study will proceed with sampling a wider equine population, including the retesting of any animals that may test positive during initial screening. Strengthening surveillance adapted to Albania's veterinary infrastructure is crucial to document and maintain the country's EIA-free status.

Acknowledgments. We would like to express our sincere gratitude to the laboratory team at the Faculty of Veterinary Medicine in Skopje for their invaluable support in conducting the ELISA and AGID tests on equine samples. Their efforts played a crucial role in ensuring the reliability and accuracy of the findings presented in this study.

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