2022 WORKSHOP OF THE NRLS FOR EQUINE DISEASES

EQUINE INFECTIOUS ANEMIA IN ARGENTINA: Surveillance Program and Epidemiological Situation

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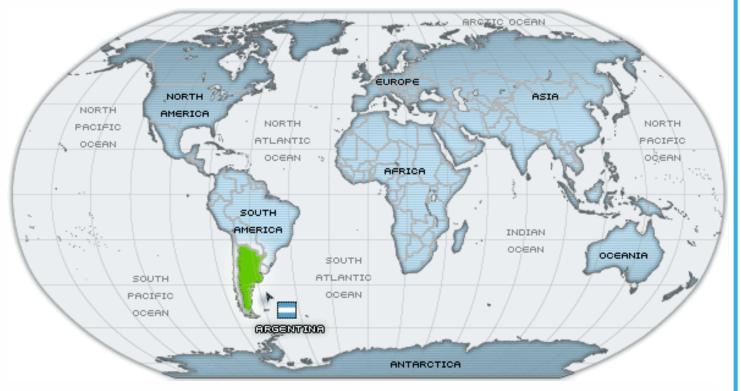




Instituto Nacional de Tecnología Agropecuaria Argentina

Republic of Argentina

- 44 million inhabitants
- Continental surface: 2,780,092 km²
- From north to south: 3,700 km long
- Climate: predominantly temperate, but with extremes ranging from subtropical in the north to subpolar in the far south
- Official language: Spanish







Instituto Nacional de Tecnología Agropecuaria Argentina

National Organizations



Ministerio de Economía Secretaria de Agricultura, Ganadería y Pesca



SERVICIO NACIONAL DE SANIDAD Y CALIDAD AGROALIMENTARIA



Instituto Nacional de Tecnología Agropecuaria

O 44 experimental units

240 extension units



13 research institutes

11 in Castelar (Bs. As) & 1 in Córdoba + 1 Economy & Sociology Institute

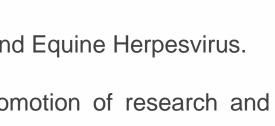






INTA - Equine Virology Lab

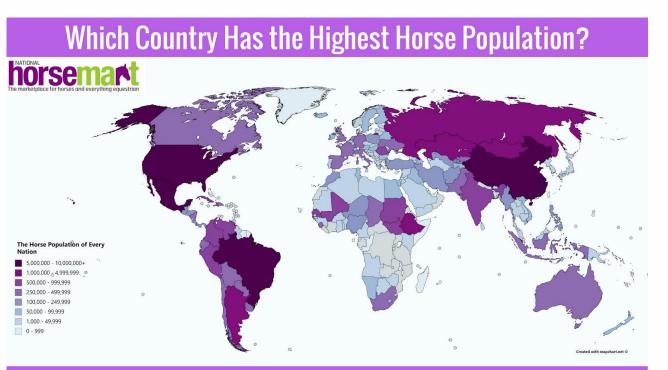
- Specialized diagnostic service on equine viral diseases.
- Permanent epidemiological surveillance on Equine Arteritis, Equine Influenza, West Nile Virus, Equine Rotavirus, Equine Herpesvirus, etc.
- Representation before national and international organizations:
 - National Commission of Equine Health and Wellness (SENASA)
 - Expert Surveillance Panel on Equine Influenza (WOAH).
- Technology transfer for the production of equine viral vaccines.
- Equine Infectious Anemia Network Lab (L032 SENASA).
- Lines of research in Equine Viral Diseases, focused mainly on Equine Rotavirus and Equine Herpesvirus.
- Since 1992: agreement between INTA and Thoroughbred breeders for the promotion of research and diagnosis on Equine Viral Diseases.







Equine Production





2.599.882 heads

• Argentina is among the 10 countries with the largest horse populations in the world.

 It occupies the 4th place amongst Thoroughbred horse producing countries (after the United States, Australia and Ireland).

- It is the main polo horse producer in the world.
- Horse live and purebred exports in 2020: 5.94M USD.





Equine Infectious Anemia (EIA) and the Equine Industry

The first report of clinical signs associated with EIA occurred in 1843 (Lignee, 1843).

EIA has an almost worldwide distribution.

EIA is of considerable importance to the equine industry, being one of only eleven notifiable equine specific diseases listed by the World Organization of Animal Health (WOAH).

It is most commonly transmitted by large hematophagous insect vectors (horse flies and deer flies). Thus, the prevalence is higher in regions with warm climates.

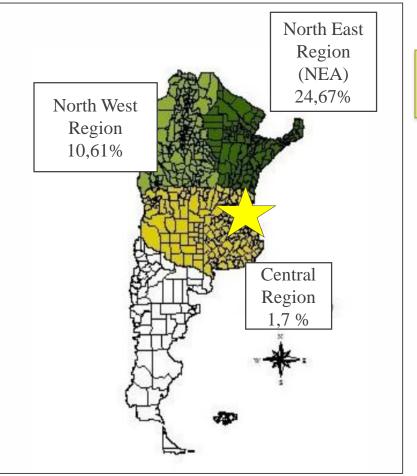
The virus could be also transmitted via iatrogenic route.

There are currently no vaccines against EIA virus in clinical use (China: live attenuated vaccine 1975-1990).

EIA is still **a threat to equine health** throughout the world and continues to impact domestic and international equine movement and trade.



EIA in Argentina



* E. Durante, A. Chiricosta, B. Cosentino, A. Marcos, A. Perez, E. León. **"Estimación de la prevalencia de Anemia Infecciosa Equina en tres zonas infectadas de la República Argentina".** La Especie Equina, 2015.

EIA is endemic in the northern area of Argentina.



The epidemiological situation varies across the country, with a **low prevalence in the central region** and a disease-free status in Patagonia (southern region).

The **main horse breeding area** (Buenos Aires and Córdoba provinces) is located in the **Central Region of Argentina**; thus the importance of mantaining a low prevalence in this region.





National Legislation

- ✓ Control and Eradication of Equine Diseases Programme (*Res. SENASA 617/05*)
- ✓ Adaptation to the International Animal Health Code (Res.SENASA 422/03)
- ✓ 230 Network Labs (accredited by SENASA)
- ✓ 3965 Accreditted Veterinarians
- Health Control Regulations:



World Organisation for Animal Health Founded as OIE

- Terrestrial Animal Health Code
 Terrestrial Manual
- All horses that are mobilized must be tested for EIA and transit with the corresponding certification;
- The officially recognized test for diagnosis is AGID, only performed by Network Labs, and certification is valid for 60 days from sampling date;
- Mandatory notification to SENASA of EIA positive horses;
- Horses that test positive for EIA and present clinical signs must be euthanized within 48 hours. In the endemic area, those horses that test positive for EIA without clinical signs can be euthanized or permanently isolated.
- Mandatory interdiction and analysis of the premise in which EIA positive equid was found.



National Legislation

- <u>2017:</u> Official EIA-free Status in Patagonia Region (*Res. SENASA 386/17*)
- During 2014-2015, 1235 animals from 136 production units, including racetracks, equestrian clubs and stud farms, all with a high rate of movement of horses or that had entered horses from areas other than Patagonia, were analyzed.
- In total, between 2012 and 2015, 4,346 samples from horses located in North Patagonia, South Patagonia and South Buenos Aires Regional Centers, were analyzed
- All resulted negative.







National Legislation

Control Zone for Equine Infectious Anemia Draft Resolution

Involving the region comprised of the Provinces of Misiones, Corrientes and Entre Ríos.

In the Northeast area of Argentina, working equids are still important agricultural animals and EIA is endemic (24,7%).

Then, strategies other than euthanasia without compensation should be considered in this region.

Premise infected

Premise undergoing sanitation

Premise sanitized

Sports and recreation premises

Premise with unknown status

EIA virus infection is still a big concern in Argentina as the intense movement of horses is a permanent threat to the purebred horse population.



What happens in the central region?

The central region is comprised of the Provinces of Buenos Aires, Córdoba, Mendoza, La Pampa and south of Entre Ríos. All these have a template climate.

Main area of breeding activities of Thoroughbred, Jumping and Polo horses (Embryo transfer centers), and sports facilities: racetracks, jumping clubs, polo fields, shows, amongst others

Frequent movement of horses from northern area to central area of Argentina.

Outbreaks in this region are of great concern for the horse industry.



Above: main breeding área Below: sports activities





From research to a diagnostic kit





Available online at www.sciencedirect.com

ScienceDirect

Veterinary Microbiology 121 (2007) 344-351

veterinary microbiology

CICVYA

Instituto de Virología

www.elseviet.com/locate/vetmic

Short communication

Standardization and validation of an agar gel immunodiffusion test for the diagnosis of equine infectious anemia using a recombinant p26 antigen

I. Alvarez*, G. Gutierrez, A. Vissani, S. Rodriguez, M. Barrandeguy, K. Trono Instituto de Virología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas, INTA, C.C. 1712 Castelar, Argentina Received 11 July 2006; received in revised form 5 January 2007; accepted 9 January 2007

CLINICAL AND VACCINE IMMUNOLOGY, Dec. 2007, p. 1646–1648 1556-6811/07/\$08.00+0 doi:10.1128/CVI.00293-07 Copyright © 2007, American Society for Microbiology. All Rights Reserved. Vol. 14, No. 12

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journal homepage: www.elsevier.com/locate/jviromet

Journal of Virological Methods

Western Blot Assay Using Recombinant p26 Antigen for Detection of Equine Infectious Anemia Virus-Specific Antibodies[⊽]

I. Alvarez,¹* G. Gutierrez,¹ E. Ostlund,² M. Barrandeguy,¹ and K. Trono¹ Instituto de Virología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas, Instituto Nacional de Tecnología Agropecuaria, C.C. 1712, Castelar, Argentina,¹ and National Veterinary Services Laboratories, P.O. Box 844, Ames, Iowa 50010²

Received 17 July 2007/Returned for modification 18 September 2007/Accepted 16 October 2007

We analyzed the performance of a single-band Western blot (WB) test using recombinant p26 (rp26) capsid protein of equine infectious anemia virus. According to the results obtained, the rp26 WB test is a reliable confirmatory diagnostic tool to be used as a complementary test after an enzyme-linked immunosorbent assay or agar gel immunodiffusion test yielding doubtful results. Immunochromatographic lateral flow test for detection of antibodies to Equine infectious anemia virus

I. Alvarez*, G. Gutierrez, M. Barrandeguy, K. Trono

Instituto de Virología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas, INTA, CP 1686, Hurlingham, Buenos Atres, Argentina





Techonology transfer: KIT EIA rp26 IDGA IncuINTA



10 years of comercialization by INTEA SA Approved by SENASA 18 series produced Used by 25 SENASA Network labs with 570.000 samples analyzed (SENASA L032 – INTA EQUINE VIROLOGY LAB)

EURL Interlaboratory proficiency test 2018 EURL Interlaboratory proficiency test 2022

Kit IncuINTA







Detection of Equine Infectious Anemia virus by insulated isothermal RT-PCR (iiRT-PCR) assay using the POCKIT TM Nucleic acid analyzer

M. Barrandeguy, G. Espasandin, I. Alvarez, <u>A. Vissani</u>, F. Cipolini, D. Martinez, S. Chung, Y. Tsai, and others. *10th International Equine Infectious Diseases Conference (IEIDC X).* Buenos Aires, Argentina, April 4-8, 2016. Journal of

Equine Veterinary Science, Vol. 39, S7-S8

- Clinical samples included serum, whole blood and buffy coat collected from 165 horses distributed in an endemic (n: 53), a sporadic (n: 92), and a free (n: 20) EIA zone based on previous prevalence studies performed in Argentina.
- The sensitivity of the EIAV iiRT-PCR assay to detect infected horses was assessed on whole blood and buffy coat samples, and compared with the AGID test.

		AC			
	Buffy coat samples	Positive	Negative	TOTAL	
iiRT-PCR	Positive	31	1	32	
	Negative	25	108	133	
	TOTAL	56	109	165	-

Total agreement was 84.24%



Outbreak in San Nicolas - 2012

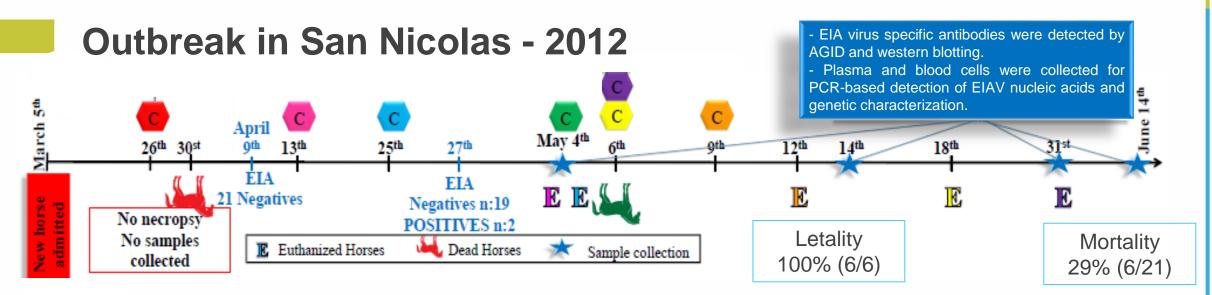
• The facility is located on the banks of the Ramallo stream, 260 km northwest of the city of Buenos Aires, Argentina.

- Horseflies are abundant, mainly in the summer season (December to March).
- The premises have a barn with stalls for all the animals (n:21) and encompase 18 hectares of land.
- Clinical signs: fever, depression, apathy, weight loss, anorexia.
- Test for EIA was performed every 60 days in the whole population.

An outbreak of Equine Infectious Anemia at a horse riding center in Argentina underscores the limitations of serological testing. D Duclós, MA Vissani, I Alvarez, C Olguín Perglione, SJ Cook, F Cook, ME Barrandeguy. International *Conference on Equine Infectious Diseases IX.* 21 al 26 de Octubre de 2012,

Lexington, Kentucky, USA.





	May 4th		May 14 th		May 31 ^{at}		June 14 th	
HORSE	AGID	WB	AGID	WB	AGID	WB	AGID	WB
Baral Hiorito	-	weak	-	weak	-	weak	-	weak
Noble Rxpression	-	weak	-	weak	-	weak	-	weak
La Nena	-	-	-	-	-	-	-	-
El Gordo	-	weak						
Pegasus Sumatra	-	-	-	-	-	-	-	-
Noble Harold	-	-	-	-	-	-	-	-
Rainy City	-	-	-	-	-	-	-	-
Baral Martina	-	-	-	-	-	-	-	-
Noble Harry	-	-	-	-	-	-	-	-
Tiny Mister Conclusive	-	weak						
Pegasus Paris	-	-	-	-	-	-	-	-
Alfil	-	-	-	-	-	-	-	-
Aquiles	-	-	-	-	-	-	-	-
Santino	-	-	+	+				
Mini	-	-	-	-	-	-	-	-
Corcho	-	-	-	-	-	-	-	-
Ce Efe Erre Cluy	+	+						
Bilbao	+	+						
Noble Heralty	+	+						

ELAV sequences within the viral genome (U5 of the LTR to TAT) from the 3 clinical cases

The timeline of events coupled with the fact that a stable population of horses/ponies had
previously and repeatedly tested negative for EIA strongly suggests the riding center
outbreak was caused by the horse introduced on March 5th.

- The fact this horse possessed a negative EIA certificate illustrates a deficiency of indirect serological testing, namely the delay between pathogen exposure and production of detectable antibodies. Therefore, horses can be infected with EIAV and pose a considerable threat for transmission long before the production of antibodies measurable by AGID.
- Transmission was facilitated by high horsefly vector numbers in the summer months (December to March) caused by proximity of the riding center to water.
- Preliminary results on molecular characterization showed that the strains isolated from this
 outbreak are different from the molecular clone ELAV UK.

This outbreak of ELA in Argentina's main horse-breeding region is an infrequent event and is of great concern for the industry in general.

Antibody detection by AGID and western blotting

Outbreak in Baradero - 2015

• The facility is located on the banks of the Ramallo stream, 150 km northwest of the city of Buenos Aires, in Argentina.

- Horseflies are abundant mainly in the summer season (December to March).
- 124 crossbred horses (Criollo, Arabian and Quarter horses); 109 adults, 15 foals.
- Not tested for EIA in the previous 3 years.
- No EIA control on animals admitted into the farm.
- Clinical signs: death, with no attributable causes, of 10 horses during 2014-2015.

Diagnosis and control of Equine Infectious Anemia in a horse farm located in Buenos Aires province, Argentina. <u>MA Vissani, J</u> Reynal O'Connor, C Olguin Perglione, S Traverso , G Gutierrez, I Alvarez, M Barrandeguy. *10th International Equine Infectious Diseases Conference (IEIDC X)*. Buenos Aires, Argentina, April 4-8, 2016

Journal of Equine Veterinary Science, Vol. 39, S13







Outbreak in Baradero - 2015

Number of animals analyzed and AGID results since the first detection of EIA in the farm.

	March 21 st	May 15 th	June 23 rd	July 30 th	August 28 th	September 17 th	October 5 th	November 11 th	
Horses tested	Adults				Adults and sucklings				
	109	83	67	68	83	82	82	82	
AGID Positives	24	20	5	3	1	0	0	0	
Prevalence	22%	24%	7%	4%	1%	0%	0%	0%	
		Contracted the infection dur period or were already inf when introduced into the			fected	The farm was quarantined for 60 days after the last negative result, as required by SENASA legislation.			

*Samples of serum and buffy coat, and the spleen of one horse corresponding to this outbreak were sent to Dr. Hans Aymeric, Laboratoire de Pathologie Equine de Dozulé, ANSES (2015) for a collaborative proyect.



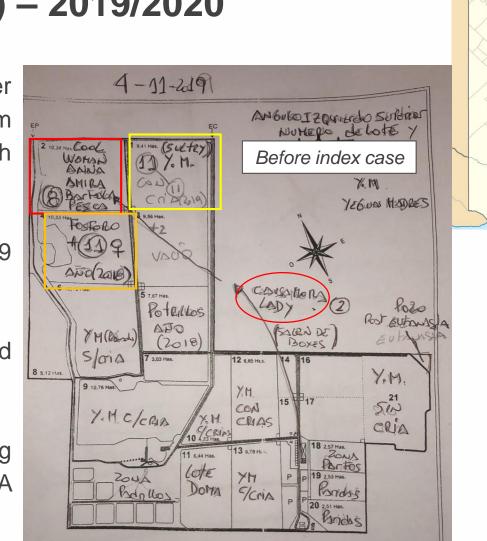
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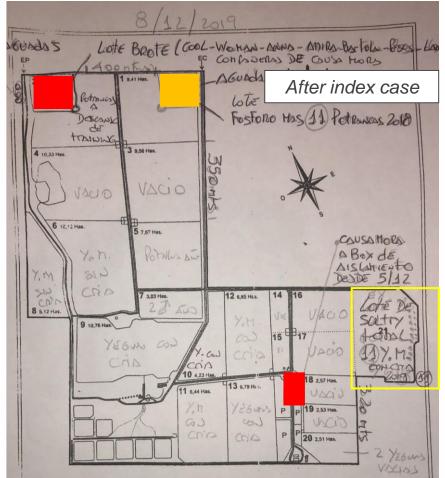
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Outbreak in Solís (Bs. As.) - 2019/2020

- The outbreak took place between December 2019 and June 2020, in a Thoroughbred farm located in Solís, 100 km from Buenos Aires, with regular entry and exit of horses.
- 170 hectares, with 160 adult horses and 37 2019 season foals.
- A training mare from the city of Campana tested negative to EIA by AGID on October 28th, 2019.
- On December 3rd, this mare started showing fever and depression and tested positive to EIA by AGID.



Outbreak in Solís (Bs. As.) – 2019/2020



Mares at **paddock 2** were restringed in a smaller place, were tested weekly by iiPCR and isolated if hyperthermia appeared.

Yearlings at **paddock 4** were relocated to a smaller place in paddock 1 and were tested by AGID every 15 days.

Mares at **paddock 1** were relocated to paddock 21 and were tested by AGID every 15 days.

	Paddock	Date of EIA diagnosis	Clinical signs
Causa Mora	2	5/12/2019	+
Cool	2	18/12/2019	+
Fósforo	4	4/1/2020	-
Lady Pop	2	3/1/2020	+
Woman	2	3/1/2020	+
Anna	2	16/1/2020	+
Amira	2	28/1/2020	+
Sultry con cria	1	7/2/2020	-
Bartola	2	9/3/2020	-
Pesca	2	16/3/2022	-
Malavita con cría	1	11/4/2020	-



Other outbreaks with devastating consequences

Alerta en Córdoba por la presencia de anemia infecciosa equina

El Senasa procedió al aislamiento de los caballos en un establecimiento de Villa María y se aguarda el resultado oficial de los test.

Por Agrofy News





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Clarin **Rural**

Palermo Racetrack, 2019

Se confirmó un caso de anemia infecciosa equina en el hipódromo de Palermo

Se trata de un caballo proveniente de Entre Ríos que, luego de proceder a un nuevo muestreo del animal, se confirmó el diagnóstico positivo por lo que debió ser sacrificado



senasa

💿 Instagram

SENASA COMUNICA	
REPORTE RADIO	

SENASA EN LOS MEDIOS INFOGRAFÍAS

2015

Se detectaron 5 casos de anemia infecciosa equina en el hipódromo de San Isidro Agentes de la Dirección Nacional de Sanidad Animal del Senasa interdictaron 233 caballos de polo para realizar los análisis serológicos correspondientes.

El Servicio Nacional de Sanidad y Calidad Agroalimentaria (Senasa) informa que se detectaron 5 casos positivos de anemia infecciosa equina [AIE] en el hipódromo de San Isidro de Buenos Aires. Agentes de la Dirección Nacional de Sanidad Animal del Senasa interdictaron 233 caballos de polo para realizar los análisis serológicos correspondientes a la totalidad de los animales.

El Senasa enviará las muestras tomadas al Laboratorio, con el objetivo de determinar si existen más

Anemia Infecciosa Equina: Senasa detectó un brote en Lincoln

El organismo confirmó a comienzos de agosto que 54 equinos dieron positivo y fueron enviados a faena, como marca el protocolo. Se encontró un nexo epidemiológico con un campo de Teodelina, en Santa Fe. Lincoln, Bs. As., 2020

Por Miguel Vencius - EL ABC RURAL - 17 agosto, 2020







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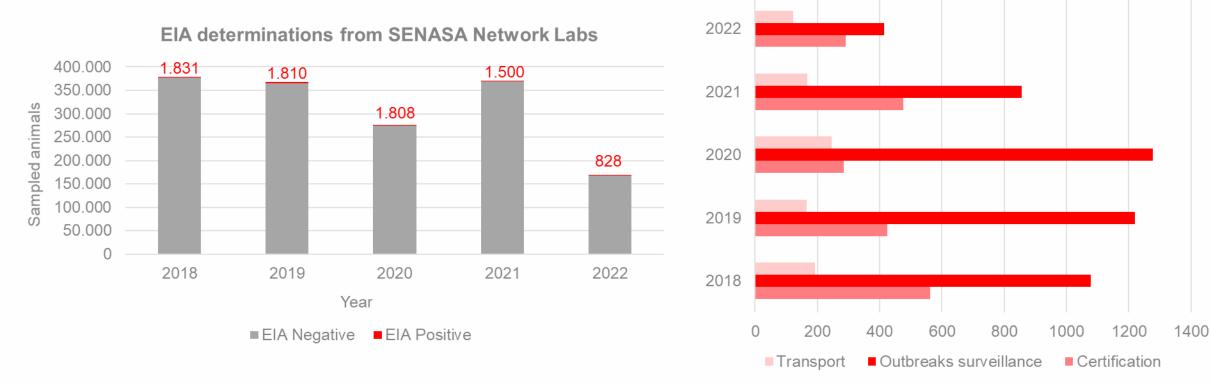
También cabe mencionar que simultáneamente se está investigando el origen del problema para actuar rápidamente y evitar la difusión de la enfermedad.

animales enfermos entre los interdicatados

Todo este procedimiento se realiza en cumplimiento de la normativa sanitaria vigente San Isidro Racetrack



EIA Results of SENASA Network Labs 2018-2022

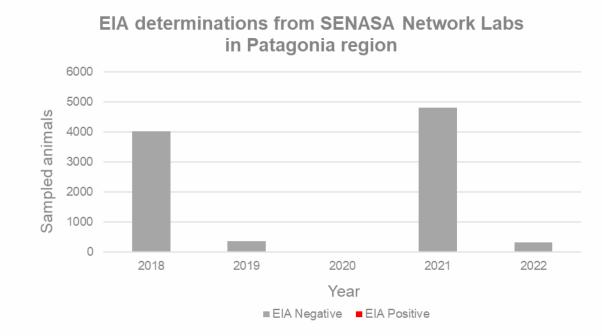


EIA positive results: reasons for sampling

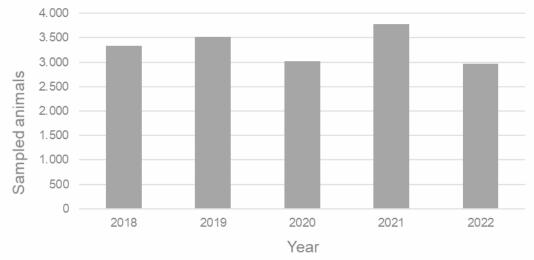




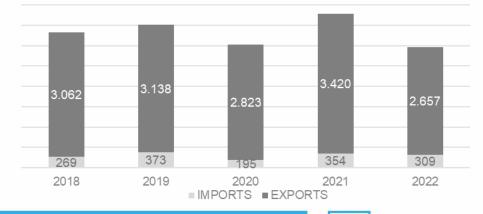
EIA Results of SENASA Network Labs 2018-2022



EIA determinations from imports and exports



■ EIA Negative ■ EIA Positive





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Final remarks

This presentation emphasizes the importance of sustaining EIA surveillance in the area considered as "low prevalence" in Argentina, as this area is where the most valuable horses are bred in Argentina.

The Thoroughbred, Polo Pony and Jumping horse populations are controlled periodically, and the prevalence of EIA in this valuable horse population is extremely low.

However, it is necessary to bear in mind the fact that it is mandatory to strictly comply with legislation, mostly for crossbred horses and for horses moving from high EIA prevalence areas to a low prevalence area.

All those horses that have been exported tested negative for EIA, and Argentina has never had a rejection in another country due to a positive animal at destination.

Horses can be infected with EIAV and pose a considerable threat for transmission long before the production of antibodies is measurable by AGID. Then, in EIA outbreaks in the low prevalence area of our country, Western blot and iiPCR could be considered alternative diagnostic tools in the implementation of a control strategy.





EQUINE VIROLOGY LAB

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SENASA Control and Eradication of Equine Diseases Programme Mario Iturria

Ximena Melón



Investigate, evaluate, protect

Stéphan Zientara José Valle-Casuso Gaëlle Gonzalez Delphine Gaudaire



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