Serological evidence of equine infectious anaemia, West Nile fever, surra and equine piroplasmosis in a herd of horses in northern Argentina

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Running title: Serological survey of a herd in Argentina

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Abstract

Northern Argentina hosts equine populations living under preserved natural areas and extensive breeding conditions, with limited access to veterinary care. Horses can be in contact with *i*) wildlife considered to be a potential reservoir of horse pathogens (e.g. capybara, coatis and pampas deer) and/or *ii*) potential disease vectors such as ticks, horse flies, *Culicidae* and vampire bats. In this context, the aim of this study was to assess the exposure of horses from a herd in northern Argentina to different vector-borne pathogens.

Serum samples were collected from 20 horses on a farm in Chaco province. Most of these horses were in good health, but a few showed clinical signs such as fever, neurological signs or emaciation. Potential vectors (ticks, horse flies and *Culicidae*) were present and a fresh bite of a vampire bat (*Desmodus rotundus*) was observed on one horse. This serological survey revealed that 100% (20/20) were positive for equine infectious anaemia (EIA), 100% (18/18) for West Nile fever (WNF), 53% (10/19) for surra and 45% (9/20) for equine piroplasmosis (*Babesia equi*). Among these horses, four were found seropositive for all four infections. On the other hand, all the tested horses were seronegative for equine viral arteritis (EVA), Eastern equine encephalomyelitis (EEE), Venezuelan equine encephalitis (VEE), Western equine encephalomyelitis (WEE) and glanders.

The data from this survey conducted on a small number of animals illustrate the need for an effective application of surveillance programmes and control measures for equine diseases in northern Argentina and constitute, to our knowledge, the first report of horses simultaneously seropositive for EIA, WNF, surra and equine piroplasmosis.

Keywords

Argentina, equine infectious anaemia, piroplasmosis, surra, West Nile fever

Short communication

The province of Chaco in Argentina is characterised by subtropical weather with a dry season in a landscape composed of humid forest, savannas, gallery forests and flooded forests. In this rural environment, the economy is based on agriculture and extensive livestock activities (predominantly cattle and horses), but access to veterinary care such as vaccinations and treatment or confirmatory diagnosis remains limited for some horse populations. This farming method implies that domestic animals are frequently in contact with wild animals and exposed to numerous vectors. The reported wildlife includes numerous animals considered to be potential reservoirs for horse pathogens, including capybara (*Hydrochoerus hydrochaeris*) (Herrera and Castro, 2017), ring-tailed coati (*Nasua nasua*) (Santos et al., 2018), pampas deer (*Ozotoceros bezoarticus*) (Silveira et al., 2013), black howler monkeys (*Alouatta caraya*) (Martínez et al., 2016) and various bird species. The presence in this geographical area of disease vectors such as ticks (*Ixodidae*) (Nava et al., 2006), horse flies (*Tabanidae* and *Stomoxys* flies), *Culicidae* (Stein et al., 2013) and vampire bats (*Desmodus rotundus*) (Hoare, 1965) is also documented. Although the number of field studies dedicated to the pathogens circulating in this province remains low, it may be noted that numerous vector-borne diseases have already been reported. These include equine infectious anaemia (EIA) (De la Sota et al., 2005), West Nile fever (WNF) (Oria et al., 2018), surra (Santa Cruz et al., 2013), equine piroplasmosis (*Babesia equi*) (Holman et al., 1998), Eastern equine encephalomyelitis (EEE), Venezuelan equine encephalitis (VEE) and Western equine encephalomyelitis (WEE) (Monath et al., 1985).

In a context where domestic and wild animals cohabit with pathogens and vectors, our objective was to evaluate the prevalence of various major equine diseases notifiable to the OIE (World Organisation for Animal Health) in a herd of horses from the northern

Argentinean province of Chaco. The diseases investigated were EIA, WNF, surra, equine piroplasmosis, glanders, EVA, EEE, VEE and WEE.

The study was conducted on a 3,000-hectare farm in the municipality of Laguna Limpia in the province of Chaco, Argentina (Fig. 1). The area comprises native forest, ponds and flooded areas, and a diverse fauna including capybaras, coatis, deers, howler monkeys and cattle, together with the presence of common vectors including ticks, tabanids, stomoxes, *Culicidae* and vampire bats. Following concerns expressed by farmers about the general health of their animals, serum samples were collected in June 2018 from 20 horses randomly selected in a herd in preserved natural areas and extensive breeding condition made up of 48 mixed-breed horses of all ages (Fig. 2). These horses have regular access to food but limited access to veterinary care. The only pharmaceutical products administered to our knowledge are mebendazole, and vitamin B12 and iron supplements. None of the horses were vaccinated. They were sampled regardless of their physical condition. Their general health, rectal temperature and clinical signs such as oedema and ataxia were recorded. Blood samples were collected aseptically by jugular venipuncture and either collected in glass tubes and centrifuged at 1600 xg for 15 min for serum collection or in EDTA tubes to monitor the packed cell volume (PCV), white blood cells (WBC), neutrophils and lymphocytes (Supporting Information Table S1).

The serological methods used in this study are listed in Table 1, and were performed in accordance with the corresponding chapters in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2015). Inconclusive results (cytotoxicity and anticomplementary) were excluded for calculation of seropositivity rates.

Of the 20 horses sampled in this study, 60% (12/20) were of average or poor general health, and 90% (7/8; the temperature of the other animals was not recorded) had a fever ($\geq$ 38.5 °C) (Supporting Information Table S1). The observed clinical signs (Fig. 2) included oedema, nasal discharge, emaciation, depression, recumbency and neurological signs (Fig. 2 and Supporting Information Table S1). Moreover, cases of abortion had previously been reported on this farm. An abundant population of haematophagous flies (*Tabanidae* and *Stomoxys*), *Culicidae* and ticks (*Amblyomma cajennense*) were observed at the same time in the horses' environment, and a fresh vampire bat bite was observed on one horse's neck (Fig. 2F).

In this environment conducive to the dissemination of vector-borne diseases, we evaluated the prevalence of antibodies against EIA, WNF, surra, equine piroplasmosis, glanders, EVA, EEE, VEE and WEE. Results are presented in Supporting Information Table S1 and summarised in Fig 3.

The seroprevalence values were 100% for EIA (20/20) and WNF (18/18), 53% (10/19) for surra and 45% (9/20) for equine piroplasmosis. Interestingly, four horses were seropositive for all four diseases. All the animals tested were seronegative for glanders, EVA, EEE, VEE and WEE (Fig. 3A and Supporting Information Table S1).

EIA is widespread in northern Argentina, with infection rates of 39% and 76% reported in the provinces of Chaco and Formosa, respectively (De la Sota et al., 2005). However, this is the first time to our knowledge that a seroprevalence value for EIA of 100% is reported for a given herd. This potential EIA reservoir should be of great concern for the horse-breeding industry in a context where EIA is covered by strict regulatory control measures in many countries.

WNF was first detected in Argentina from diseased and dead horses in 2006 (Chancey et al., 2015) and, since then, its circulation has been reported in different provinces in Argentina, including Chaco (Oria et al., 2018). Given the abundance of *Culicidae* (vectors of WNF) and birds (hosts, natural reservoir) in this region (Campbell et al., 2002), there appears to be a major risk of this virus being transmitted to incidental dead-end hosts represented by horses (and potentially humans). This concern is illustrated by our results, which revealed seroprevalence for WNF of 100% within our panel. Even if such a level of seroprevalence has already been reported for WNF in equids in Morocco, Pakistan, Palestine and Iran (Eybpoosh et al., 2019), this is, to our knowledge, the first time that a whole group of equids have been detected as seropositive for WNF in Argentina. Besides, even if the WNF microneutralization test is highly specific for WNF, WNF belonging to Japanese encephalitis serocomplex, serological cross reaction with Saint Louis encephalitis virus can't be totally ruled out.

Surra (formerly referred to as "mal de caderas" in South America), caused by *T. evansi*, has been circulating in Argentina for decades. It not only affects horses but also wildlife such as capybaras and domestic animals such as dogs (Aregawi et al., 2019). In this study, we determined the percentage of horses seropositive for surra by immune trypanolysis (IT) RoTat 1.2, which detects only antibodies that recognise a single multi-copy epitope at the surface of a *T. evansi* type A clone expressing the RoTat 1.2 variant surface glycoprotein (Verloo et al., 2001). This test is the reference test for surra antibody detection due to its very high specificity and thus positive prediction value (Holland et al., 2002). Given the severity of this disease and the absence of treatment or a vaccination strategy, the high level of seropositivity detected in this herd (53%) is a finding of serious concern not only for this herd but for the whole equine industry in the province of Chaco.

Equine piroplasmosis is known to be endemic in Chaco (Holman et al., 1998). As complement fixation test (CFT) signals generally disappear 2 to 3 months post infection in infected horses (Weiland, 1986) the (CFT) results obtained in this study, with 9/20 animal tested positive, indicate that the disease is currently circulating in this area. Furthermore, we can assume that we would have obtained much higher prevalence rates if we had tested the sera with the indirect fluorescent antibody test, a method that gives positive results during latent infection (Weiland, 1986), and we could even expect 100% seroprevalence as previously described (Holman et al., 1998).

No obvious correlation between a specific disease and the general health of these horses was detected (Supporting Information Table S1).

The main findings of our study were the confirmation of the presence of antibodies against EIA, WNF, equine trypanosomis and equine piroplasmosis in horses indigenous to the province of Chaco, Argentina. In this context, the presence of vectors such as ticks, horse flies, *Culicidae* and vampire bats constitute an important potential source of pathogen dissemination that should raise significant concern among the Argentinean horse-breeding industry. Interestingly, four horses appeared simultaneously seropositive for EIA, WNF, surra and equine piroplasmosis. All the animals tested were seronegative for glanders, EVA, EEE, VEE and WEE. Despite the small scale of our study, our results illustrate the need for effective implementation of surveillance programmes and control measures to tackle equine diseases in northern Argentina.

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Conflict of interest statement

The authors declare no potential conflicts of interest with respect to the research, authorship, publication of this article and/or financial and personal relationships that could inappropriately influence this work.

Ethical statement

The authors confirm their adherence to the ethical policies of the journal, as noted on the journal's author guidelines page, and that the appropriate ethical review committee approval has been received. Procedures involving horses were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The protocol for the culture of trypanosomes in mice was approved by the Veterinary Ethical Committee of the Institute of Tropical Medicine Antwerp, Belgium (ITM) (BM2013-7).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aregawi, W.G., Agga, G.E., Abdi, R.D., Büscher, P., 2019. Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasit Vectors 12, 67.

Campbell, G.L., Marfin, A.A., Lanciotti, R.S., Gubler, D.J., 2002. West Nile virus. Lancet Infect Dis 2, 519-529.

Chancey, C., Grinev, A., Volkova, E., Rios, M., 2015. The global ecology and epidemiology of West Nile virus. Biomed Res Int 2015, 376230.

De la Sota, M.D., Gonzáles, R., Chiricosta, A., 2005. Contribución a la prevalencia de la Anemia Infecciosa Equina en la República Argentina. Revista Colegio de Médicos Veterinarios de la Provincia de Buenos Aires 10, 52-60.

Eybpoosh, S., Fazlalipour, M., Baniasadi, V., Pouriayevali, M.H., Sadeghi, F., Ahmadi Vasmehjani, A., Karbalaie Niya, M.H., Hewson, R., Salehi-Vaziri, M., 2019. Epidemiology of West Nile Virus in the Eastern Mediterranean region: A systematic review. PLoS neglected tropical diseases 13, e0007081.

Herrera, E.A., Castro, Y., 2017. *Trypanosoma evansi* (Kinetoplastida: Trypanosomatidae) in capybaras (*Hydrochoerus hydrochaeris*, Rodentia: Hydrochoeridae): prevalence, effect and sexual selection. Rev Biol Trop 65, 229-237.

Hoare, C.A., 1965. Vampire bats as vectors and hosts of equine and bovine trypanosomes. Acta tropica 22, 204-216.

Holland, W.G., Thanh, N.G., My, L.N., Magnus, E., Verloo, D., Büscher, P., Goddeeris, B., Vercruysse, J., 2002. Evaluation of whole fresh blood and dried blood on filter paper discs in serological tests for *Trypanosoma evansi* in experimentally infected water buffaloes. Acta tropica 81, 159-165.

Holman, P.J., Becú, T., Bakos, E., Polledo, G., Cruz, D., Wagner, G.G., 1998. *Babesia equi* field isolates cultured from horse blood using a microcentrifuge method. J Parasitol 84, 696-699.

Lazić, S., Lupulović, D., Gaudaire, D., Petrovic, T., Lazić, G., Hans, A., 2017. Serological evidence of equine arteritis virus infection and phylogenetic analysis of viral isolates in semen of stallions from Serbia. BMC Vet Res 13, 316.

Martínez, M.F., Kowalewski, M.M., Salomón, O.D., Schijman, A.G., 2016. Molecular characterization of trypanosomatid infections in wild howler monkeys (*Alouatta caraya*) in northeastern Argentina. Int J Parasitol Parasites Wildl 5, 198-206.

Monath, T.P., Sabattini, M.S., Pauli, R., Daffner, J.F., Mitchell, C.J., Bowen, G.S., Cropp, C.B., 1985. Arbovirus investigations in Argentina, 1977–1980. The American Journal of Tropical Medicine and Hygiene 34, 966-975.

Nava, S., Caparros, J.A., Mangold, A.J., Guglielmone, A.A., 2006. Ticks (*Acari*: *Ixodida*: *Argasidae*, *Ixodidae*) infesting humans in Northwestern Cordoba Province, Argentina. Medicina (B Aires) 66, 225-228.

Oria, G.I., Spinsanti, L.I., Pirota, V.L., Martinez, F., Stechina, O.S., Etchepare, E., Contigiani, M.S., Stein, M., 2018. Seroprevalence of *Flavivirus* in horses in Chaco, Argentina. Circulation during 2013-2014. Revista Brasileira de Medicina Veterinaria 40.

Santa Cruz, A., Comolli, J., Ortiz, J., González, J., González, A., 2013. Morphometric data of *Trypanosoma evansi* in capybaras (*Hydrochaeris hydrochaeris*) from Chaco, Argentina, Vol 24, 60-62 pp.

Santos, F.M., de Macedo, G.C., Barreto, W.T.G., Oliveira-Santos, L.G.R., Garcia, C.M., Mourão, G.M., Edith de Oliveira Porfírio, G., Domenis Marino, E., Rogério André, M., Perles, L., Elisei de Oliveira, C., Braziliano de Andrade, G., Jansen, A.M., Miraglia Herrera, H., 2018. Outcomes of *Trypanosoma cruzi* and *Trypanosoma evansi* infections on health of Southern coati (*Nasua nasua*), crab-eating fox (*Cerdocyon thous*), and ocelot (*Leopardus pardalis*) in the Brazilian Pantanal. PLoS One 13, e0201357.

Silveira, J.A., Rabelo, E.M., Lacerda, A.C., Borges, P.A., Tomás, W.M., Pellegrin, A.O., Tomich, R.G., Ribeiro, M.F., 2013. Molecular detection and identification of hemoparasites in pampas deer (*Ozotoceros bezoarticus Linnaeus*, 1758) from the Pantanal Brazil. Ticks Tick Borne Dis 4, 341-345.

Stein, M., Zalazar, L., Willener, J.A., Almeida, F.L., Almirón, W.R., 2013. Culicidae (Diptera) selection of humans, chickens and rabbits in three different environments in the province of Chaco, Argentina. Mem Inst Oswaldo Cruz 108, 563-571.

Verloo, D., Magnus, E., Büscher, P., 2001. General expression of RoTat 1.2 variable antigen type in *Trypanosoma evansi* isolates from different origin. Veterinary parasitology 97, 183-189.

Weiland, G., 1986. Species-specific serodiagnosis of equine piroplasma infections by means of complement fixation test (CFT), immunofluorescence (IIF), and enzyme-linked immunosorbent assay (ELISA). Veterinary parasitology 20, 43-48.

Table 1. Methods used for the serological diagnosis of equine diseases from the sera collected on a farm in the municipality of Laguna Limpia, province of Chaco, Argentina.

Disease	Serological method	Antigen source	Reference or provider	OIE Terrestrial Manual chapter
WNF	MNT[a]	WNV IS-98-ST1	AF481864	3.1.24.
EEE	MNT	NCPV689 strain H178/99	ICTV catalog number 0407041v	3.5.5.
VEE	MNT	Trinidad Donkey strain TC-83	L01443.1	3.5.5.
WEE	MNT	NCPV691, strain H160/99	ICTV catalog number 0407043v	3.5.5.
EVA	VNT[b]	EAV Bucyrus (ATCC VR - 796)	(Lazić et al., 2017)	3.5.10.
EIA	AGID[c]	Recombinant p26 protein	VMRD[d]	3.5.6.
Surra	IT[e]	*Trypanosoma evansi* Rotat 1.2	(Verloo et al., 2001)	3.1.21.
Equine piroplasmosis	CFT[f]	*Babesia caballi*	USDA[g]	3.5.8.
Glanders	CFT	*Burkholderia mallei* Ivan/NCTC10230 strain -	Ccpro GmbH, Germany	3.5.11.

[a] Virus-specific microneutralization tests.

[b] Virus neutralisation test.

[c] Agar gel immunodiffusion assay.

[d] Veterinary Medical Research & Development (Pullman, Washington, USA).

[e] Immune trypanolysis.

[f] Complement fixation test.

[g] United States Department of Agriculture (Ames, Iowa, USA).

Figure captions

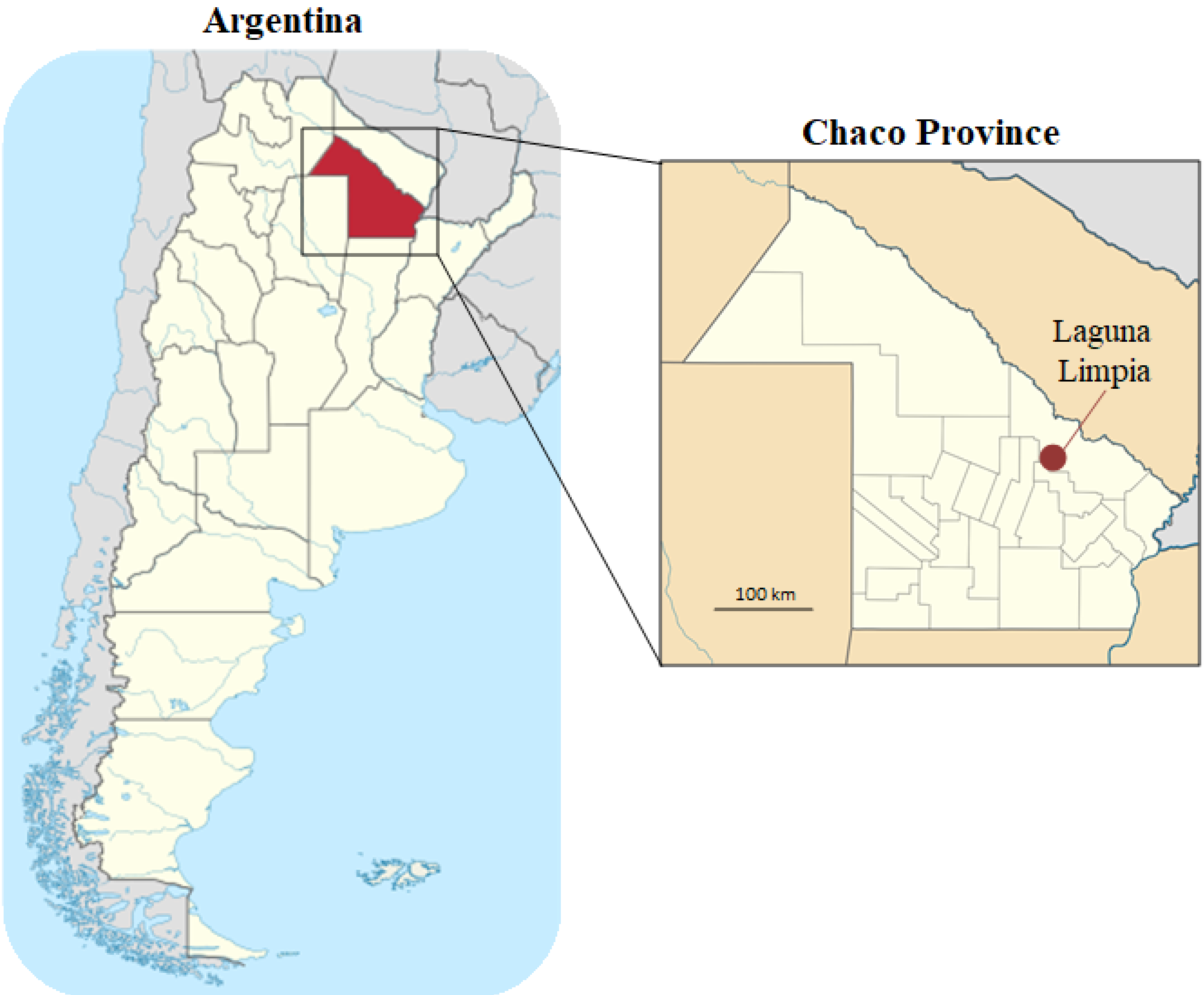
Figure 1. Geographical situation of the Laguna Limpia farm (province of Chaco, Argentina) where the horse samples were collected.

Figure 2. Illustration of the sampled horse herd, clinical signs and evidence of haematophagous vectors. Photographs of the herd during the serum sampling campaign (A and B). The clinical signs observed included weight loss (C) and nasal discharge (D). The evidenced haematophagous vectors included ticks (E) and vampire bats (*Desmodus rotundus*), as shown by the fresh bite (F) on the neck of one of the horses.

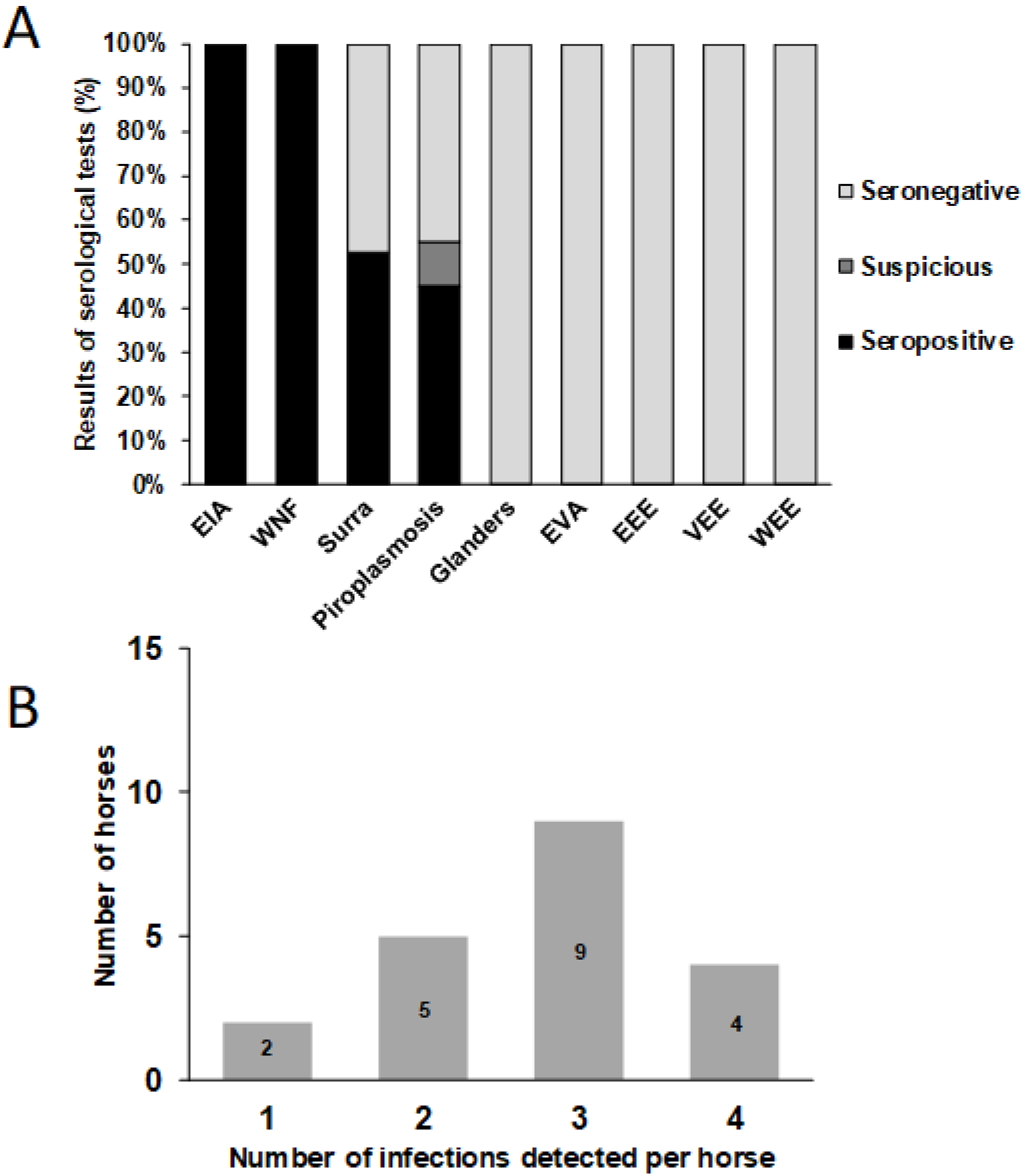
Figure 3. Results of the serological tests performed on the sera collected from 20 horses on a farm in the province of Chaco, northern Argentina. (A) Percentage of positive results obtained in serological tests for equine infectious anaemia (EIA), West Nile fever (WNF), surra, equine piroplasmosis, glanders, equine viral arteritis (EVA), Eastern equine encephalomyelitis (EEE), Venezuelan equine encephalitis (VEE) and Western equine encephalomyelitis (WEE). (B) Frequency distribution of the number of seropositive results obtained for each infection per horse.

Supporting information

Table S1. Data recorded for 20 horses on a farm located in the province of Chaco, northern Argentina.


Argentina
Chaco Province
Laguna
Limpia
100 km


A
B
C
D
E
F



A
Results of serological tests (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Seronegative
Suspicious
Seropositive
EIA
WNF
Surra
Piroplasmosis
Glanders
EVA
EEE
VEE
WEE
B
Number of horses
15
10
5
0
2
5
9
4
1
2
3
4
Number of infections detected per horse

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This study describes, for the first time, horses simultaneously seropositive for equine infectious anaemia, West Nile fever, surra and equine piroplasmosis in northern Argentina.