ARTICLES
Archives of Veterinary Science
UFPR
https://www.ufpr.br/

Comparison of three commercial enzyme-linked immunosorbent assays for the diagnosis of Classical Swine Fever

Fernando Aguilar Vargas[1], Fabián Carvajal[2], Mónica Guzmán[2], Bernal León[2]

[1]*Servicio Nacional de Salud Animal, Ministerio de Agricultura y Ganadería. Heredia, Costa Rica. https://orcid.org/0000-0001-5739-2437.*
[2]*Laboratorio de Bioseguridad, Servicio Nacional de Salud Animal, Ministerio de Agricultura y Ganadería, Costa Rica.*

Submitted: *19/04/23*
Accepted: *16/05/23*

* *Corresponding author: Bernal León, bernal_leon@yahoo.com.*

Abstract: Classical swine fever (CSF) is a highly contagious viral disease that affects both pigs and wild boars. The disease can cause significant economic losses in the pig industry and poses a threat to food security. Therefore, it is crucial to have effective surveillance programs for detecting and controlling the disease. The aim of this study was to compare three commercial ELISA kits for detecting CSF antibodies. The results showed that all three kits had 100% congruence in undiluted samples, indicating that they are highly reliable for use as a screening test on routine samples. Additionally, the study found good reproducibility between technicians with no significant influence on the variation of results. However, the study also identified a low consistency of positive results (37.7%) for the kit IDvet Screen E2 in diluted samples, with significant variations in results from all three kits. This finding suggests that the other two kits, Herdchek E2 and Priocheck E2, may be better suited for detecting animals with low levels of antibodies, allowing for earlier detection of infected animals and implementation of relevant control measures. In conclusion, the study demonstrates the importance of using high-quality commercial ELISA kits for CSF surveillance. The findings suggest that any of the three kits tested could be used as a reliable screening test on routine samples. However, for detecting animals with low levels of antibodies, the Herdchek E2 and Priocheck E2 kits may be more effective.
Keywords: Classical swine fever; ELISA; Costa Rica.

1. Introduction

Classical swine fever (CSF) is one of the most significant transboundary viral diseases affecting swine species and wild boars worldwide. The disease causes significant economic losses in pig production due to its high morbidity and mortality. While vaccination campaigns and strict control measures led to the eradication of the disease in domestic pigs in the European Union and North America, it remains a significant threat in the Asian and American continents, where large annual outbreaks are reported (Shevel and Nychyk 2022; “On Community Measures for the Control of Classical Swine Fever (Text with EEA Relevance)” 2001).

In February 1994, the authorities of Costa Rican Animal Health Service diagnosed the first case of the CSF virus. The cost of controlling and eradicating the disease was approximately 700 million colones, and three years later, after the sacrifice of 26,000 pigs, the country was declared free (Quiros 2002). Despite being located in a region with a high transmission rate, Costa Rica was recognized by the World Organization for Animal Health (WOAH) as a CSF-free state in 2018. The country relies on prevention and control measures based on surveillance, rapid detection, and quarantine of possible cases, without the use of vaccination (Servicio Nacional de Salud Animal 2009).

Identification of the disease is complex due to the wide range of clinical syndromes observed in both domestic pigs and wild boars. Clinical signs are dependent on the CSF strain, pig age, and immune system determinants (Petrov et al., 2014). In general, during the first two weeks after infection, the acute phase is characterized by unspecific, often referred to as "atypical," clinical signs such as high fever, anorexia, gastrointestinal signs, general weakness, and conjunctivitis (Blome et al., 2017). Moreover, the incubation period can last anywhere from 4 to 10 days, and neutralizing antibodies against E2, the most immunogenic CSFV protein, are produced between 10 and 20 days after natural infection (Ganges et al., 2020). Due to this clinical variability, surveillance and diagnosis must be confirmed through highly sensitive and specific laboratory assays that can identify the presence of the virus or virus-induced antibodies (Wang et al., 2020).

Among the diagnostic methods available at the National Veterinary Services Laboratory (LANASEVE), responsible for diagnosing CSF in Costa Rica, are molecular methods, commonly used for the detection and/or typing of pestiviruses, including the virus responsible for Classical swine fever (CSFV). However, they are used less frequently as a screening test, especially during active surveillance (Paton et al, 2000; Servicio Nacional de Salud Animal, 2009).

Serological tests are utilized for detecting specific antibodies, with enzyme-linked immunosorbent assays (ELISAs) being the most commonly used tests for routine surveillance in Costa Rica, due to their advantages. ELISAs are quick, simple tests that do not require sophisticated equipment (Greiser-Wilke, Blome, and Moennig 2007). Commercial ELISAs are designed to detect antibodies directed against the E2, Erns, or NS3 glycoproteins of CSFV and are available worldwide (Greiser-Wilke, Blome, and Moennig 2007). The Erns or NS3 ELISAs can differentiate between natural and vaccine antibodies. However, in regions where vaccination is not practiced, such as Costa Rica, E2-ELISAs are used as conventional screening tests for CSFV infection in herds (Schroeder et al., 2012)

There are several commercial E2 CSFV ELISA kits available, and they differ in terms of simplicity, cost, sensitivity, and specificity. The purpose of this study was to compare three commercial ELISAs for the detection of IgM and IgG antibodies against CSFV glycoprotein E2, using positive and negative reference controls, as well as field negative samples. The ELISAs evaluated in this study were Priocheck® CSFV Ab 2.0 (Prionics, Lelystad B.V, the Netherlands), HerdChek® CSFV Ab (IDEXX, Laboratories B.V. Schiphol-Rijk, the Netherlands), and IDVet Screen® CSF E2 Competition (ID Screen Classical Swine Fever E2 Competition, Grabels, France).

2. Material e Methods

2.1. Serum samples used in the study

We used sera from swine that were positive for CSFV reference strains, including Alfort/187, Glenfort, Congenital Tremor, and Paderborn, as well as field negative sera. These samples were obtained from reference laboratories, such as Centro Nacional de Sanidad Agropecuaria in Cuba (six positive and fourteen negative sera), the National Center for Foreign Animal Disease in the Canadian Food Inspection Agency (nine positive and one negative serum), and the IRTA CRESA laboratory in Spain (eight positives and two negative samples). Additionally, we included 32 swine serum samples from active and passive surveillance, resulting in a total of 72 samples. Refer to Table 1 for the distribution of the samples.

n[a]	Strain	Reference Laboratory	known result	Analyzed by
6		Centro Nacional de Sanidad Agropecuaria, Cuba	Positive	Analyst 1
14	NA[c]	Centro Nacional de Sanidad Agropecuaria, Cuba	Negative	Analyst 1
9	Alfort/ Glentorf/ Cong. Tremor/ Paderborn	National Centre for Foreign Animal Disease, Canada	Positive	Analyst 2
1	NA	National Centre for Foreign Animal Disease, Canada	Negative	Analyst 2
8		Centro de Investigación en Sanidad Animal, España	Positive	Analyst 1
2	NA	Centro de Investigación en Sanidad Animal, España	Negative	Analyst 1
32	NA	Field surveillance, Costa Rica	Negative	Analyst 2
17[b]	Alfort/ Glentorf/ Cong. Tremor/ Paderborn	Selected samples from the Interlaboratory	Positive	Analyst 1 y 2

Table 1 *– Classification of the sera used for the comparison of the commercial ELISA kits. a number of sera; b Samples selected for dilution; c information not available.*

2.2. Study design

For this study, we used sera from pigs that were obtained from interlaboratory comparison tests with known results (reference controls) and sera from pigs that were obtained from routine surveillance in the field. Since our laboratory lacked the necessary conditions to inoculate pigs and establish the number of days post-infection at which these ELISAs could detect the first antibodies in the inoculated pigs, we decided to dilute some of the positive controls to simulate these conditions. We made two-fold serial dilutions of 17 selected positive sera, ranging from 1:16 to 1:128. The samples were processed in duplicate by two technicians, and the readings were taken using two different absorbance readers, namely Epoch 2 Microplate Spectrophotometer (Epoch™2 microplate spectrophotometer, Model number - EPOCH 2, Company- BioTek Instruments, USA) and BioTek ELx800 microplate reader (BioTek Instruments, Inc., Winooski, USA), both of which were used at a wavelength of 450 nm. A total of 136 determinations per analyst were made, and each commercial kit was compared.

2.3. Commercial ELISA methods

All methods were carried out following the manufacturer's instructions. The kits included positive and negative controls (one strong positive, one weak positive, and one negative). The absorbance level cut-off values recommended by the manufacturers were used to interpret the results. Doubtful results were considered positive based on the manufacturer's manual. Table 2 displays the main characteristics of each ELISA.

Abbreviated name	HerdChek E2	Priocheck E2	IDvet Screen E2
Full name	HerdChek CSFV Ab	PrioCHECK CSFV Ab 2.0	ID Screen CSF E2 Competition
Producer/supplier	IDEXX laboratories	Prionics Lelystad B.V.	Innovative Diagnostics laboratories
Antigen detected	E2	E2	E2
No. of steps	3	3	3
Incubation time (short protocol)	160 min	110 min	90 min
Reagents that have to be prepared	Wash buffer	Wash buffer	Wash buffer
Filter wavelength in nm	450	450	450
Clear instructions	Yes	Yes	Yes

Table 2 *– Characteristics of the three Enzyme-linked immunosorbent assays used in this study.*

2.4. Statistical analysis

Reproducibility and the percentages of positive and negative correlations of valid results were calculated for all ELISA kits. The reproducibility and agreement percentages were computed from the results of the 136 determinations, including the diluted positive samples. The reproducibility of each test is presented as the percentage of the 136 determinations that produced the same outcome on that test by different technicians. The agreement between the tests was assessed using the kappa value and the McNemar test (Trajman and Luiz 2008; Chmura Kraemer, Periyakoil, and Noda 2002).

3. Results

The agreement among the results of the three ELISA kits was very good when tested on undiluted samples, with 100% agreement among them. However, when dilutions were made, particularly from 1:32 dilutions, the agreement decreased, especially with the IDvet Screen E2 kit. This suggests that the sensitivity of the IDvet Screen E2 kit may be lower than that of the other two kits. However, the results of both technicians showed no significant differences, indicating good reproducibility. The specificity of all three kits was 100%, with no variations in the results of the known negative samples.

Dilution	Kits									
	Priocheck E2		Positive % agreement	IDvet Screen E2		Positive % agreement	Herdchek E2		Positive % agreement	% overall specificity
	+	-		+	-		+	-		
NDS[a]	23	13	100	23	13	100	23	13	100	100
1:16	34	0	100	27	7	79,4	34	0	100	NA[b]
1:32	27	7	79,4	8	26	23,5	32	2	94,1	NA
1:64	16	18	47	5	29	14,7	26	8	76,4	NA
1:128	7	27	20	0	34	0	14	20	41,1	NA

Table 3 *– Results of the determinations made by analyst 1 for diagnosis of CSF by commercial ELISA E2. a non-diluted samples; b Applicable because the samples used were positive.*

Dilución	Kits									
	Priocheck E2		Positive % agreement	IDvet Screen E2		Positive % agreement	Herdchek E2		Positive % agreement	% overall specificity
	+	-		+	-		+	-		
NDS[a]	9	33	100	9	33	100	9	33	100	100
1:16	34	0	100	30	4	88,2	34	0	100	NA[b]
1:32	24	10	70,5	8	26	23,5	32	2	94,1	NA
1:64	12	22	35,2	4	30	13,3	22	12	64,7	NA
1:128	7	27	20,5	0	34	0	10	24	29,4	NA

Table 4 *– Results of the determinations made by analyst 2 for diagnosis of CSF by commercial ELISA E2. a non-diluted samples; b Applicable because the samples used were positive.*

The analysis of the results obtained by analyst 1 showed a positive correlation of 37.7% and 94% among the different ELISAs. The congruence between the Priocheck E2 and Herdchek E2 kits was good at 93.5%. In contrast, the IDvet Screen E2 showed a lower correlation of 37.7% compared to the Herdchek E2. Table 5 presents the comparative results of the different E2 ELISAs obtained by analyst 1.

Kits		IDvet Screen E2		Positive % agreement	Priocheck E2		Positive % agreement
		+	-		+	-	
Herdchek E2	+	40	66	37.7%	72	26	93.5%
	-	0	30		5	33	
Kappa (95% CI)		0.21			0.52		
Mcnemar test		0,000000001			0.000162		

Table 5 – *Comparative results of the Elisa E2 kits obtained by analyst 1.*

Kits		IDvet Screen E2		Positive % agreement	Priocheck E2		Positive % agreement
		+	-		+	-	
Herdchek E2	+	42	56	42.9%	84	22	100%
	-	0	38		0	30	
Kappa (95% CI)		0.30			0.63		
Mcnemar test		0.000001			0.000003		

Table 6 *– Comparative results of Elisa E2 kits obtained by Analyst 2.*

No significant discrepancies were observed when comparing the absorbance readings obtained from both microplate readers. The results showed 100% agreement for both the IDvet Screen E2 and Priocheck E2 kits. For the Herdchek E2 kit, the agreement was also very good, with a Kappa value greater than 81. Table 7 summarizes the results obtained using the two different absorbance microplate readers.

Kit	Reader				Kappa (95% CI)	Mcnemar test
	Elx800		EPOCH2			
	+	-	+	-		
Herdchek E2	105	31	106	30	0.85	0.14
Priocheck E2	84	52	84	52	1	NA[a]
IDvet Screen E2	42	94	42	94	1	NA

Table 7 *– Comparison of the determinations obtained by absorbance microplate Reader. a Not Applicable because the samples used were positives.*

4. Discussion

Due to the significant morbidity and economic impact that Classical Swine Fever (CSF) has on the swine industry, early detection and rapid diagnosis are critical. This study aimed to compare three ELISAs specifically designed to detect IgM and IgG class antibodies against CSFV E2 glycoprotein. Our results indicate that all three commercial kits perform similarly, with an overall percentage agreement of 100% in routine (undiluted) samples while maintaining good specificity. CSFV belongs to the genus Pestivirus, which comprises 11 proposed species within the family Flaviviridae, including Pronghorn antelope pestivirus, Bungowannah virus, Giraffe pestivirus, Rat pestivirus, Bovine viral diarrhea virus type 1 and type 2, HoBi-like pestivirus, Border disease virus, Aydin-like pestivirus, and Atypical porcine pestivirus. However, ELISA tests are known to have low specificity due to cross-reactivity with other pestiviruses (Wang et al., 2020; van Rijn, 2007b; Postel, Smith, and Becher, 2021).

Previous studies have reported similar results of concordance between Herdchek E2 and Priocheck E2, with sensitivity and specificity greater than 98% for some of these kits. However, the positive agreement was significantly lower (ranging from 37.7% to 54.5%) for IDvet Screen E2, and the agreement was consistently low (kappa < 0.40) when compared to the Herdchek E2 ELISA on the diluted samples. Although pestiviruses are genetically and immunologically related, there are differences between CSFV and other pestiviruses, including the antigenic structure of the Envelope glycoprotein E2, which has four antigenic domains. The three kits compared in this study use E2 antigen to coat the plates in their ELISAs, but they may use different domains, which could explain the differences in sensitivity observed between the kits (van Rijn, 2007a).

Experimental tests conducted on pigs infected with CSF have shown that the antibody response during the incubation period varies depending on the days-post-infection (dpi). The first antibodies are typically detected at an average of 14 dpi, and the antibody titer increases on average at 20 dpi, with antibody titers ranging from 1/5 to 1/80. This can even occur in animals with subclinical infections that can last for extended periods (Leavens et al., 1998). The lower sensitivity of IDvet Screen E2 in diluted samples may indicate a reduced capacity to detect animals with low antibody titers, which could affect the primary objective of active surveillance, which is to detect infected animals as early as possible to establish control measures (Leavens et al., 1998; Oscar Cabezón et al., 2017).

Pigs infected with low or moderate virulence strains or congenitally infected pigs can develop chronic CSF and continue shedding the virus for several months, which can lead to reinfection (O. Cabezón et al., 2017; Weesendorp et al., 2011). Clinical diagnosis is not effective in detecting such infections, and animals that are sick can produce antibodies that are only present intermittently or in low titers, leading to equivocal or false-negative ELISA results, as measured by percentage of blocking values (Bohórquez et al., 2020; Muñoz-González et al., 2015). In this study, the IDvet Screen E2 kit showed lower ability to detect low antibody titers (in diluted samples) compared to the Priocheck E2 and Herdchek E2 kits, indicating that this kit might also have lower ability to detect animals with persistent infections (Coronado et al., 2019; Bohórquez et al., 2020).

The reproducibility of the three kits was good, with overall agreement ranging from good to very good (Kappa > 60). No significant differences were observed between technicians or when comparing microplate readers (Mcnemar test > 0.05). IDvet Screen E2 showed some advantages over the other kits, such as a shorter incubation period and lower cost. However, this study has some limitations, including the inability to assess the specificity of the kits due to the lack of a negative "gold standard," as well as the absence of positive field samples to evaluate sensitivity due to the disease-free status of the country. Nonetheless, the use of reference controls allowed for a general evaluation and comparison of the diagnostic capacity of the different kits, as has been done in previous studies (Colijn, Bloemraad, and Wensvoort, 1997).

5. Conclusions

In conclusion, the Herdchek E2 and Priocheck E2 kits showed a higher percentage of positive agreement and greater detection of positives in the diluted samples, so they seem to be better for detecting exposed animals with low levels of antibodies. However, the comparison of the commercial Herdchek E2, Priocheck E2, and IDvet Screen E2 kits showed a high percentage of general consistency of the results in routine samples and without significant differences in reproducibility. In addition, IDvet Screen E2 presents greater practicality and lower cost, so this kit is a good candidate to be used as a disease detection test, in regions free of CSF and without vaccination, such as Costa Rica.

Acknowledgments: Dr. John Pasick for reviewing the manuscript and his valuable comments. This study was supported by the Regional Project for the Eradication of Classical Swine Fever of the International Regional Organization for Agricultural Health (OIRSA).

6. References

Blome, Sandra, Christoph Staubach, Julia Henke, Jolene Carlson, and Martin Beer. 2017. “Classical Swine Fever —an Updated Review.” Viruses 9 (4): 1–24. https://doi.org/10.3390/v9040086.

Bohórquez, José Alejandro, Sara Muñoz-González, Marta Pérez-Simó, Iván Muñoz, Rosa Rosell, Liani Coronado, Mariano Domingo, and Llilianne Ganges. 2020. “Foetal Immune Response Activation and High Replication Rate during Generation of Classical Swine Fever Congenital Infection.” Pathogens 9 (4): 285. https://doi.org/10.3390/pathogens9040285.

Cabezón, O., A. Colom-Cadena, S. Muñoz-González, M. Pérez-Simó, J. A. Bohórquez, R. Rosell, I. Marco, M. Domingo, S. Lavín, and L. Ganges. 2017. “Post-Natal Persistent Infection with Classical Swine Fever Virus in Wild Boar: A Strategy for Viral Maintenance?” Transboundary and Emerging Diseases 64 (2): 651–55. https://doi.org/10.1111/tbed.12395.

Cabezón, Oscar, Sara Muñoz-González, Andreu Colom-Cadena, Marta Pérez-Simó, Rosa Rosell, Santiago Lavín, Ignasi Marco, et al. 2017. “African Swine Fever Virus Infection in Classical Swine Fever Subclinically Infected Wild Boars.” BMC Veterinary Research 13 (1): 227. https://doi.org/10.1186/s12917-017-1150-0.

Chmura Kraemer, Helena, Vyjeyanthi S. Periyakoil, and Art Noda. 2002. “Kappa Coefficients in Medical Research.” Statistics in Medicine 21 (14): 2109–29. https://doi.org/10.1002/sim.1180.

Colijn, E.O., M. Bloemraad, and G. Wensvoort. 1997. “An Improved ELISA for the Detection of Serum Antibodies Directed against Classical Swine Fever Virus.” Veterinary Microbiology 59 (1): 15–25. https://doi.org/10.1016/S0378-1135(97)00178-8.

Coronado, Liani, Jose Alejandro Bohórquez, Sara Muñoz-González, Lester Josue Perez, Rosa Rosell, Osvaldo Fonseca, Laiyen Delgado, Carmen Laura Perera, Maria Teresa Frías, and Llilianne Ganges. 2019. “Investigation

of Chronic and Persistent Classical Swine Fever Infections under Field Conditions and Their Impact on Vaccine Efficacy." BMC Veterinary Research 15 (1): 247. https://doi.org/10.1186/s12917-019-1982-x.

De, Comunicación, and Costa Rica. n.d. "Comité de Medidas Sanitarias y Fitosanitarias Original: español declaratoria sobre el reconocimiento del estatus sanitario de Costa Rica como país libre de peste porcina clásica."

Ganges, Llilianne, Helen R. Crooke, Jose Alejandro Bohórquez, Alexander Postel, Yoshihiro Sakoda, Paul Becher, and Nicolas Ruggli. 2020. "Classical Swine Fever Virus: The Past, Present and Future." Virus Research 289. https://doi.org/10.1016/j.virusres.2020.198151.

Greiser-Wilke, Irene, Sandra Blome, and Volker Moennig. 2007. "Diagnostic Methods for Detection of Classical Swine Fever Virus—Status Quo and New Developments." Vaccine 25 (30): 5524–30. https://doi.org/10.1016/j.vaccine.2006.11.043.

Leavens, H., H. Deluyker, A. de Kruif, and D. Berkvens. 1998. "An Experimental Infection with Classical Swine Fever Virus in Weaner Pigs: I. Transmission of the Virus, Course of the Disease, and Antibody Response." Veterinary Quarterly 20 (2): 41–45. https://doi.org/10.1080/01652176.1998.9694836.

Moser, Christian, Nicolas Ruggli, Jon Duri Tratschin, and Martin A. Hofmann. 1996. "Detection of Antibodies against Classical Swine Fever Virus in Swine Sera by Indirect ELISA Using Recombinant Envelope Glycoprotein E2." Veterinary Microbiology 51 (1–2): 41–53. https://doi.org/10.1016/0378-1135(96)00019-3.

Muñoz-González, Sara, Nicolas Ruggli, Rosa Rosell, Lester Josué Pérez, Maria Teresa Frías-Leuporeau, Lorenzo Fraile, Maria Montoya, et al. 2015. "Postnatal Persistent Infection with Classical Swine Fever Virus and Its Immunological Implications." PLOS ONE 10 (5): e0125692. https://doi.org/10.1371/journal.pone.0125692.

"On Community Measures for the Control of Classical Swine Fever (Text with EEA Relevance)." 2001.

Paton, D.J, A McGoldrick, S Belak, C Mittelholzer, F Koenen, H Vanderhallen, M Biagetti, et al. 2000. "Classical Swine Fever Virus: A Ring Test to Evaluate RT-PCR Detection Methods." Veterinary Microbiology 73 (2–3): 159–74. https://doi.org/10.1016/S0378-1135(00)00142-5.

Petrov, Anja, Ulrike Blohm, Martin Beer, Jana Pietschmann, and Sandra Blome. 2014. "Comparative Analyses of Host Responses upon Infection with Moderately Virulent Classical Swine Fever Virus in Domestic Pigs and Wild Boar." Virology Journal 11 (1): 1–6. https://doi.org/10.1186/1743-422X-11-134.

Postel, Alexander, Donald B. Smith, and Paul Becher. 2021. "Proposed Update to the Taxonomy of Pestiviruses: Eight Additional Species within the Genus Pestivirus, Family Flaviviridae." Viruses 13 (8): 1542. https://doi.org/10.3390/v13081542.

Quiros, Ligia. 2002. "Proyecto Regional de Prevención de La Fiebre Porcina Clásica En Centro América, Belice y Panamá (PREFIP)." Heredia, Costa Rica.

Rijn, P. A. van. 2007a. "A Common Neutralizing Epitope on Envelope Glycoprotein E2 of Different Pestiviruses: Implications for Improvement of Vaccines and Diagnostics for Classical Swine Fever (CSF)?" Veterinary Microbiology 125 (1–2): 150–56. https://doi.org/10.1016/j.vetmic.2007.05.001.

Rijn, P.A. van. 2007b. "A Common Neutralizing Epitope on Envelope Glycoprotein E2 of Different Pestiviruses: Implications for Improvement of Vaccines and Diagnostics for Classical Swine Fever (CSF)?" Veterinary Microbiology 125 (1–2): 150–56. https://doi.org/10.1016/j.vetmic.2007.05.001.

Schroeder, S., T. von Rosen, S. Blome, W. Loeffen, A. Haegeman, F. Koenen, and Å Uttenthal. 2012. "Evaluation of Classical Swine Fever Virus Antibody Detection Assays with an Emphasis on the Differentiation of Infected from Vaccinated Animals." OIE Revue Scientifique et Technique 31 (3): 997–1010. https://doi.org/10.20506/rst.31.3.2173.

Servicio Nacional de Salud Animal, 2009. 2009. "Costa Rica país libre de peste porcina clásica."

Shevel, Yu. v., and S. A. Nychyk. 2022. "Analysis of the Epizootic Status of Classical Swine Fever in the World for the Period 2000–2009 and 2012–2020." Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies 24 (107): 88–93. https://doi.org/10.32718/nvlvet10715.

Trajman, A., and R. R. Luiz. 2008. "McNemar χ 2 Test Revisited: Comparing Sensitivity and Specificity of Diagnostic Examinations." Scandinavian Journal of Clinical and Laboratory Investigation 68 (1): 77–80. https://doi.org/10.1080/00365510701666031.

Wang, Lihua, Rachel Madera, Yuzhen Li, David Scott McVey, Barbara S. Drolet, and Jishu Shi. 2020. "Recent Advances in the Diagnosis of Classical Swine Fever and Future Perspectives." Pathogens 9 (8): 658. https://doi.org/10.3390/pathogens9080658.

Weesendorp, Eefke, Jantien Backer, Arjan Stegeman, and Willie Loeffen. 2011. "Transmission of Classical Swine Fever Virus Depends on the Clinical Course of Infection Which Is Associated with High and Low Levels of Virus Excretion." Veterinary Microbiology 147 (3–4): 262–73. https://doi.org/10.1016/j.vetmic.2010.06.032.