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PERFORMANCE OF RAPID TESTS FOR CANINE VISCERAL LEISHMANIASIS DIAGNOSIS IN ARGENTINA

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Abstract To diagnose dogs infected by *Leishmania infantum* rK39 rapid diagnosis test is widely used in the Americas, while dual path platform (DPP) was recently adopted by Brazil. In this study we assessed the performance of rK39-RDT and DPP tests in recent urban transmission scenarios of Argentina. The sensitivity and specificity were evaluated with a sera panel and field samples, taken as true infected those from parasitological and/or PCR positive tests. Since none of these tests can be taken as a gold standard, the performance was also evaluated using Latent Class Analysis, a statistical modeling technique which allows to estimating sensitivity and specificity defining a latent class variable as the reference standard. The sensitivity of both tests in the panel was around 92% (symptomatic dogs 96%, asymptomatic 83%), while the sensitivity in field samples of rK39-RDT was 77%, and DPP 98% (mean in symptomatic dogs 89%, asymptomatic 82%). The specificity was similar for both tests and samples, around 98%. Therefore, these tests are acceptable for program dog population-based studies, as spatial stratification, focus intervention and follow up, and they could be used for individual screening and confirmation of clinical presumptive diagnosis in polysymptomatic dogs. The inability to discriminate between immunity and actual infectiousness suggest that a combination with other non-immunological based tests will be required for highly sensitive/specific diagnosis in order to targeting control measures in individual reservoirs from public health perspective, as for individual management from animal health perspective.

Key words: diagnosis, immunologic test, population surveillance, canine leishmaniasis

Resumen ***Desempeño de pruebas diagnósticas rápidas para leishmaniosis visceral canina en Argentina.*** Para diagnosticar perros infectados por *Leishmania infantum*, en las Américas se utiliza ampliamente la prueba rápida rK39, mientras que DPP fue adoptado recientemente por Brasil. En este estudio se evaluó el desempeño de las pruebas rK39-RDT y DPP en escenarios de transmisión urbana reciente en Argentina. La sensibilidad y especificidad se evaluaron con un panel de sueros y muestras de campo, considerando muestras infectadas verdaderas aquellas con pruebas parasitológicas y/o de PCR positivas. Como ninguna de estas pruebas puede considerarse estándar de oro, el desempeño también se evaluó mediante análisis de clases latentes, una técnica de modelado estadístico que permite estimar sensibilidad y especificidad definiendo una variable de clase latente como estándar. La sensibilidad de ambas pruebas en el panel fue de alrededor del 92% (perros sintomáticos 96%, asintomáticos 83%), mientras que la sensibilidad en muestras de campo fue rK39-RDT: 77%, y DPP 98% (media en perros sintomáticos 89%, asintomáticos 82%). La especificidad fue similar para ambas pruebas y muestras, cerca de 98%. Por lo tanto, estas pruebas son aceptables para estudios programáticos caninos de base-poblacional, como estratificación espacial, intervención de foco y seguimiento, y podrían utilizarse para el tamizaje individual y la confirmación del diagnóstico clínico presuntivo en perros poli-sintomáticos. La incapacidad de discriminar entre inmunidad e infectividad real sugiere que se requerirá una combinación con otras pruebas, de base no inmunológica, para un diagnóstico suficientemente sensible/específico que permita definir las medidas de control en reservorios individuales, tanto para salud pública, como para la gestión individual en salud animal.

Palabras clave: diagnóstico, prueba inmunológica, vigilancia poblacional, leishmaniasis canina

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Visceral leishmaniasis is a neglected vector-borne disease with an estimated worldwide annual incidence of 200 000 to 400 000 cases, and a case-fatality rate of 10%[1]. *Leishmania infantum* is the etiological agent of the disease in the Americas, with *Lutozmyia longipalpis* as its main vector. The cases of Brazil account for 96% of the human visceral cases of the Americas, but an increasing incidence and expansion of transmission in Argentina, Colombia, Paraguay and Venezuela were reported during the last decades[2]. In Argentina the urban vector was recorded for the first time in 2004, the first human case in 2006, and after that the vector spread to five provinces, and human cases to four provinces. This trend is due to the process of urbanization and expansion that started in Brazil during 1970-1980[3]. The dog, despite its clinical status (asymptomatic to polysymptomatic), is the main urban reservoir of parasites transmitted both to dogs and humans through the bite of an infected vector. But there is also a dog to dog reservoir by dog vertical and sexual transmission. Therefore, the accurate diagnosis of canine visceral leishmaniasis (CVL) became a critical 'One Health' issue for human public health, and for individual and collective animal health.

Many immuno-serological and molecular diagnostic tests have been proposed and even are commercially available[4]. However, in order to assess its performance, the aim of any new test should be defined: a) for control programs: population-based surveillance screening of reservoirs, cohort studies and impact assessment, individual-based management decision, identification of super-spreaders; b) for the veterinarian perspective and research: diagnosis for case management, biomarkers of disease prognosis, molecules for vaccine development and evaluation of effectiveness of treatment.

Since December 2011, the Brazilian Ministry of Health, requires for CVL immunoserodiagnosis, in the Surveillance and Control Program of Leishmaniasis, the dual-path platform DPP® rapid test for screening, with an enzyme-linked immunosorbent assay (ELISA) using soluble antigens of *L. infantum* as confirmatory test [5]. Argentina Leishmaniasis' Program use Kalazar Detect™ for CVL diagnosis, a rK39 dipstick rapid test commercially available with a recombinant protein of *L.infantum*, which is also contained in the modified rK28 DPP test. Both rapid tests were compared in Brazil showing that the results could depend on the transmission scenario intensity[6].

Therefore, we performed a study to assess the performance of both tests in the relatively new foci of Argentina, in the southern latitudes of CVL spread, with a controlled sera panel and with field sampled dogs from an endemic city, so with a broad spectrum of immune responses to infection. The results can be useful to compare the Brazilian studies with those of other countries, mainly in border areas, to evaluate the usefulness of the DPP outside Brazil, but also to analyze the advantages and limitations of an immune-serological rapid tests for CVL diagnosis.

Materials and methods

Serological Qualitative Rapid Diagnostic Tests**:** a) rK39-RDT**:** Kalazar Detect™ (InBios, Inc., Seattle, WA, USA) kit for CVL. It is based on a 39 amino acid repeat immunodominant B-cell epitope in a kinesin-related protein, which is conserved between *L. infantum* and *L. donovani*. b) DPP: Dual-path platform fast test (TR DPP®kit for CVL (Bio-Manguinhos, Rio de Janeiro, Brazil). It is a colloidal gold-based immunochromatography assay with a recombinant chimeric protein (rK28) multi-epitope from the fusion of *L. infantum* genes: k9, single repeat units of k39 and k26[7].

Parasitological test: Samples were taken by a popliteal lymph node puncture with a hypodermic needle; smears were fixed with methanol and stained with May-Grünwald-Giemsa. Routine analysis involved the observation of two hundred microscopical fields, or until the identification of at least one amastigote.

PCR tests: a) Sample genome quality was evaluated with protocol targets the CytB gene of vertebrates (CytB1 5´-CCC CTC AGA ATG ATA TTT GTC CTC A-3´ and CytB2 5´-CCA TCC AAC ATC TCA GCA TGA TGA AA-3´). Human genome of a healthy donor was employed as positive control. b) *Leishmania* was detected using a standardized protocol targeting the ribosomal internal transcribed spacer (ITS-1) of *Leishmania* sp. (LITSR 5´-CTG GAT CAT TTT CCG ATG-3´ and L5.8S 5´-TGA TAC CAC TTA TCG CAC TT-3´)[8]. *Leishmania (V.) braziliensis* reference strain (MHOM/BR/1975/2903) was employed as positive control. PCRs were carried out with 5 µl of extracted DNA in a final volume of 50 µl containing 1× PCR buffer (200mM Tris-HCl, pH 8), 0.1mM EDTA, 1mM DTT, 50% glycerol (v/v) (Invitrogen™), 2 mM MgCl2 (Invitrogen™), 2.5% DMSO (SIGMA™), 0.2 mM dNTP Mix, 0.5 µM of each primer, and 1.4 U Taq polymerase (Invitrogen™). Up to 10 µl of the amplified products were analyzed by 2% agarose gel electrophoresis at 5 V/cm, stained with SYBR Safe™ (0.5 µg/ml) and visualized with a Safe Imager™ 2.0 Blue-Light Transilluminator (470 nm). For ITS-1 positive samples PCR was followed up by a RFLP assay for *Leishmania* strain identification, or amplicons sequenced using Macrogen Inc. service (Korea). Edition and alignment of the sequencer AB1 files were performed with Codon Code™ v 3.0.1 (CodonCode Corporation). *Leishmania* strain sequence homology was considered when the value retrieved by Blast (Basic Local Alignment Tool) was over 99% (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The panel (n = 431) involves the following sera samples categories: i) *Infected* dogs from the endemic area with positive parasitological test from popliteal lymph node smear or positive PCR (Origin: Posadas city, Argentina). ii) *Positive inconclusive* dogs from visceral leishmaniasis endemic area with negative parasitological test of popliteal lymph node smear but positive rK39-RDT (Posadas city). iii) *Negative,* dogs from the endemic area with negative parasitological test from popliteal lymph node smear, negative PCR, and negative rK39-RDT (Posadas city). iv) *Non-infected*, clinically healthy dogs from a non-endemic VL area (Buenos Aires city, Argentina). v) *Non-infected, other infections* dogs from non-endemic areas, with infections of *Trypanosoma cruzi* (n = 50), *Leptospira* sp. (n = 13), *Dirofilaria* sp (n = 10), demodicosis by *Demodex* sp. (n = 3) and *Brucella* sp. (n = 1) (Table 1). Samples were further discriminated according the dog donor clinical status as: a) *Asymptomatic*, not presented any clinical sign of CVL disease. b) *Symptomatic*, presented lymphadenopathy together with other clinical sign, like onychogryphosis, cutaneous

TABLE 1.– *Canine sera panel for performance evaluation of DPP and rK39-RDT tests for canine visceral leishmaniasis diagnosis*

Group	Area of origin	Characteristics	n	Symptomatic n (%)
Infected	Endemic	Parasitological or PCR positive	89	60 (67.4%)
Positive inconclusive	Endemic	Parasitological negative, rK9-RDT positive	73	34 (46.6%)
Negative	Endemic	Parasitological, PCR and rK39-RDT negative	91	45 (49.5%)
Non-infected	non-endemic		101	
	non-endemic	Potential cross-reactivity	77	
Total			431	

lesions, weight loss, conjunctivitis, alopecia or apathy. None dogs received leishmaniasis vaccine. The dogs were tested by optical parasitological diagnosis from popliteal lymph node smears and PCR from splenic aspirates as described in the section parasitological test, and DPP and rK39-RDT diagnostic test kits were used in accordance with manufacturer's recommendations. Test readers were blinded to the clinical status of the dog, and the results of other diagnostic tests.

Dogs were sampled during September 2013, in Oberá city (Argentina), an endemic area for CVL with antecedents of human visceral cases, in the neighborhoods of *Villa Erasmie* and *Cien hectáreas*, identified by the local agent of zoonosis as sectors with high density of canine presumptive cases. Dwellings were selected by systematic random sampling, and in each dwelling all the dogs were sampled (n = 563). All sampled dogs were owned, and no samples from stray dogs were collected. The dogs were examined for clinical signs of CVL (categories as in the section above) and the symptoms recorded. None dogs received leishmaniasis vaccine. Popliteal lymph node aspirates and blood samples (venipuncture of the jugular or the cephalic) were collected regardless the clinical status, using disposable syringes and needles, sera were separated by centrifugation, and processed 3-4 h after collection for the rapid tests. DPP and rK39-RDT diagnostic test kits were used in accordance with manufacturer's recommendations. In addition, when the volume of the sample obtained from popliteal lymph node aspirates allowed it (n = 118), smears-parasitological direct tests and PCR were carried out. For this last purpose the samples in 200 µl of phosphate buffer solution pH7.0 were kept cool in the field until they were frozen at -20 °C.

We compared DPP and rK39-RDT results of the sera panel between infected and non-infected groups (n = 267). In order to compute the sensitivity and specificity we used a positive parasitological and/or PCR results as true infected. However, as a gold standard with high specificity but low sensitivity could underestimate the true specificity of the tests evaluated, we performed also a Latent Class Analysis (LCA), a statistical modeling technique recommended in absence of an acceptable gold standard, and previously used for canine leishmaniasis[9-11]. We used the results of the DPP, rK39-RDT and parasitological tests (n = 431) to define a non-observable (latent) variable indicating the true disease status of the sample[10]. The goodness of fit of the statistical model was evaluated using the likelihood-ratio statistic G^2 and entropy R^2. DPP was also evaluated using rK39-RDT as reference standard (n = 431). The sensitivity and specificity for DPP and rK39-RDT of the sera panel and samples for field performance were estimated using 95% confidence interval (CI). In order to compare sensitivities and specificities the McNemar's test was employed, with a significance level of 0.05. The agreement between the results obtained with the rapid tests was assessed using the Cohen's Kappa statistic: no agreement (< 0), slight (0-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), and perfect-almost perfect agreement (0.81-1). The statistical analyses were performed using Stata™ software (version 13.1; Stata Corp, College Station, TX). Latent class analysis was performed using the LCA Stata Plugin™ (Version 1.2; University Park: The Methodology Center, Penn State).

Field procedures and handling of dogs were performed according to the protocol and informed approved by the Ethics Committee of the Fatala Chaben Institute and complies with National regulations and OIE recommendations. All dog owners agreed to include their dogs in the study and signed an informed consent form before sample collection.

Results

Table 2 summarizes the sera panel results of the two evaluated RDTs discriminated by the clinical status with the parasitological and/or PCR results as gold standard, while in Table 3 we used latent class analysis (LCA) as reference standard. Table 4 shows the results of DPP performance using rK39-RDT results as standard. Regarding to cross-reactivity, just one of sera diagnosed previously with *Leptospira* sp. infection reacted with DPP.

Table 5 shows the sensitivities and specificities of the sera sampled in Oberá city according to the clinical status and with the parasitological and/or PCR result as reference standard. The clinical status of the infected dogs (n = 143) were asymptomatic, (n = 77, 53.9%), symptomatic (n = 56, 39.2%), while 10 dogs (7.0%) were not clinically evaluated (n = 10, 7.0%). The PCR-RFLPs positive from splenic aspirates (n = 21) or sequenced positive PCR from splenic aspirates (n = 14) were identified as *Leishmania infantum.*

The kappa indexes between rK39-RDT and DPP was 0.838 in the sera panel (Table 4), while in field conditions it was 0.685 (Table 5).

The estimated overall prevalence of CVL in dogs from dwellings of two neighbors of high transmission in Oberá city was 28% (95% CI: 24.0-32.1). This value was computed assuming as 'infected' sero-reactive dogs those with positive rK39-RDT and DPP, or dogs with one

TABLE 2.– *Sensitivities and specificities of DPP and rK39-RDT tests for detection of anti-*Leishmania *antibodies in canine sera panel using parasitological or PCR test positive samples as standard reference for sensitivity (n = 267)**

	DPP		rK39-RDT	
	Estimate	(95%CI)	Estimate	(95%CI)
Sensitivity				
overall	93.3	(85.9; 97.5)	89.9	(81.7; 95.3)
symptomatic	98.3	(91.1; 100)	93.3	(83.8; 98.2)
asymptomatic	82.8	(64.2; 94.2)	82.8	(64.2; 94.2)
Specificity				
overall	97.8	(94.3; 99.4)	98.9	(96.0; 99.9)
other infections, non VL	93.3	(85.9; 97.5)	100.0	(95.3; 100)
without other infections	98.0	(93.0; 99.8)	98.0	(93.0; 99.8)

**McNemar´s test to compare sensitivity and specificity between DPP and rK39-RDT was not significant (p>0.05) in all cases*

TABLE 3.– *Sensitivities and specificities of DPP and rK39-RDT tests for detection of anti-*Leishmania *antibodies in canine sera panel using Latent Class Analysis as standard reference (n=431)**

	DPP		rK39-RDT	
	Estimate	(95%CI)	Estimate	(95%CI)
Sensitivity				
overall	100	(97.4; 100)	99.3	(96.0; 100)
symptomatic	100	(95.8; 100)	98.8	(93.7; 100)
asymptomatic	100	(93.2; 100)	100	(93.2; 100)
Specificity				
overall	95.6	(92.5; 97.6)	93.9	(90.5; 96.3)
other infections, non VL	97.4	(90.9; 99.7)	100	(95.3; 100)
without other infections	95.3	(91.3; 97.8)	99	(96.3; 99.9)

**McNemar´s test to compare sensitivity and specificity between DPP and rK39-RDT was not significant (p>0.05) in all cases*

TABLE 4.– *Sensitivities and specificities of DPP test for detection of anti-*Leishmania *antibodies in canine sera panel using rK39-RDT as standard reference (n = 431)*

	Estimate	(95%CI)
Sensitivity		
overall	88.4	(82.3; 93.0)
symptomatic	94.4	(91.6; 97.2)
asymptomatic	80.0	(68.2; 88.9)
Specificity		
overall	94.9	(91.6-97.2)
other infections, non VL	97.4	(90.9; 99.7)
without other infections	95.3	(91.2; 97.8)

Kappa index = 0.838

of them positive and positive by parasitological or PCR tests, and 'non-infected' dogs with both negative rK39 and DPP, or dogs with one of them negative and negative by parasitological and PCR tests (inconclusive results as rK39-DPP results/no parasitological nor PCR, negative parasitological without PCR, or negative PCR without parasitological were excluded (n = 52)).

Discussion

Using a panel of well characterized sera and parasitological or molecular diagnosis as reference standards, both rapid diagnostic tests evaluated showed an overall good sensitivity and specificity (Table 6). DPP and rK39-RDT had a very good agreement between them with a Kappa

TABLE 5.– *Sensitivities and specificities of DPP and rK39-RDT tests for detection of anti-Leishmania antibodies in canine sera from field sampling (Oberá city) using parasitological or PCR test positive samples as standard reference (n = 511)*

	DPP Estimate (95%CI)	rK39-RDT Estimate (95%CI)	p*
Sensitivity			
overall	93.7 (88.4-97.1)	76.9 (69.1-83.6)	< 0.001
symptomatic	98.2 (90.4-100)	80.4 (67.6-89.8)	< 0.001
asymptomatic	92.2 (83.8-97.1)	71.4 (60.0-81.2)	0.003
Specificity	95.9 (93.4-97.7)	98.6 (96.9-99.6)	0.03
Kappa index = 0.685			

**McNemar´s test to compare sensitivity and specificity between DPP and rK39-RDT*

agreement index of 0.83, although the sensitivity of DPP taking into account rK39-RDT positives was 88.4% (95%CI: 82.3; 93.0).

When the performance of the RDT tests was evaluated in the field, with a broader spectrum of cases, the specificity remains in the same range than the results with the sera panel (Table 6), but the overall sensitivity dropped due to the low performance of rK39-RDT ≈ 77%, and so the Kappa agreement index between the tests was 0.68, barely substantial. The results with dog populations in actual field scenarios are sensitive to the uncontrolled variables as the prevalence rate; in Brazil the Kappa index between DPP and rK39-RDT was 0.87 in a low transmission area (Spírito Santo State), but 0.54 in a high endemic area (Piauí State)[6]. Further, the interpretation of tests by multiple operators, taking as negative some weak signals mainly from asymptomatic dog samples, could decrease the sensitivity of the tests. This fact highlights the operator-dependent risk of bias when RDTs are used by agents in wide program activities, and so the need for standardized procedures, capacitation and quality control.

The performances obtained in this study are comparable with the results obtained by other authors (Table 6). However, the sensitivity of RK39-RDT in much lower in some reports due to protocol differences as cohort studies or convenience sampling, and sera conservation procedures[15], or the tests were performed with different brands of dipsticks[16, 17, 19]. The disparate results reported for DPP were explained by cross-reactions mainly in asymptomatic dogs[7,21] and possible false positives in the gold standard[25]. As it was discussed above for the Kappa agreement index the specificity varies according the transmission scenarios from 74% to 98% for rK39-RDT and from 60% to 98% for DPP (Table 6)[6].

Therefore, the difference in the literature about CVL RDTs sensitivity, specificity and confidence interval amplitudes may be related both to methodological issues[13-16] and biological ones, besides the possible differences in parasite species/strains-geographical settings. According to the gold standard or the tests taken as true positive the sensitivity could vary from 15% to 26%[22]. On the other hand, among the biological-based differences between studies, population and individual variables may modulate the performance of the test as genetic profile of dogs, socio-epidemiological context (life quality and immunocompromise), prevalence rate, time since infection, antibody titre, parasite load, clinical score and infectiousness[8, 15].

The cross-reactions reported are: rK39-RDT DiaMed-Vet-IT with *Neospora caninum* in 1 out of 9 sera, *Hepatozoon canis* 1/2[26], and human malaria 1/55[27]; rK39-RDT InBios *Ehrlichia* sp. 1/3 and *Trypanosoma cruzi* 3/12[28]; DPP with *L. braziliensis* 1/2 and 3/9, *L. amazonensis* 2/2[7, 29], *Babesia* sp. 4/9[21], *Leptospira* sp. 1/13 in our study. Other authors did not found cross-reactions of DPP or rK39-RDT with *Ehrlichia* sp. or *Babesia* sp. [30]. Therefore, besides the lack of information in same articles about differential diagnosis to discard double infections, there are also many inconsistencies about cross-reactions that may be due to the stage of infection-immunity of the control cases and common epitopes or precipitation of unspecific immunocomplexes. Anyway, there are more reports of cross-reactions with DPP than with rK39-RDT, and thus the reactivity with other species of *Leishmania* that also infect dogs in sympatric scenarios requires further investigation.

In conclusion, the performance of rK39-RDT and DPP for CVL diagnosis is comparable and acceptable at least for symptomatic dogs when tested in scenarios of visceral leishmaniasis recent southern spread in Argentina. CVL RDTs are more portable for point of care diagnosis, have lower costs and are simpler, have quicker results, improve

TABLE 6.– *Reported overall sensitivities, sensitivities in symptomatic (S symp) and asymptomatic (S asympt) populations, and specificities (Spec) of DPP and rK39-RDT tests for detection of anti-*Leishmania *antibodies*

	Sensit	S symp	S asymp	Spec		Ref.
Rk39-RDT/DPP						
	92-100	96.0	83.0	98-95	Argentina	*
	87.5	89.0	82.0	97.0	Argentina	**
Rk39-RDT/						
Human VL	93.9			95.3	World	15
	84.7			96.8	Brazil	12,13
Canine VL	89.6				World	15
	64.7	66.7	55.6		Argentina	15 a
	88.0	90.0	77.0	74-98	Brazil	6
	96.0			100	Brazil	18
	46.3	76.9	33.3	100	Brazil	15 b
	72-77			61-75	Brazil	16 c
	55.2	60.0	33.3		Brazil	17d
	91.5			94.7	Brazil	19 e
DPP						
Canine VL	93.0			92.0	Brazil	20
	86.0			94.0	Brazil	9
	90.6	89.4	92.1	95.1	Brazil	21
	21.4			92.6	Brazil	23
	81.3	76.2	23,8	72.4	Brazil	24
	87.5-88	100	72.7	68.2-73.3	Brazil	25
	72.5	98.0	47.0	96.0	Brazil	7
	89.0	89.9	75.0	70.2	Brazil	5
	98.0	98.0	100	60-98	Brazil	6

** This study: Sera panel, mean of DPP and rK39-RDT results*
*** This study; Field performance, mean of DPP and rK39-RDT results*
[a]*Cruz I, Acosta L, Gutierrez MN, Nieto J, Canavate C, et al. (2010) A canine leishmaniasis pilot survey in an emerging focus of visceral leishmaniasis: Posadas (Misiones, Argentina). BMC Infect Dis 10: 34, re-analized by 15*
[b]*Cohort study*
[c]Leishmania *RAPYDTEST; Intersep, Wokingham, United Kingdom*
[d]*Sensit* Leishmania *rK39 Ubio Biotechnology Systems Pvt Ltd, Cochin Kerala, India.*
[e]*rK39 immunochromatography non specified brand*

the compliance of dog owners and the relationship with the communities[12]. However, the statement of acceptability should be contextualized according the purpose of the test, from clinical diagnosis (individual-based), to seroprevalence studies (population-based) or outbreak control tools (decision making). In this sense, the evaluated RDTs do not discriminate between infection and immunity, involving pre-clinical and recently infected dogs, and chronic individuals who solved the infection but remain with immune memory [6]. Therefore, both RDTs, are still acceptable for program dog population-based studies as spatial stratification, focus intervention and follow up, dog individual screening and confirmation of clinical presumptive CVL diagnosis in polysimptomatic dogs. However, the sensitivity to detect asymptomatic and even to discriminate among symptomatic the most infectious ones (core-transmitters/super-spreaders) is not enough for operational programs[15,31] , in order to target reservoir-based control interventions, mainly in high prevalence settings. Immunological tests in combination with other non-immunological based tests will be required for highly sensitive/specific diagnosis of infected dogs with *L. infantum*, for reservoir management both from public health and individual animal health perspectives.

Conflict of interest: None to declare

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LA TAPA

***Colchicum autumnale* -** Hans-Simon Holtzbecker (m. 1671)

***Colchicum autumnale*.** Del *Gottorfer Codex* (1649 al 1659). Hans-Simon Holtzbecker (m. 1671). *Gouache* sobre pergamino. Fuente: *https://commons.wikimedia.org/wiki/File:Gc23_colchicum_autumnale.jpg*

Libro encargado a Holtzbecker por el Duque de Gottorp para ilustrar las flores del jardín de su castillo (Palacio de Gottorp, Schleswig**,** Alemania). Son cuatro volúmenes, 365 páginas ilustran 1180 plantas (50 × 38 cm). El *Codex* se encuentra en la *Royal Collection of Graphic Art* de la *SMK - National Gallery of Denmark*, Copenhague, Dinamarca. La restauración para exhibir y digitalizar las páginas puede apreciarse en: *https://www.youtube.com/watch?v=JLtWrWT0mAs*. El artista era nativo de Hamburgo y se le atribuyen cuatro florilegios, uno de ellos, el *Moller Florilegium*, se remató en Christie's, Londres, por 551 500 libras (GBP) en 1999.

El bulbo del cólquico (*syn*: mataperros, azafrán bastardo, etc.), se usó desde tiempo inmemorial para tratar la inflamación, los dolores articulares, y la gota. Ahora sus usos son más amplios (Dasgeb B, Kornreich D, McGuinn K, Okon L, Brownell I, Sackett DL. Colchicine: an ancient drug with novel applications. *Br J Dermatol* 2018; 178: 350-6. doi:10.1111/bjd.15896). Más aún, se han publicado los resultados del *Colchicine Cardiovascular Outcomes Trial* (COLCOT), que probó dosis bajas de colchicina en la prevención secundaria después del infarto de miocardio (Tardif J-C, Kouz S, Waters et al. Efficacy and safety of low-sose xolchicine after myocardial infarction. *N Engl J Med* 2019; 381: 2497-505). Como sugiere una editorialista, mantengamos la cautela. (Newby LK. Inflammation as a treatment target after acute myocardial infarction. *N Engl J Med* 2019; 381: 2562-63). Y más cuando leemos la declaración de conflictos de interés.

El agente activo del cólquico es la colchicina, remedio o veneno, según la dosis. La colchicina bloquea las mitosis en metafase porque cuando se une a las tubulinas, altera su conformación e impide el ensamblaje de los microtúbulos y el huso mitótico. Este efecto sobre las células fue descubierto por R. Pernice, patólogo siciliano que publicó su trabajo en una inalcanzable revista siciliana. Al lector curioso puede interesarle el editorial de Gerald Weissmann titulado *Medea and the Microtubule: Research has been translational ever since colchis,* erudito, entretenido y pleno de información. (*Faseb J* 2009; 23: 2761-94).