Original

Immunochromatographic rapid test in diagnosis of canine distemper

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ABSTRACT

Objective. To evaluate the use of a rapid immunochromatographic test, associated with a complete blood count and the search for viral inclusions as a method of diagnosing distemper in dogs. **Materialsand methods.** Nineteen dogs, males and females, aged 2 to 120 months, with clinical suspicion of distemper, were examined at the UFFS Veterinary Hospital. Conjunctival swabs were collected to perform the rapid immunochromatographic test and 3-5 ml bloodsamples were drawn for complete blood count and Lentz corpuscle investigation. After performing the blood count, the blood was centrifuged, and the plasma was used to perform a second test. **Results.** Among the 19 suspect animals, seven were positive in the rapid test, both in blood plasma and conjunctival swab material; in two of these, Lentz bodies were observed. **Conclusions.** The association of the rapid immunochromatographic test with the complete blood count in dogs with clinical suspicion of distemper improves the chances of diagnosing the disease in the acute phase and the test can also be performed with blood plasma, using the same sample sent for complete blood count. The hematological study revealed that anemia and lymphopenia are the main changes in positive animals.

Keywords: Blood cell count; immunochromatography; lymphopenia; Morbillivirus (*Source: MeSH, DeCS*).

RESUMEN

Objetivo. Evaluar el uso de una prueba inmunocromatográfica rápida, asociada con el recuento sanguíneo completo y la búsqueda de inclusiones virales como método de diagnóstico de moquillo en perros.**Materiales y métodos.** Diecinueve perros, machos y hembras, de 2 a120 meses, con sospecha clínica de moquillo fueron examinados en el Hospital Veterinario de la UFFS. Se recogieron hisopos conjuntivales para realizar la prueba inmunocromatográfica rápida y también se sacaron muestras de 3 a 5 ml de sangre para el recuento sanguíneo completo y la investigación del corpúsculo de Lentz. Después de realizar el recuento sanguíneo, la sangre se centrifugó y el plasma se usó para realizar una segunda prueba. **Resultados**. De los 19 animales sospechosos, 7 fueron positivos en

la prueba rápida, tanto enplasma sanguíneo como en material de torunda conjuntival, en 2 de estos se observaron cuerpos de Lentz. **Conclusiones.** La asociación de la prueba inmunocromatográfica rápida con el recuento sanguíneo completo en perros con sospecha clínica de moquillo mejora las posibilidades de diagnóstico de la enfermedad en la fase aguda y la prueba también se puede realizar con plasma sanguíneo, utilizando la misma muestra enviada para el recuento sanguíneo completo. El estudio hematológico reveló que la anemia y la linfopenia son los principales cambios en los animales positivos.

Palabras clave: Inmunocromatografía; linfopenia; *Morbillivirus*; recuento de células sanguíneas (*Fuente: MeSH, DeCS*).

INTRODUCTION

Canine distemper is a cosmopolitan viral disease that can result in death for dogs and other carnivores. Encephalitis caused by the canine distemper virus, an RNA virus of the Morbillivirus genus, is the leadingcause of death. It is a highly contagious disease, with the second highest mortality rate among dogs, which is why it is of utmost epidemiological importance (1).

The virus has a tropism for lymphoid, nervous, and epithelial tissue, and the affected animals may have different symptoms (2,3). Specifically in domestic canids, the age of the animal, as well as its immunologicalstatus and virulence of the strain, will define the severity and duration of the course of the disease (4).

The first contact that will cause an infection is usually inhaling the virus. In the respiratory tract of the host, the virus will replicate in the lymphoid tissue and will later be engulfed by macrophages, with the consequent spread to other organs. After the incubation period, which varies from seven to 28 days, the animals develop a characteristic biphasic fever, compatible with viremia and generalized infection of the lymphoid tissues, with lymphoid depletion and lymphopenia. Profound immunosuppression is a consequence of apoptosis and spinal dysfunction. The second viremia is associated with high fever and infection of parenchymal tissues, such as the digestive tract, the skin, and the central nervous system (CNS) (4,5).

The clinical manifestations are directly related to the tissue predilection of the virus. They are characterized by nasal discharges, rhinitis, conjunctivitis, anorexia, diarrhea, secondary dermatitis, hyperkeratosis of the cushions and snout, and with a higher incidence, the most varied neurological signs, depending on the degree of involvement and viral distribution of the CNS. Hyperesthesia, cerebellar and vestibular signs, lack of motor coordination, tetraparesis, myoclonus, and ataxia are common signs (6).

Countless methods for diagnosing canine distemper exist. In the past, viral isolation in cell cultures was attempted; however, the study proved to be slow and prone to false negative results when the animal was not in the acute phase of the disease (7). Once in the central nervous system, in the case of persistent infections, the virus generates a local antibody response, and its detection can be carried out through the cerebrospinal fluid, proving to be an effective method for virological diagnosis in cases of encephalitis (8). There are also indirect methods that detect and quantify specific viral antibodies with their advantages and disadvantages (9). The reverse transcription-preceded polymerase chain reaction (RT-PCR) technique has been used successfully in animals with clinical signs of the disease for some time (10); however, since this technique is not available in all veterinary service facilities, there is a need to send the biological material to specialized laboratories, which leads to a delay in diagnosis.

The most punctual and specific diagnosis can be made from the blood smear, where Lentz inclusion bodies can be seen in red blood cells andleukocytes. Other types of cells may also contain the corpuscle, suchas cells associated with exudates (eye secretions) and epithelial cells. It is worth mentioning that this visualization is not always possible since the viral presence is closely related to the viremia period. Even in the absence of inclusion bodies, infection with distemper virus should never be ruled out (11). Sousa et al (12) pointed out that 21% of infected animals will have inclusion bodies.

Chromatographic immunoassay tests offer a quick and accurate diagnosis. The test detects protein F, a protein that makes up the virus, common to all existing strains. It can be performed with

samples of conjunctival mucosa, serum, plasma, whole blood, or feces from an infected dog (9). Evolution using the immunochromatographic method should, when possible, be associated with other findings (13). According to Sousa et al (12), leukocyte morphology and inclusion corpuscles should always be evaluated in animals with suspected distemper.

There are studies that prove that immunochromatographic tests are closely dependent on the biological sample used and the degree of infection of the disease, since a high sensitivity was found in 100% of conjunctival swab samples (14). Therefore, this study aimed to evaluate the use of a rapid immunochromatographic test associated with the complete blood count and looking for viral inclusions as a method of rapid diagnosis of distemper in dogs with clinical suspicion of the disease. The use of the conjunctival swab test was also compared with the use of blood plasma, according to the manufacturer's instructions.

MATERIAL AND METHODS

Animals and study site. The study was carried out at the Superintendency of the University Veterinary Hospital Unit (SUHVU) of the Federal University of Fronteira Sul, Campus Realeza-PR. Nineteen dogs, whose main clinical suspicion was canine distemper, were evaluated regardless of their sex, breed, or age, from July 2016 to July 2017.

Sampling procedures. Samples were collected after local antisepsis with a gauze soaked in 70% alcohol, 3 to 5 ml of blood were collected from the jugular vein using a 5 ml syringe and a 25 x 7.5 mm needle. Blood was placed in a tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for complete blood count and plasmaperformance. Biological material was collected from the ocular conjunctiva with a sterile swab for immunochromatographic testing in the practice.

Rapid immunochromatographic test. After the clinical evaluationof the animal, the immunochromatographic test was performed with a commercial kit (SensPERT Cinomose-Vencofarma Ltda, Londrina, Brazil) (Figure 1), using the material collected from the conjunctival swab, following the manufacturer's

guidelines. The kit consists of a sterile swab, a tube containing a buffer solution, a pipette, and the test cassette, which contains specific antibodies to detect the F protein of the canine distemper virus. The sample was placed in the buffer solution and 100 μL of the solution was placed in the test cassette. The buffer solution was moved by capillary action, and although there were no antigens in the sample, a first control line (C) was marked, which guarantees the viability of the test. If antigens are present, they bind to the antibodies present on the nitrocellulose membrane, marking as positive on the test line (P). The animal was considered positive when the mark on the test line occurred within five to ten minutes after applying the sample to the test cassette, as recommended by the manufacturer.

Figure 1. Immunochromatographic test with positive result. Line control (C) and test line (P).

Lab tests. Blood count was performed in an automatic cell counter (Bio - 2900 Vet - Bioeasy Diagnostical), recording the total leukocyte count, the number of red blood cells, the hemoglobin concentration, the mean corpuscular volume (CMV), the hemoglobin concentration, corpuscular mean (MCHC), and the coefficient of variation of the erythrocyte diameter distribution (RDW-CV - Red Cell Distribution Width). The hematocrit was determined by the microhematocrit method (NI1807 hematocrit centrifuge, Nova Instruments, Piracicaba-SP, Brazil). Blood smear was stained with a rapid hematological stain of the Romanowsky type (Panoptic Rapid LB- Laborclin Ltda, Pinhais, Brazil), the differential examination of leukocytes in 100 cells, the morphological evaluation, and the investigation of the intra-erythrocyte body / Lentz intra-leukocyte. After performing the blood count, the blood was centrifuged to obtain plasma and 200 μl was diluted in 200 μl of buffer solution. After homogenization, 100 μl of the dilution was transferred to the test cassette, which was used for qualitative investigation of viral particles by the same rapid immunochromatographic test.

Statistical analyses. From the results of the rapid test and theobservation of the inclusion corpuscles, the animals were reorganized into two groups: positive group (PG), with animals with a positive result in the rapid test; and suspicious group (SG), with animals that are still suspect, that is to say, they were negative in the rapid test and did not have Lentz corpuscles. The Mann-Whitney test was applied to evaluate the difference between the PG and SG hematological data, with a significance level of 5%.

Ethical considerations. The owners agreed with the sampling, since the procedure would not cause any damage or alteration to their dogs. The experimentation has the approval of the Brazilian Ethics and Animal Experimentation (23205.003857 / 2016-56).

RESULTS

The results of the immunochromatographic tests were the same in the two biological materials tested, ocular secretion and blood plasma. Seven animals (33.3%) showed positive results in both samples (PG). The rest of the evaluated

animals remained suspicious (SG). In thePG, the age ranged from four months to six years, 57.14% were female and, in relation to the breed, purebred animals predominated (85.7%).

The results of the hematological evaluation are shown in Table 1. Statistical analysis of the hematological changes showed a significant difference between the groups. In the erythrogram, the PG presented lower values for the hematocrit, and in the leukogram, lower values for the lymphocytes and monocytes. There were no significant differences between the groups for platelet count and plasma protein concentrations. Thrombocytopenia was observed in 28.5% of the animals in the PG, while no animal in the SG presented thrombocytopenia.

In the morphological evaluation, reactive lymphocytes were observed in 28.6% of the positive animals and in 50% of the suspect animals. Intra-erythrocyte and intra-leukocyte Lentz corpuscles were observed in two PG animals, representing 9.5% of the total samples and 28.6% of the PG animals. No rapid test negative dog showed Lentz corpuscle.

Table 1. Results of the hematologial evaluation and concentration of total serum proteins (TSP) of 19 dogs in the city of Realeza/Paraná with clinical suspicion of distemper, grouped based on the result of the immunochromatographic test as positive, the reactive, the suspects, and the non-reactive animals.

Parameters	Median (25th percentile; 75th percentile)			
	RR	Positives (n=7)	Suspects (n=12)	p value
Erythrocytes (x 10^6 μL)	5.5 – 8.5	5.0 (4.05; 5.3)	5.4 (4.33; 6.6)	0.259
Hemoglobin (g/dL)	12 – 18	9.4 (8.4; 12.4)	13.1 (10.3; 16.2)	0.091
Hematocrit (%)	37 – 55	30 (26.2; 38)[b]	44 (33; 48)[a]	0.035
MCV (fL)	60 – 77	65.4 (63.8; 73.9)	66.5 (62.6; 70.8)	0.710
MCHC (%)	32 – 36	31.8 (29.3; 33.3)	32.3 (28.2; 33.3)	0.767
RDW-CV (%)	11 - 15φ	14.9 (14.3; 15.8)	14.4 (13.9; 15.3)	0.762
TSP (g/dL)	6 – 8	6.4 (6.2; 6.85)	5.8 (4.6; 7.1)	0.297
Blood Platelets (x 10^3 cells μL)	200 - 500	324 (140; 376)	320 (269; 458)	0.536
Leukocytes (x10^3 cells μL)	6 – 17	10.1 (8.9; 12.6)	17.5 (11.3; 21.1)	0.099
Neutrophils (x 10^3 cells μL)	3 – 11.5	8.3 (5.5; 11.7)	12.1 (6.4; 15.7)	0.277
Lymphocytes (x 10^3cells μL)	1 – 4.8	0.55 (0.38; 2)[b]	2.6 (1.8; 2.9)[a]	0.019
Eosinophils (x 10^3cells μL)	0.1 – 1.25	0.1 (0.02; 0.2)	0.52 (0.11; 1.28)	0.070
Monocytes (x 10^3cells μL)	0.15 – 1.35	0.4 (0.32; 0.69)[b]	1.8 (1; 3.1)[a]	0.005
Basophils (x 10^3cells μL)	0 – 300	0.0	60.4*	-

RV= Reference ranges (15);
φ Hematology analyzer baseline value;
[ab] In the same line, they indicate a significant difference by the Mann-Whitney test (p<0,05);
MCV= mean corpuscular volume; MCHC= mean corpuscular haemoglobin concentration;
RDW= Red Cell Distribution Width; TSP= Total Serum Protein; *Average.

DISCUSSION

The orientation of the manufacturer responsible for the kit for the detection of the distemper virus was to carry out the test with material collected with a swab from the nasal and ocular discharge in dogs. In the present study, it can be seen that the use of blood plasma showed the same safety in the result. The use of plasma allows the clinician who does not have the kit at the time of the consultation to send the sample to another veterinarian or laboratory for its performance.

Regarding the frequency of dogs detected as positive in the present study, the results were superior to previous studies that evaluated dogs with clinical suspicion of distemper. Santos et al (13) detected canine distemper antigens by immunochromatographic assay in 19.9% of the dogs with clinical suspicion, using conjunctival mucosa swabs as biological material.

In the study by Curti et al (9), conjunctival mucosa samples were also collected from dogs suspected of distemper, with 21.2% of animals positive for distemper antigens in the immunoassay kit. It is worth noting that in the study made by Curti et al (9), the diagnosis of distemper was confirmed by RT-PCR. There was no false positive in the rapid test; however, in six animals with systemic or neurological signs or both, the Immunoassay test was negative, but there was confirmation of distemper by RT-PCR. This demonstrates that in distemper, the sensitivity of rapid tests is inferior to molecular diagnosis, and the possibility of disease in non-reactive animals should not be excluded.

In the study by Curti et al (9), conjunctival mucosa samples were also taken from dogs suspected of distemper, with 21.2% of animals positive for distemper antigens in the immunoassay kit. It may be noted that in the study by Curti et al (9), the diagnosis of distemper was confirmed by RT-PCR. This means that there was no false positive in the rapid test; however, in six animals with systemic or neurological signs or both, the Immunoassay test was negative, but there was confirmation of distemper by RT-PCR, thus demonstrating that in distemper, the sensitivity of rapid tests is inferior to molecular diagnosis, and the possibility of disease in non-reactive animals should not be excluded.

An et al (14) performed an immunochromatographic assay in 66 animals with clinical symptoms suggestive of distemper, from blood samples, nasal discharge, and a conjunctival swab. Most of the dogs were young, with systemic and acute clinical signs. All animals were positive for the test and in two animals, the result was considered a false positive compared to PCR. The study revealed greater specificity when analyzed from conjunctival mucosa samples.

In the present study, Lentz corpuscles were observed in 28.6% of the animals in the SG. According to Silva et al (16), these inclusions represent the cytopathic effect in cells, their visualization in erythrocytes and leukocytes confers a definitive diagnosis. The frequency of observation of the Lentz corpuscle in the GS was similar to the study by Sousa et al (12), who found 22.2% of the animals with the inclusion corpuscle. It can be observed that the inclusion of Lentz, although confirmatory, may not be present in most animals with distemper, since its presence is restricted to the viremic phase that occurs in the acute phase of the disease (16).

Among the negative animals in the rapid test, two showed clinical signs compatible with a chronic condition of distemper. The immunoassay kit works by detecting antigens and not antibodies; therefore, the fact that they were in the chronic phase of the disease cannot be excluded (9). In the rest of the animals in the GS, the diagnosis was not conclusive and pathologies that have similar symptoms cannot be ruled out; a variety of signs are common in the inflammation of the central nervous system and infectious diseases (9).

The ages of the PG animals were different from that found in the study by Santos et al (13), in which the animals had an average age of seven years. Regarding breed, purebred animals predominated in Santos et al (13); however, Budaszewski et al (2) and Sousa et al (12) observed a higher prevalence in mixed breed animals. The frequency of the gender was similar, which is in confirmation with that studied by Freitas-Filho et al (17).

In the hematological evaluation (Table 1), anemia was the predominant change in the positive group (85.7%), with a statistical difference only for the hematocrit. There was no statistical difference for VCM, MCHC, and RDW. Anemia was classified predominantly as regenerative. Anemia is a frequent finding in distemper, determined by the destruction of erythrocytes by the virus or by the formation of immune complexes in their membrane, which cause hemolysis (18). It is observed that the

hematological findings of different studies reveal the high prevalence of anemia in animals positive for canine distemper (19). Silva et al (16) found 61% anemia and Santos et al (13) 48.32%.

For total serum protein, there was no difference between the groups. The values were close to the lower limit of reference in both. Silva et al (16) found hypoalbuminemia in 100% of the animals studied and an elevation of alpha 2 globulin in 54%. The authors justify that the low level of serum albumin is frequent in animals with distemper, due to intestinal epithelial damage with consequent diarrhea, apathy, and food rejection, and also due to vomiting, which results in less intake and absorption of proteins. The plasma globulin elevation is common in several inflammatory reactions; in particular, the alpha 2 component is significantly increased in viral and bacterial infections, such as distemper.

In the positive group, the total leukocyte count was within the normal range and the main change was lymphopenia, with a significant difference between the groups. Lymphopenia is related to the viral predilection for lymphoid tissue, which causes its atrophy and subsequent decrease in cell production (20). Barbosa et al. (21) also confirm immunosuppression due to the tropism of the virus for lymphoid tissue, which causes transient lymphopenia, infects mature lymphocytes, and causes apoptosis. Reactive lymphocytes were observed in 28.6% of the positive animals and in 50% of the animals in the suspicious group. Souza et al (12) observed this change in 72.2% of the animals with suspected distemper.

In suspicious animals, leukocytosis was observed, due to neutrophilia and monocytosis, with a significant difference between the groups only for monocytes, indicating a chronic inflammatory process, which may be due to distemper and other diseases (22). In distemper, neutrophilia occurs in response to a secondary bacterial infection, especially in the chronic phase (20). Eosinophil and basophil counts were similar between groups.

Thrombocytopenia has been observed in 28.5% of the PG animals and there were no significant differences between the groups for the number of platelets. The reduction in the number of platelets is an important change between the hematological changes in dogs with distemper, which may occur more frequently than in the present study. Silva et al (16) found in 69% of the dogs, Santos et al (13), in 50.33%, and Sousa et al (12), in 38.8%.

Analyzing the most frequent changes, the findings of the present study corroborate with previous studies. Tudury et al (23), Almeida et al (20), and Barbosa et al (21) describe anemia, lymphopenia, and leukocytosis as the most common changes. Thrombocytopenia is described by Almeida et al (20) and Souza et al (12). Souza et al (12) also describe anemia as the most common disorder, followed by reactive lymphocytes, monocytosis, and lymphopenia.

In conclusion, the association of the rapid immunochromatographic test with the complete blood count in dogs with clinical suspicion of distemper improves the chances of diagnosing the disease in the acute phase. Additionally, the test can also be performed on blood plasma, using the same sample submitted for the complete blood count. The hematological study revealed that anemia and lymphopenia are the main changes in positive animals.

Conflict of interests.

The authors of this study declare that there are no conflicts of interest with the publication of this manuscript.

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