Parvovirus and distemper - the serious gastroenteritis viral

Parvovirose e cinomose – as graves gastroenterites virais

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ABSTRACT
Viruses, in their great majority, have the serious capacity to be easily transmitted through countless carriers. The great concern is due to the fact that they are able to promote critical conditions that threaten animal body homeostasis, inducing serious pathological conditions such as vomiting and diarrhea. Parvovirus is characterized by being a serious disease that affects a large part of the young animal population due to numerous reasons such as the nonexistence or lack of immunity right when they are born. Affected animals have very serious enteric conditions, which often lead the animal to death quickly. Canine distemper, caused by the canine distemper virus, causes enteric conditions and mainly neurological conditions, promoting diarrhea and nervous symptoms such as cases of paralysis and incoordination of the affected members. Diseases, if detected in time, enable effective therapeutic, control and prevention measures that can increase the life expectancy of the affected animals and, in some cases, cure patients.

Keywords: death, disease, dogs, virus

RESUMO
Os vírus, em sua grande maioria, possuem a grave capacidade de serem facilmente transmitidos por meio de inúmeros veiculadores. A grande preocupação, se deve ao fato de serem capazes de promover quadros críticos que ameaçam a homeostase corpórea animal, induzindo graves quadros patológicos como vômitos e diarreias. A parvovirose, se caracteriza, por ser uma doença grave que afeta grande parte da população animal

jovem devido a inúmeros motivos como pela a inexistência ou falhas da imunidade logo quando nascem. Os animais afetados apresentam quadros entéricos gravíssimos, os quais muitas das vezes levam o animal ao óbito rapidamente. A cinomose, causada pelo vírus da cinomose, acarreta quadros entéricos e principalmente quadros neurológicos promovendo quadros de diarreia e sintomatologia nervosa como casos de paralisia e incoordenação dos membros dos afetados. As doenças, se detectadas a tempo, possibilitam medidas terapêuticas, de controle e prevenção eficazes que podem aumentar a expectativa de vida dos animais acometidos e em alguns casos, a cura dos pacientes.

Palavras-chave: óbito, doença, vírus, cães

1 INTRODUCTION

Due to the high rates of morbidity and mortality caused by gastric and enteric disorders in dogs, and the high prevalence of such disorders in veterinary clinics and hospitals, the need for an ever more in-depth and updated knowledge of veterinarians in these diseases is notable. The lack of information often compromises the diagnosis of the disease and the knowledge of its etiopathogenesis, negatively influences the treatment processes, prophylaxis, and even the information passed on to the tutors. Due to the scarcity of epidemiological surveys on the occurrence of gastroenteric diseases in dogs, there is a need to improve research in this area, in view of the great importance and relevance of these conditions for the health of animals.

2 VIRAL GASTROENTERITIS

Gastroenteritis are diseases that occur frequently and among the various causes of gastroenteritis are viral, bacterial, parasitic, medicated, immune-mediated and idiopathic. The most common clinical signs presented are vomiting and diarrhea, which are potential sources of dissemination of contaminating particles, thus being able to affect a large number of animals (Bichard e Sherding, 2003; Abd El-Baky et al., 2017). Viral gastroenteritis is the most relevant disease for small animal clinicians, due to its high incidence and pathogenicity, ease of transmission between animals and maintenance in the environment. After the contagion has occurred, the entire manifestation of the disease will be determined by a series of factors, such as the age of the animal, the virulent power of the viral strain, the pathways of infection, body condition, stress and the possibility of secondary diseases being associated (Correa e Correa, 1992; Pereira, 2014).

Among the most common agents to promote viral gastroenteritis are parvovirus and canine distemper virus, which are almost always related to cases of diarrhea and

vomiting (Greene e Decaro, 2015; Pereira, 2015). Animals infected with parvovirus can have extensive symptoms due to the virus affecting the gastroenteric tract, bone marrow, myocardium, skin and nervous system. Yellowish hemorrhagic diarrhea, sudden onset vomiting, anorexia, lethargy, apathy, fever, changes in hematological patterns such as leukopenia due to lymphopenia due to immune destruction and, in some cases, anemia, among other secondary diseases (Moraes e Costa, 2007; Greene e Decaro, 2015; Abd El-Baky et al., 2017; Degene e Zebene, 2019). Distemper clinically promotes those affected, multifocal encephalopathies, head tilting, nystagmus, leukopenia, imbalances, paralysis, myoclonus, loss of appetite, depression, seizures in severe cases, eye, nasal discharge purulent, tonsillitis, feverish conditions. Diarrhea, vomiting, dry cough tending to become wet, dyspnoea, purulent dermatitis, blindness. Coinfections can promote symptomatic variation (Negrão et al., 2007; Moraes et al., 2013; Greene e Valdevelde, 2015).

2.1 PARVOVIROSIS

Parvovirus is a disease capable of promoting one of the most serious and common enteritis in the clinical routine, being extremely contagious, deleterious, and often lethal. Parvoviruses belong to the family *Parvoviridae*, subfamily Parvovirus, having a viral DNA structure (Abd El-Baky et al., 2017). They do not have an envelope, thus hindering their elimination through common disinfectants and guaranteeing their survival in the environment for up to 5 months (Pereira, 2015; Grano et al., 2019). It affects dogs of all ages and breeds, preferably puppies aged between 6 weeks and 6 months, as they have an immune system still in development (Monti, 2004; Silva, 2010; Vieira, 2011; Gimenes, 2016).

Among the breeds of greater predisposition, first, mixed breed dogs with 31.25% of involvement, followed by Poodle (12.5%), Rottweiler (10.4%) and Shih-Tzu (6.25 %) (Oliveira, 2007). The greater predisposition of mixed breed dogs can be understood due to the breed being in greater quantity in an errant manner in urban centers, in addition to being animals with less vaccination coverage because they are not vaccinated most of the time, thus enabling greater susceptibility of them (Glickman et al., 1985; Mouzin, 2004; Oliveira et al., 2009). Breeds such as Pinscher, Dobermann, German Shepherd, Labrador and Pit Bull are also mentioned in different studies as breeds with a high degree of involvement (Glickman et al., 1985; Isola, 2014). Only about 9% of the puppies survive without medical care, and about 90% recover after starting the appropriate therapeutic

protocol (Balvedi et al., 2015). The occurrence of parvovirus in dogs ranges from 30.7% (Granados, 2015) to 34.6% (Gizzi, 2014).

Parvovirus can be classified into two types, CPV-1 and CPV-2. Type 1 does not manifest pathological importance for animals, type 2 being the strain that causes the disease in dogs. CPV-2 has three mutant variants, CPV-2a, CPV-2b, and CPV-2c (Vannamahaxay e Chuammitr, 2017; Grano et al., 2019; Goddard e Leisewitz, 2019) with new mutations (De Oliveira et al., 2019). The CPV-2 incubation period can be from 7 to 14 days, however, in cases of mutant strains, it can be reduced to 4 to 6 days (Greene e Decaro, 2015). Most infections originate through contaminated feces released by sick dogs, around the third and fourth day of infection, which spread around 10^7 to 10^9 viral particles per fecal gram (Paes e Mangia, 2016). The contagion of healthy dogs occurs via oronasal or parenteral (Pereira, 2014). The infection begins when the virus binds to surface receptors, especially glycoproteins, replicating in lymphoid tissues of the oropharynx, mesenteric lymph nodes and thymus. Through the hematogenic and lymphatic pathways, they carry out the viremia process and spread to other regions, such as other lymph nodes, small intestine and bone marrow. In the medullary region, the virus can promote a deleterious action in the precursors of erythroid, myeloid and megakaryocytic cell lines, promoting medullary hypoplasia (Arns et al., 2012; Hoskins, 2014).

Most of the time, the intestinal epithelium is the most affected, especially the crypts of the small intestine, which give rise to the villus cells. As the disease progresses, other regions are also affected, such as the jejunal region. Viruses affect the germinative base of cells, impairing epithelial renewal and causing extensive areas of crypt and villus necrosis, which causes a severe inflammatory condition with changes in the absorption/regulation of nutrients and electrolytes and the occurrence of mucous diarrhea and/or bloody (Sherding, 2013; Greene e Decaro, 2015).

The clinical picture is manifested by the presence of frequent vomiting in association with yellowish diarrhea, tending to gray, with streaks of blood or melena (Abd El-Baky et al., 2017; Grano et al., 2019). Affected animals show marked emaciation and dehydration, requiring rapid fluid replacement. Changes in the hematological profile, such as neutrophilia and leukopenia, may be present in the most severe cases, leading to the occurrence of secondary bacterial infections (Wang e Wang, 2019), endotoxemia and septicemia (Arns et al., 2012; Abd El-Baky et al., 2017). In addition to gastroenteric changes, which are the most prevalent, occurrences of neurological diseases due to sepsis,

cutaneous diseases, such as multifocal erythema, myocarditis (Ford et al., 2017; Molesan et al., 2019), thrombosis, cystitis (due to ascending contamination by feces), among others (Greene e Decaro, 2015).

2.2 DISTEMPER

Distemper is an infectious disease caused by viruses belonging to the genus *Morbilivirus* and the family *Paramyxoviridae* which is characterized by being simple, enveloped RNA (Tozato et al., 2016), composed of H and F glycoproteins (Carvalho et al., 2012; Rendon-Marin et al., 2019), fundamental for the entry and fusion of the virus to the host cells, causing various types of clinical conditions within the same disease (Greene e Valdevelde, 2015). Among the affected species, canids are the most vulnerable to distemper (Mendes et al., 2000; Degene e Zebene, 2019).

The age group most affected is around 03 to 06 months, precisely because it is the period of reduction of maternal protection obtained through colostrum (Larson et al., 2006; Greene e Valdevelde, 2015). Among the most affected breeds are the mixed breed dogs corresponding to 65.5% of the cases, followed by the Poodle breed (12.5%), Pinscher (8.7%) and Pit Bull (2.9%) (Brito et al., 2016). Similar results were found in different studies classifying mixed breed, Poodle and Pinscher dogs as the breeds most affected by distemper (Frade e Dantas, 2011). The higher occurrence of mixed breed dogs is due to the greater susceptibility presented by being in greater contact with sources of contamination and less vaccine protection (Glickman et al., 1985; Mouzin et al., 2004; Larson et al., 2006; Oliveira et al., 2009).

The virus begins to be eliminated about seven days after the animal's contact with transmissible sources, including respiratory and ocular secretions, spreading through contaminated aerosols or droplets (Morães e Costa, 2007; Degene e Zebene, 2019). It is possible for transplacental transmission to occur, so the puppies can already be born infected. In addition, asymptomatic cases serve as sources of viral replacements in clusters of dogs, ensuring an increased spread of the disease (Greene e Valdevelde, 2015).

After contact of the animals with contaminated secretions, the virus reaches the epithelium of the cranial aerial ways. Within 24 hours after contact, viral multiplication in regional macrophages begins and, through lymphocytes, are disseminated to the tonsils and lymph nodes of the bronchial tree. After 2 to 4 days from contact until the onset of symptoms, the lymph nodes and tonsils become organs filled with viral loads (Alves et al., 2020). After 4 to 6 days, these viruses are disseminated and multiply in splenic

lymphoid follicles, in liver Kupffer cells, affect the lymphoid tissue associated with the stomach and intestines, in addition to the mesenteric lymph nodes. The temperature rises and the change in the hematological pattern can be observed through leukopenia, due to lymphoid destruction (Morães e Costa, 2007; Greene e Valdevelde, 2015).

The virus reaches the central nervous system, via hematogen, about 8 to 9 days after the onset of symptoms, causing acute and/or subacute encephalitis (Rendon-Marin et al., 2019), with lesions in the white and gray substances of the brain, which can evolve to polioencephalomalacia (gray substance necrosis). In addition, the virus also multiplies in the choroid plexus, a structure responsible for the production of cerebrospinal fluid, being easily disseminated to regions that come into contact with the liquid, such as the optic nerves (Carvalho et al., 2012; Greene e Valdevelde, 2015). It also affects the anterior region of the olfactory bulbs, altering the entire olfactory cortex and the limbic system, which is responsible for emotions and social behavior. The severity and number of lesions are variable due to immunological and characteristic factors of each animal (Greene e Valdevelde, 2015).

Upon contact with the epithelium of the gastrointestinal tract, the virus can penetrate and multiply in the lymphatic tissue referring to the stomach and small intestine lamina, inducing regional lymphocytes to apoptosis, compromising the animal's immunity (Carvalho et al., 2012; Greene e Valdevelde, 2015**)**. The presence of the virus causes degradation of the epitheliums and lymphocyte destruction, causing a severe inflammatory response, resulting in gastroenteritis (often bloody), which usually come with vomiting (Freitas, 2019). After epithelial colonization by the virus, secretions with viral particles can now be eliminated through the skin, glands, urine, feces, vomiting and eye and respiratory secretions (Greene e Valdevelde, 2015; Santos et al., 2020). The signs can be resolved as the animal's immunity rises, however, the distemper virus has the ability to induce latency in the tissues, and may be stagnant for long periods in organs and tissues, making detection difficult in tests, but it is enough that there is an immunosuppressive event, such as stress, for the disease to reignite. Animals can recover from the disease, but the fact is related to long periods of immunity and excretion of the virus. In addition, secondary bacterial infections can intensify the clinical status of the affected animal, due to the high degree of immunosuppression (Greene e Valdevelde, 2015).

Puppies affected by systemic disease may have thymus atrophy, rhinitis, conjunctivitis, tracheitis, bronchitis, pneumonia, catarrhal enteritis and even problems

with dental coverage (Greene e Valdevelde, 2015). Due to the virus's ability to induce latency and take shelter in the integument, as in digital cushions, these can present hyperkeratosis. Viral inclusions, acidophilic in color, can be present in various tissues and cells such as neurons, digestive mucosa, cardiac region (Molesan et al., 2019; Bastos et al., 2020; Kim et al., 2020), urine, among others (Greene e Valdevelde, 2015). In addition, it is possible to observe the presence of giant cells (Moraes et al., 2013) in the white matter of the brain, in the uveas, lymph nodes and in the lungs (Greene e Valdevelde, 2015).

3 VIRAL RECONTAMINATIONS

Due to the vast deleterious power of parvovirus and distemper virus for the general health of animals, it is necessary to pay attention to their occurrences, mechanisms of origin and pathogenesis, in addition to the possibility of reinfections. Reinfections are observed in cases where there is no effective sanitary control, agglomerations of animals (Sellon e Crawford, 2010), failures and lack of vaccine protocols (Vieira, 2011) structured in 3 initial doses and annual boosters, immunity reductions, in addition to specific and individual factors of each animal (Maes et al., 2003; Sprea, 2005). Animals that do not present clinical symptoms, that is, asymptomatic ones, provide viral dispersion, which contributes to the spread of the diseases reported in the study (Greene e Valdevelde, 2015).

Cases of recontamination by parvovirus are due to continuous exposure to the infecting agent, due to the viruses remaining in the environment for a long period of time after being eliminated, which is related to the lack of viral envelope (Prittie, 2004; Grano et al., 2019). In the case of reinfection by the distemper virus, the high dispersion of the virus through, mainly, the airway, added to the intense contact between animals, allows new contamination to occur. All of the factors described above are of great value for the health-disease process, however, for new diseases to occur it is necessary that there is an imbalance of the animal organism and that it becomes susceptible (Souza et al., 2002; Guyton, 2017). Through recontamination, the organism is subject to numerous damages to the physical and cellular integrity of the animal. Changes in morphology and structure are observed, even in the physiological processes performed by them, such as the mechanisms of absorption and secretion of substances essential for survival, in addition to general balance and defense against possible pathogens (Hamura, 2017).

4 DIAGNOSIS

To establish the diagnosis of parvovirus and distemper, one must take into account all the symptoms presented by the animal (Tozato et al., 2016), the history and blood tests performed for the general analysis of the patient's condition, in addition to the differential diagnoses such as other enteric viruses (Pelisari et al., 2010). To obtain the definitive diagnosis, however, specific tests are required for the detection of the virus or antibodies, such as immunochromatographic tests, electron microscopy, immunohistochemistry, immunofluorescence, isolation of the virus from infected tissues, PCR and ELISA, among others (PRITTIE, 2004). Among the various forms of diagnosis, it is possible to perform immunochromatographic tests, such as the Alere Parvovirose Ag Test Kit® and Alere Cinomose Ag Test Kit®. They are low cost and can be performed due to the ease of execution and allow a diagnosis to be made quickly, however, they can give false and/or dubious results, so that, depending on the animal's clinic, it is necessary to perform other tests for proof (Silveira, 2015; Suătean et al., 2020).

The Alere Parvovirose Ag Test Kit® is an immunochromatographic test, with a specificity equal to 98.8% and sensitivity of 100%, for the detection of parvovirus antigens by means of biological samples from rectal swab or even fresh feces (Alere, 2019). With high precision, they detect antigens by means of a staining mechanism that allows a quick reading of the presented result. In the test there are 02 lines, the test and the control, which remain inapparent before use.

Upon the start of the use and the analysis in a period between 5 - 10 minutes, the lines are colored and both can be colored (positive animal), only one is colored (negative animal), but, in some cases, failures occur. Among them, none of the lines may be colored; in these cases, the result must be disregarded and the test redone. Other failures in detection can result from numerous factors such as, for example, in cases where there is an insufficient amount of sample. For the test to be efficient in determining the result, it is necessary that there is an adequate amount of biological sample deposited, in order to dye the line efficiently and with a strong color. When there is insufficiency or reduction of the tested portion it is possible that there will be doubtful results, as they also depend on the reading of the performer (Esfandiari e Klingeborn, 2000; Desario et al., 2005).

The Alere Cinomose Ag Test Kit® can also be considered an immunochromatographic test for quantitative detection of the antigen as well as that of parvovirus, made from biological materials such as ocular, nasal, salivary, urine or even plasma samples. It has a specificity of 97.7% and a sensitivity of 98.8% (Alere, 2019a).

It works in a similar way to the parvovirus test, in which one or two lines will stain in a period between 5 - 10 minutes. Detection failure cases are likely to happen due to the factors discussed above (Ranno e Leseux, 2018).

5 TREATMENT

For parvovirus, the main means of treatment is the restoration of the hydroelectrolytic balance of these animals, with fluid therapy determined according to the degree of dehydration and assessment of the levels of electrolytes present. Generally, the fluid of choice is lactated ringer, and depending on the severity of the condition, the addition of glucose solution and potassium replacement is necessary. It is also extremely important that the treatment is aimed at combating possible secondary infections (Wang e Wang, 2019), which must take into account the role of gram-negative bacteria present in the intestine, due to the production of lipopolysaccharides for the correct choice of antibiotics (Pereira, 2015).

The treatment must also be symptomatic, according to the symptomatic picture that the affected animal presents over the course of the disease. In cases of vomiting, antiemetics should be present in the treatment protocol and in cases of blood loss, blood transfusion is an option to be considered, when large red blood cell and protein losses are evident. Among the antiemetics used are metoclopramide (0.2-0.4 mg/kg, subcutaneously (SC), every 6-8 hours; 1 to 2 mg/kg/day intravenous (IV), continuous infusion). The use of prokinetics is avoided due to the peristaltic effect they promote, and, in some cases, it can promote intussusception conditions such as metoclopramide, unless it is, in a slow continuous infusion. Unresponsive pictures can be controlled with chlorpromazine (0.3-0.5 mg/kg, intramuscularly (IM)/SC, every 8 hours). Ondansetron (Suătean et al., 2020) can be used in severe cases at a dose of 0.5 mg/kg IV, diluted in 0.9% saline solution, every 8 or 12 hours. In cases of acute and continuous vomiting, maropitant citrate (1 mg/kg/day, SC) becomes an important choice. To correct gastric ulcerations, the use of ranitidine and omeprazole has shown excellent efficacy (Pereira, 2015; Willard, 2015, Balvedi, 2015; Paes, 2016; Suătean et al., 2020).

For the treatment to be more efficient it is very important that the diagnosis and identification of the causative agent is as early as possible. The diet must be modified in such a way that, in the first days, nasogastric catheterization is performed with the administration of liquid food and, after the reduction of vomiting and diarrhea, the diet can be gradually changed from pasty to dry. The use of probiotics and pâtés becomes an

adjunct to the treatment of parvovirus, ensuring improvement of intestinal function and food source (Pereira, 2015). Monitoring during hospitalization is very important to determine the patient's prognosis (Suătean et al., 2020).

Regarding distemper, there is no specific treatment (Degene e Zebene, 2019), and supportive and symptomatic therapy is established, which aims to strengthen the affected animal's organism and inhibit and/or treat secondary infections in which the patient is susceptible due to immunosuppression, caused by the virus (Monteiro et al., 2010; Freire e Morães, 2019). Supportive treatment consists of broad-spectrum antibiotic therapy to control or treat opportunistic agents in the respiratory and gastrointestinal tracts (Freitas, 2017). Fluid therapy with lactated ringer or 5% glucose solution for energy supply, antipyretics, antiemetics, immunostimulants and gastric protectors if necessary. Use of B vitamins to stabilize affected neurotransmitters and stimulate appetite. Vitamins A and E can also be recommended for their antioxidant action to protect the nervous system due to the formation of free radicals (Freire e Morães, 2019).

Therapy of animals that already have neurological signs does not always show satisfactory recovery. In the case of seizures, diazepam (2-4 mg/kg intramuscularly) and phenobarbital as maintenance drugs (2 mg/kg oral or intravenous every 12 hours) are indicated (Greene e Valdevelde, 2015; Freitas, 2017). The use of glucocorticoids is only indicated in patients with chronic infections of the virus, with prednisolone being commonly chosen (2-4 mg/kg/day orally for 15 days), its use may be beneficial for the control of pupillary dilation that may occur due to encephalitis or optic neuritis. Corticosteroids such as dexamethasone (2.2 mg/kg intravenously) are recommended in case of cerebral edema, being maintained in anti-inflammatory doses (Monteiro et al., 2010; Freitas, 2017).

In order to decrease viral replication, ribavirin is used (30 mg/kg/day orally for 15 days) and may be associated with dimethyl sulfoxide (DMSO) (20 mg/kg/day intravenously for 15 days) diluted in 10 to 20% sodium chloride solution (0.9% NaCl) aiding in the diffusion of ribavirin in tissues and enhancing its action (Freire e Morães, 2019). Other supportive treatments are important to be applied aiming at the improvement and well-being (Duarte e Afonso, 2020) of the affected animal is the hygiene of the animal, leaving it free from nasal and ocular secretions in addition to providing an adequate nutritional support (Freitas, 2017). Unconventional treatments are being widely used to treat sequelae left by the virus, contributing to the rehabilitation of these animals and greatly improving their quality of life. Acupuncture and physiotherapy are used

successfully in the treatment of paresis, paralysis, myoclonus and other sequelae caused by the disease (Freire e Morães, 2019).

6 CONTROL AND PREVENTION

The control and prevention of viral diseases occurs through the use of vaccines in an appropriate manner (De Oliveira et al., 2019), focusing mainly on young dogs, so that it can fulfill its mission of promoting an adequate level of antibodies for the animal to be able to remain immunocompetent to the contact viral agent (Degene e Zebene, 2019). It is known that the use of the vaccine decreases the incidence of these infections, but it is also important to target control measures for such agents (Dezengrini et al., 2007). The vaccination protocol is very similar for all viral diseases; it must be started after 45 days of life, where the following two boosters are applied at an interval of 21. In adult animals, booster is recommended at an interval of 1 year (Pereira, 2015).

It is extremely important that animals are in good health, making it difficult for them to be affected by viral diseases, among other secondary agents that can also lead to being affected by a virus. For this, it is important to have an adequate diet associated with an environment that provides quality of life for the animal, clean, always focusing on the hygiene of the place and removal of feces and secretions, and restriction of free access to the street of these animals, where they may have relied on various types of infectious agents (Pereira, 2015)

7 FINAL CONSIDERATIONS

The veterinary medical clinic is subject to numerous losses caused by viral agents, in particular, the parvovirus and distemper virus. Affected animals develop severe changes in body balance, ranging from gastric to neurological changes, mild or severe, which can lead the animal to death if effective therapeutic measures are not carried out in a timely manner. Agents can be easily acquired by dogs due to numerous factors such as through intimate contact with the transmitting sources and even due to failures or lack of vaccine protocols. Knowing the distribution of these diseases, the susceptible animals and the most affected breeds, allows the clinician a better knowledge about the problems, thus enabling the establishment of effective control and prevention measures, such as the importance of vaccination during the young phase of the animals and reinforcements in the following years. Thus, it is essential to carry out more in-depth studies on canine gastroenteropathies, as a way to improve the clinical and therapeutic approach, in addition

to alerting tutors about the conditions and severities to which animals may be subjected, as a way to reduce involvement and ensure well-being.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the manuscript. They also report that they have no relationship with the company responsible for the production and dissemination of the tests mentioned in the material.

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