

SEROLOGICAL EVALUATION OF CANINE CORONAVIRUS ANTIBODIES

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ABSTRACT - Canine enteric coronavirus (CECoV) is an infectious disease, affecting dogs and being responsible for the occurrence of diarrhoea with various degrees of severity in puppies. Research has covered two main directions: the establishment of canine coronavirus carriers among healthy animals and those with digestive troubles (diarrhoea, vomiting and weight loss), and the assessment, after vaccination with canine anticoronavirus vaccine, of specific antibody titer type IgG and IgM. The values of optical density, determined by indirect ELISA for anticoronavirus antibodies type IgM, have varied from 93 to 1004, showing the specific immune response after infection and the establishment of canine coronavirus carriers and disposers. The values of optical density, determined by indirect ELISA for anticoronavirus antibodies type IgG, have varied after 14 days, from 89 to 556, while after 21 days, from 312 to 723. In case of anticoronavirus antibodies type IgM, after 14 days, the same values varied from 43 to 267, while after 21 days, from 45 to 253, showing the specific immune response after vaccine. The obtained results allowed us to assess the importance of knowing the carrier and removal status of canine coronavirus, as

a source of infection for other animals, not necessarily belonging to the same breeds. These could develop very severe diseases, even lethal ones.

Key words: coronavirus, dogs, antibodies

REZUMAT - **Evaluarea serologică a anticorpilor anticoronavirus canin.** Coronavirusul enteric canin (CECoV) este un agent patogen infecțios, ce afectează, pe scară largă, câinii, fiind responsabil de apariția unei diaree cu grade diferite de severitate. Cercetările efectuate au vizat două direcții importante: stabilirea purtătorilor de coronavirus canin în rândul animalelor sănătoase și cu afecțiune digestivă (diaree, vărsături și pierdere în greutate) și evaluarea, după vaccinare cu vaccin anticoronavirus canin, a titrului de anticorpi specifici, de tip IgG și IgM. Valorile densității optice, determinate de ELISA indirectă pentru anticorpi anticoronavirus de tip IgG, au variat de la 121 la 1426, iar în cazul anticorpilor anticoronavirus de tip IgM au variat de la 93 la 1004, indicând răspunsul imun specific după infecție și suspectarea animalelor ca fiind purtătoare și eliminatoare de coronavirus canin. Valorile

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densității optice, determinate de ELISA indirectă pentru anticorpi anticoronavirus de tip IgG, au variat, la 14 zile, de la 89 la 556, iar la 21 de zile, de la 312 la 723. În cazul anticorpilor anticoronavirus de tip IgM, la 14 zile, aceleași valori au variat de la 43 la 267, iar la 21 de zile, de la 45 la 253, indicând răspunsul imun specific postvaccinal. Rezultatele obținute ne-au permis să apreciem importanța cunoașterii statusului de purtător și eliminator de coronavirus canin, fiind sursă de infecție și pentru alte animale, nu neapărat din aceeași specie, și care pot dezvolta afecțiuni foarte severe, chiar letale.

Cuvinte cheie : coronavirus, câini, anticorpi

INTRODUCTION

Coronaviruses belong to *Coronaviridae* family, *Coronavirus* genus and include three distinct antigenic groups (Spaan, 1988; Lai, quoted by Gorbalenya *et al.*, 2004), each having genetically related species. Canine coronavirus (CCoV), feline coronavirus (FCoV), transmissible gastroenteritis virus in pigs (TGEV) and human coronavirus 229E belong to the first group. Viruses are large (27-32 kb), enveloped, with unsegmented genome, positive RNA monocatenar type and responsible for the production of various diseases in humans, fowls and domestic animals (Volker, 2007).

Respiratory tract, gastrointestinal organs and nervous tissue are the most common target organs for coronaviruses, but other organs are equally affected (Buonavoglia, 2006; Decaro, 2008). Canine coronavirus

may cause very contagious enteritis (Ruggieri, 2007). It was discovered in 1971 in Germany, during an outbreak in sentry dogs (Binna *et al.*, 1974, quoted by Pratelli, 2006).

In puppies, alone or in combination with other pathogens, coronavirus is responsible for the occurrence of diarrhoea, vomiting, dehydration, loss of appetite and occasional death. It was noted that the carrier and disposer status of coronavirus in faeces might be more lasting than reported. Pratelli *et al.* (2003) detected canine coronavirus in the faeces of naturally infected puppies, for a period of up to 180 days. Therefore, it can be concluded that these asymptomatic animals could serve as a continuous source of virus that can induce an immune response in dogs.

Research has covered two main directions: the establishment of canine coronavirus carriers status among healthy animals, or with poor digestive symptoms (diarrhoea, vomiting and weight loss), and the evaluation of anticoronavirus specific antibodies type IgG and IgM, after vaccination with canine anticoronavirus vaccine.

MATERIALS AND METHODS

Investigations were conducted during 2008 - 2009 on both sexe 50 dogs, of different breeds and ages; 15 of them were shown for consultation in different clinics of Iași for the modification of health condition (especially, digestive symptoms). 15 samples were taken from the Miroslava paddock of Iași. 20 subjects

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were evaluated for determining the degree of carrier and disposer of coronavirus; they were clinically healthy.

As concerns the vaccination, there were two groups of 10 clinically healthy animals, a group being vaccinated with canine anticoronavirus vaccine. The evaluation of postvaccinal titer was done after 14 and 21 days. We also mention that the healthy status was based on clinical examination and testing anticoronavirus antibodies.

The materials were subjected to the pathological examination in the Microbiology-Immunology Laboratory of the University Centre for Veterinary Research of Iași (financing the materials was done through the IDEI project, code 1129/2008, CNC SIS) and TRITEST medical analysis laboratory of Iași.

The pathogenic material was represented by blood samples collected without anticoagulant for serum expression. Serums were stored at -20°C until testing. The obtained serum samples were then examined serologically for canine anticoronavirus antibodies type IgG and IgM, using indirect enzyme immunoassay tests ELISA: DRG® Canine Corona Virus IgG ELISA (EIA-2482), DRG® Canine Corona Virus IgM ELISA (EIA-2483).

The test was based on canine coronavirus glycoprotein reaction with dog polyclonal antibodies. Microplate wells of the kit were coated with these proteins. Negative control was reconstituted in 1 ml of deionised water. For the quantitative test, three dilutions were done for each sample, from 1:30 (90, 270, 810), three dilutions from 1:10 for positive control and negative control.

There were transferred 100 ml of each dilution in plates, incubated for 60 minutes at 37°C , washed four times with wash solution, previously diluted 200 x; 100 ml HRPO-anti IgG were added,

conjugated to species, were incubated for 60 minutes at 37°C and washed, 100 ml substrate was added (prepared before the use by mixing equal parts of A and B buffer substrate) in each well; they were incubated 15-25 minutes at room temperature (21°C), 50 ml stop solution was added into each well and the plates were read at 450 nm.

To validate the plate, the positive control must have optical density values (OD) > 0.500 , and the negative control $\text{DO} < 0.250$. A titer of 90 and greater, obtained from sick animals, is a suggestive sign, and these are suspected of being disposers of coronavirus. As reference values, $\text{DO} < 30 =$ negative reaction, $90-270 =$ positive, probably disposer of canine coronavirus, requiring retesting after three months, $810 =$ big titer in animals after infection.

The determination of titres of IgG and IgM in serum was performed by automated analysis system CHEMWELL MANAGER 2902, an automatic analyzer for processing general chemistry analysis and immunological analysis by ELISA (in vitro techniques).

RESULTS AND DISCUSSION

According to the investigations carried out on 15 sick animals with signs of gastroenteritis as diarrhoea, vomiting, weight loss, 13 of them were positive at detecting IgG coronavirus antibodies and only one at coronavirus antibodies type IgM. In wild dogs coming from Miroslava, coronavirus antibodies type IgG were detected in all the animals. The healthy dogs, used as control, have shown antibodies, because they were vaccinated.

The values of optical density, determined by indirect ELISA for coronavirus antibodies type IgG, ranged from 121 to 1426, and in the case of IgM coronavirus antibodies, they ranged between 93 and 1004, indicating the specific immune response after infection and that animals were suspected as carriers and disposers of canine coronavirus.

Regarding the determination of IgM coronavirus antibodies, five of the positive samples for IgG were also positive for IgM.

Dog coronavirus is considered a mild or severe gastroenteritis in puppies, in which viral replication occurs primarily in the intestine epithelium. However, little is known about the immune mechanisms involved in protection. Antibodies produced by the digestive tract associated to the lymphoid tissue were the major effectors of immune response to enteric pathogens as coronaviruses.

The positive dogs for coronavirus antibodies type IgM with signs of gastroenteritis have shown recent infections of about 14 days. The dogs that were positive to IgM and negative to IgG suggested a very recent infection, of about 2-5 days, sufficient for the IgG antibody production.

The reference values of serum immunoglobulin in dogs had variations from one individual to another, for which it was examined in parallel on uniform groups of dogs, before and after vaccination.

Serological investigations of dogs prior to vaccination have shown that the limits of serum IgG (800-1800 mg/ml) and IgM (60-280 mg/ml) were close to immunogram benchmarks of the canine species.

After vaccination, the antibody levels followed an ascending curve of values, corresponding to the period from 14 to 21 days, which results in a maximum antibody titer on the 21st day. The values recorded for IgG and IgM have increased proportionally with IgG and IgM anticoronavirus antibodies, given the limits of variation of 998-2790 μ g/ml for IgG and 72-550 μ g/ml for IgM on the 14th day and the values of 1890-3200 μ g/ml for IgG and 62-450 μ g/ml for IgM, on the 21st day.

The values of optical density, determined by indirect ELISA for IgG antibodies to coronavirus on the 14th day ranged from 89 to 556 and on the 21st day, from 312 to 723. Coronavirus antibodies type IgM, after 14 days, ranged from 43 to 267 and after 21 days, from 45 to 253, indicating the specific postvaccinal immune response.

The obtained results were similar to those obtained by other researchers from different countries and geographical areas. These results allowed us to appreciate that the number of carriers and disposers of coronavirus was much larger and difficult to suspect, especially because animals did not have obvious symptoms.

Another worthy aspect was that more animals kept together, even

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belonging to different breeds (dogs, cats), were more easily exposed to cross contamination and might develop diseases that are more serious.

CONCLUSIONS

Investigations were carried out during 2008 - 2009 on both sexes 50 dogs, of different breeds and ages.

The values of optical density, determined by indirect ELISA for coronavirus antibodies type IgG, ranged from 121 to 1426, and in the case of IgM coronavirus antibodies, they ranged between 93 and 1004, indicating the specific immune response after infection and the animals suspected as carriers and dispersors of canine coronavirus.

In case of vaccination, the values of optical density, determined by indirect ELISA for IgG coronavirus antibodies after 14 days, ranged from 89 to 556 and after 21 days, from 312 to 723. Coronavirus IgM antibodies, after 14 days, ranged from 43 to 267 and after 21 days, they varied from 45 to 253, indicating the specific postvaccinal immune response.

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