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Could veterinarians be immune to contracting SARS-CoV-2?

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Abstract

Introduction: *The aim of the study was to assess the epidemic situation among veterinarians of the Świętokrzyskie Voivodeship, Poland, in relation to the control group.*

Material and methods: *The research was divided into 3 stages. Stage I involved the selection of subjects. In stage II, flow cytometry for immunophenotyping was performed and the percentage of the sub-population of CD4 cells and CD8 cells was assessed. Stage III involved collection of nasopharyngeal swab samples in order to determine the canine coronavirus CR-CoV mRNA with the rT-PCR method.*

Results: *The percentage of the CD4 and CD8 lymphocyte subpopulation in relation to the total lymphocyte population in veterinarians did not differ statistically from the percentage in the control group. The CD4/CD8 ratio in the group of veterinarians was on average 1.93, and 2.04 in the control group. There was no statistically significant difference between the groups, p = 0.591. Canine CR-CoV mRNA was not detected in any of the veterinarians or in the control group.*

Conclusions: *None of the veterinarians had a significant increase in T lymphocytes, which could be an effective defense against SARS-CoV-2.*

Key words: *SARS-CoV-2, veterinarians, canine coronavirus, immunophenotyping, flow cytometry.*

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Introduction

Veterinarians, in their day-to-day work, have direct contact with both animals and their owners, which in the time of a pandemic exposes them to a high risk of contracting the SARS-CoV-2 coronavirus.

The SARS-CoV coronavirus, like MERS-CoV and human coronaviruses that cause colds, belongs to β-coronaviruses that have a similar structure [1, 2]. The group also includes animal coronaviruses (Coronaviridae, order: Nidovirales) such as Bo-CoV (bovine coronavirus) and CR-CoV (canine coronavirus). The coronavirus that causes respiratory disease in dogs, CR-CoV, has a strong antigenic relationship with the coronaviruses that cause human colds. Presumably, CR-CoV is a bovine virus (Bo-CoV) that crossed the interspecies transmission barrier and adapted to the dog. Canine coronavirus is also spread through humans, who may be passive carriers of CR-CoV [1].

At least 40% of the human population has lymphocytes that are responsive to SARS-CoV-2 coronaviruses. It is suspected that they owe this immunity to a previous infection with other coronaviruses that cause the common cold. This is possible because pathogens – like some vaccines – can cause cross-reactivity. It is suspected that these lymphocytes were produced in the body after an earlier cold caused by coronaviruses [3]. The immune response from the common cold coronavirus against SARS-CoV-2 may be associated with a protein called spike, found on the surface of all of these microbes. They are slightly different in each type of coronavirus, but have a common component, the S2 subunit [4].

This subunit differs slightly among different coronaviruses, but the human body's immune mechanisms do not seem to distinguish them [5]. This applies at least to β-coronaviruses that cause colds, as well as SARS, MERS and COVID-19 diseases [2].

Therefore, we hypothesize that people who previously contracted the canine CR-CoV coronavirus (veterinarians) are immune to the SARS-CoV-2 virus due to the formation of cross-immunity, as there is a high antigenic relatedness of β-coronavirus surface proteins. A sign of generating non-specific immunity would be a significant increase in the titer of CD4 T cells in the blood, and the confirmation of the origin of immunity from the canine coronavirus CR-CoV would be detection of mRNA in a nasopharyngeal swab.

The aim of the study was to assess the epidemic situation among veterinarians of the Świętokrzyskie Voivodeship, Poland, in relation to the control group.

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Material and methods

Approval of the Bioethics Committee of Jan Kochanowski University No. 25/2021.

The study involved 33 practicing veterinarians from the Świętokrzyskie Voivodeship, aged 29-56, 25 women and 8 men. They were healthy volunteers, not vaccinated against SARS-CoV-2, with no comorbidities. The inclusion criteria for the study was a negative SARS-CoV-2 coronavirus test and no antibodies to SARS-CoV-2. The control group consisted of 13 randomly selected healthy, unvaccinated volunteers aged 25-60 years, 7 men and 6 women.

The research was divided into 3 stages.

Stage I involved the selection of subjects – 33 veterinarians who are not and were not infected with the SARS-CoV-2 coronavirus.

In order to exclude an infection, tests for the presence of SARS-CoV-2 coronavirus were performed. The nasopharyngeal swab samples were collected in eNAT Transport and Preservation Medium (COPAN). The RNA of SARS-CoV-2 was isolated from this medium by Viral DNA/RNA kit (A&A Biotechnology) according to the manufacturer's instructions. The real time RT-PCR detects the RdRP gene and S gene of SARS-CoV-2 and the E gene specific for SARS-CoV-1 and SARS-CoV-2 (virellaSARS-CoV-2 seqc, Gerbion). The whole procedure (isolation, amplification and interpretation) complies with the *in vitro* diagnostic requirements for diagnosis of SARS-CoV-2 and WHO recommendations.

To exclude persons who had been infected with SARS-CoV-2 asymptomatically, blood tests were performed in all subjects to detect antibodies to SARS-CoV-2. For this purpose, 2 ml of whole blood was collected from a peripheral vein into a K2EDTA tube. After centrifuging the morphotic elements, plasma was used for analysis. In order to detect antigen-specific immunoglobulin G (IgG) for SARS-CoV-2 virus, the Polycheck Anti-SARS-CoV-2 IgG test was used, where two recombinant SARS-CoV-2 virus proteins were used – the nucleocapsid phosphoprotein (N) and the S1 subunit of the spike protein (S) (Table 1).

Specificity: Nucleocapsid protein (phosphoprotein) N – 99.1%
Spike protein, S1 subunit – 99.6%
Anti SARS-CoV-2 IgG – 98.7%

Table 1. Reference range for anti-SARS-CoV-2 antibody levels

Interpretation	
IgG against phosphorylated nucleocapsid protein [kU/l]	IgG against the spike protein of the S1 subunit [kU/l]
< 0.35 negative	< 0.35 negative
0.35-< 0.7 borderline	0.35-< 0.7 borderline
≥ 0.7 positive	≥ 0.7 positive

Sensitivity: Nucleocapsid protein (phosphoprotein) N – 92.4%
Spike protein, S1 subunit – 88.6%
Anti SARS-CoV-2 IgG – 98.6%

In stage II, flow cytometry for immunophenotyping was performed and the percentage of the sub-population of Th (helper) cells with a CD4 marker and Tc (cytotoxic) cells with a CD8 marker was assessed.

For the analysis, 2 ml of whole blood was collected from a peripheral vein into a test tube with K2EDTA. The test was performed within 12 hours of blood collection. The molecular phenotypes of peripheral blood lymphocytes were analyzed using an LSR II flow cytometer (Becton Dickinson, USA). The following antibodies were used: FITC-conjugated anti-CD8, PE-conjugated anti-CD25, PerCP-conjugated anti-CD3, APC-conjugated anti-CD127, APC-Cy7-conjugated anti-CD (Becton Dickinson, USA). Compensation controls were set up using Anti-Mouse Ig, κ/Negative Control Compensation Particles Set (Becton Dickinson, USA). Data were acquired and analyzed using BD FACS DiVa (version 6.0, Becton Dickinson, USA).

Student's *t*-test was used to analyze the significance of differences between the group of tested veterinarians and the control group.

The χ^2 test was used to compare the standards of the CD4/CD8 ratio.

Stage III involved a nasopharyngeal swab samples collection in order to determine the canine coronavirus CR-CoV mRNA with the rT-PCR method at the reference laboratory of Idexx Laboratories Germany, according to the procedure described by Idexx.

Results

In the group of veterinarians, all swab samples for the SARS-CoV-2 coronavirus were negative, as in the control group.

Both in the study group and in the control group, the level of antibodies against IgG of both protein antigens of the virus was negative (below 0.35 kU/l).

The percentage of the CD4 lymphocyte subpopulation in relation to the total lymphocyte population in veterinarians did not differ significantly from the percentage in the control group.

Also, the percentage of the CD8 lymphocyte subpopulation in relation to the total lymphocyte population in veterinarians did not differ significantly from the percentage in the control group.

The CD4/CD8 ratio in the group of veterinarians was on average 1.93, and 2.04 in the control group. There was no statistically significant difference between the groups, $p = 0.591$ (Table 2).

There were no statistically significant differences between the groups for any of the studied variables.

An additional analysis was performed for the CD4/CD8 parameter, converting it to norms (0.8-1.2). The χ^2 test was used for comparison (Table 3).

There were no statistically significant differences between the studied veterinarians and the control group.

Canine CR-CoV mRNA was not detected in any of the veterinarians or in the control group. All swab samples were negative.

Table 2. Analyses of differences between groups (Th, Tc, CD4/CD8)

Dependent variable	Mean		Standard deviation		*t*(44)	*p*
	V	C	V	C		
Th (CD4)	60.92	63.37	7.39	6.03	–1.06	0.293
Tc (CD8)	33.80	31.68	6.99	4.11	1.02	0.312
CD4/CD8	1.93	2.04	0.70	0.42	–0.54	0.591

V – veterinarians, C – control group

Discussion

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease has spread worldwide, leading to an ongoing pandemic [6]. Human coronaviruses (HCoVs) were the origin of not only the current pandemic, but also of the SARS and MERS epidemics. Specific types of animals can be the source of different coronaviruses [2]. The infection is not exclusive to wild animals and there are many known strains that affect livestock and pets [7]. The animal origin of the novel coronavirus led to a discussion about the possible transmission of the disease by contact with pets [7, 8]. The empirical observations show that the presence of pets may have a positive impact on the course of COVID-19 [8]. Anecdotal evidence has suggested that veterinary doctors are rarely affected with COVID-19. A correlation between vets or pet ownership and the presence of a mild course of COVID-19 has not been found yet. The re-emerging contact with animal coronaviruses may lead to stimulation of the immunological system, thus creating an effective response to SARS-CoV-2 infection [9].

The possible positive effect of vets and pet ownership can be considered if the presence of the animal coronaviruses is high across the pet population. Dogs are the most common species taken as domestic pets, and canine coronaviruses can be transmitted easily to humans via droplets [10, 11].

The detected level of canine respiratory coronavirus (CrCoV) is from 7.5% to 54.7%. Since the infected pets may not have symptoms, the contact of owners or vets with pathogens can be unnoticed. Data show that the occurrence of CrCoV is high in dogs, which might suggest that humans who possess a pet or vets may have more frequent contact with different types of canine coronaviruses [9-11].

This is one of the reasons we tested veterinarians for canine coronavirus, looking for canine coronavirus mRNA genetic material. In the studied group of veterinarians, no one was infected with canine coronavirus.

Moreover, the coronavirus genome encodes for four structural and sixteen non-structural proteins, approximately. The spike proteins (S-proteins) are structural proteins that recognize and attach to ACE2 located on the cell membrane of the airways' epithelia and lung parenchyma [4, 5]. In their study, Tilocca and colleagues sequenced the SARS-CoV-2 amino acidic sequence and compared it to the sequences derived from other animal coronaviruses. The analysis showed that the resemblance of the whole sequence between SARS-CoV-2 and CrCoV is 36.39% [9, 12].

This is another reason why we tested veterinarians for canine coronavirus and not, for example, feline coronavirus (which belongs to α-coronaviruses and has lower antigenic compatibility with SARS-CoV-2). We assumed, in accordance with the literature, that such a high antigen similarity between SARS-CoV-2 and CrCoV may be the cause of the phenomenon of antigenic mimicry and trigger an immune response based on the cross-reactivity.

The recurrent contact with pathogens may work as immune mobilization against SARS-CoV-2 in many different paths [13].

However, further investigation of the epitope sequence shows the high homology: 57.14%, 80.00%, 83.33% and 100.00% in CrCoV epitopes: 789-799, 754-764, 424-437 and 1139-1152, respectively [4, 5, 9]. Based on the data, we suggest that recurrent contact with animal coronaviruses may lead to immunization. This effect was confirmed by the experimental studies.

Table 3. Analyses of differences between groups (CD4/CD8)

CD4/CD8	Group					
	Veterinarians		Control group		Total	
	n	%	*n*	%	*N*	%
0.8-1.2	2	6.06	0	0.00	2	4.35
Above 1.2	31	93.94	13	100.00	44	95.65
Total	33	100.00	13	100.00	46	100.00

$\chi^2(1) = 0.82$, $p = 0.364$

Furthermore, Zhao and colleagues propose one more explanation for the protective effect of the contact with zoonotic coronaviruses. Cross-reactivity with T-cells can induce the immunological response. $CD4^+$ memory T-cells on the epithelium of the airways can effectively produce interferon-gamma, which leads to activation of other cells and effective reaction [14].

Many people who have never had COVID-19 have T memory cells in their bodies that are capable of recognizing the coronavirus. This explains the phenomenon of cross-reactivity. It occurs when T cells, produced in response to a virus, respond to a similar but previously unknown pathogen. According to experts, cross-reactive T lymphocytes most likely come from earlier exposure to coronaviruses other than SARS-CoV-2, e.g. those that cause the common cold or zoonotic coronaviruses [15, 16]. People who are not exposed to SARS-CoV-2 may have T-type memory cells, which is why some have milder symptoms of the disease [17]. T cells help the immune system recognize and react to viruses with which it has already come into contact. They can reactivate quickly when they come into contact with the pathogen again, which means that subsequent infections are less severe. After the disease has ceased, a small population of long-lived T cells remains in the tissues that have been infected and continue to circulate throughout the body [15-18].

Currently, flow cytometry is the most precise and reliable tool for assessing immune status, and technological advances in this field have provided new opportunities to better define the function and phenotyping of peripheral lymphocyte cells. As the predominant subset of lymphocytes, $CD4^+$ T cells play a key role in the response to infection. Th1 cells play a key role in the defense against intracellular pathogens and autoimmune diseases by producing the key inflammatory cytokine interferon γ (IFN-γ) [19].

The percentage of the CD4 lymphocyte subpopulation in relation to the total lymphocyte population in veterinarians was not significantly different in comparison to the control group.

Memory $CD4^+$ cells are more numerous at sites of infection than $CD8^+$ T cells and play many roles in initiating and propagating the immune response. However, much less is known about how these cells provide protection and whether the location of these cells in specific areas of the tissue is of key importance [19, 20]. In the respiratory system memory $CD4^+$ T cells include cells in the airways and parenchyma, and cells adjacent to the pulmonary vessels. Airway memory $CD4^+$ cells are the first cells to encounter viral antigens during respiratory infections, suggesting a key role in protection. However, it is unclear whether the cells of the respiratory and parenchymal passages equally mediate protection during respiratory infections [16].

The percentage of the CD8 lymphocyte subpopulation in the total lymphocyte population in veterinarians was not significantly different in comparison to the control group.

The average CD4/CD8 ratio in the group of veterinarians was 1.93, and 2.04 in the control group. There was no statistically significant difference between the studied groups, $p = 0.591$.

Based on scientific data, we tried to evaluate the hypothesis that veterinarians are a special professional group that, through their daily work and contact with dogs, can be a carrier of the canine coronavirus and, therefore, create a specific immune memory based on memory T lymphocytes, which after contact with the coronavirus SARS-CoV-2, aids in fighting the virus more effectively and faster, avoiding the severe course of COVID-19. In our study, no veterinarian was a carrier of the canine coronavirus – no one was found to have Cr-CoV mRNA. None of the veterinarians had a significantly high level of T lymphocytes, and the $CD4^+/CD8^+$ ratio was not significantly different in comparison to the control group.

We are aware that the group of veterinarians participating in our study was small. Since the canine coronavirus grows mainly in the bronchi and trachea, we presume that swab samples taken from there would be more reliable. Following the report by Turner and Faber that the location of immune response cells is critical, we wonder if the number of T cells would be higher if the diagnostic material was taken from the lungs. In the respiratory system, memory $CD4^+$ T cells include cells in the airways and parenchyma, and cells adjacent to the pulmonary vessels. Airway memory $CD4^+$ T cells are the first cells to encounter viral antigens during respiratory infections, suggesting a key role in protection.

Summing up, we see the need for further studies of a specific professional group of veterinarians or, more broadly, pet owners, and the correlation between frequent contact with pets and contracting COVID-19 and its course.

Conclusions

Although veterinarians may be exposed to the canine CrCoV coronavirus, we did not detect the presence of the canine coronavirus in any of the veterinarians we studied.

None of the veterinarians had a significant increase in T lymphocytes, which could be an effective defense against SARS-CoV-2.

There is a need for research on a wider range of veterinarians or dog owners to assess whether recurrent contact with dogs can stimulate the human immune system to respond effectively to SARS-CoV-2.

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The authors declare no conflict of interest.

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