

Food quality tests and poultry diseases are just a segment of the primary scope of work performed at the Faculty of Veterinary Medicine in Skopje. Nonetheless, in the light of the current state of emergency, the laboratory is also adjusted to the 'One Health' concept. This implies that the health and wellbeing of humans and animals are interrelated and of equal importance.

The laboratory operating within the framework of the Faculty is one of the several laboratories throughout the country detecting whether given citizens are positive to Coronavirus. So far, more than 16,000 citizens have undergone testing, and the presence of the virus was detected in approximately 1,500 people. TV 21 paid a visit to the laboratory and observed the COVID-19 testing process.

Persons not wearing protective equipment may access only this corridor. Since we wished to witness the initial processing, together with Assistant Professor Iskra Cvetkovikj, PhD, we donned the compulsory protective equipment.

STATEMENT GIVEN BY: ASSISTANT PROFESSOR ISKRA CVETKOVIKJ, PhD

Several laboratories are housed in this section of the building, including two virology laboratories where we perform cultivation of different cell cultures that are used for isolation of animal viruses, some of which occur only in animals, whereas the others are zoonotic. For instance, the classical swine fever, the African swine fever virus, the bluetongue disease, the lumpy skin disease, the rabies virus, etc. The two laboratories housed here have a 2+ Biosafety Level. The last section where we handle the zoonotic pathogens has a 2+ Biosafety Level and for the time being we use this section to increase the national capacities with reference to COVID-19 testing.

This is the section where the samples of swabs taken from the citizens are brought, isn't it?

Exactly, the samples taken for COVID-19 testing purposes are brought to the rear entrance to this section and they are separated from the other samples we use in our day-to-day operation. This disables any mixing of samples and mixing of what remains as waste from our operation. When handling such samples, we wear special protective equipment.

What does this protective equipment consist of? It is actually the equipment we have on right now.

The equipment consists of a disposable protective suit, gloves, laboratory shoe covers and masks. The masks we have on at the moment are the ones we wear in our routine every day work, whereas the ones we wear inside the laboratory have a special level of protection, i.e. FFP3 masks that provide for maximum protection of the operator handling these samples.

Equipped this way, we may proceed to the laboratory where the virus is still alive. Hence, it has power of transmission.

The operations take place under high biosafety level requirements. Nevertheless, these protocols are not new to the employees here. Even before the outbreak of the Coronavirus, they worked with animal viruses that could easily be transmitted to humans.

STATEMENT GIVEN BY: ASSISTANT PROFESSOR ISKRA CVETKOVIKJ, PhD

What is actually happening in this laboratory?

The air we are breathing here right now is guided in the small laboratory via this grid system, and then it enters the laminar flow cabinet. Using a system of several filters, external engines and ventilation systems, the air is guided into the atmosphere. This means that the air does not recirculate back thus preventing the personnel working inside from any infection. This is the system we use to increase the national capacities with reference to COVID-19 diagnostics.

Namely, for the time being the personnel are working on the virus inactivation procedure. This refers to any potential virus present in the sample brought today for analysis purposes and mixed with the so-called Lysis buffer (i.e. inactivation buffer) so as to inactivate any potentially present virus.

STAND UP: JOVICA PAUNOSKI – TV21 REPORTER

This is the protective equipment that the laboratory personnel wear in their day-to-day operation when handling the Coronavirus samples and processing them pursuant to specific regulations and protocols.

Once work has been completed here, all items and surfaces are disinfected and a UV lamp is turned on so as to destroy any remaining residues.

The protective equipment we donned is disposable. Upon exiting these premises, we are to dispose of the equipment here so as to be decontaminated since the samples being analyzed could be potentially infective.

STATEMENT GIVEN BY: ASSISTANT PROFESSOR ISKRA CVETKOVIKJ, PhD

The procedure is followed by a decontamination process using an autoclave at a temperature of 120 degrees and a pressure of two atmospheres. Upon the inactivation of the waste, we have a subcontracting company that collects and disposes of the decontaminated waste. Hence, by virtue of the decontamination it is ensured that anything exiting this building or this laboratory is non-infective and this may not be a source of infection for the environment or for the people in general.

Once the virus has been inactivated, this previously disinfected transport case is used and the virus is taken to a molecular diagnostic laboratory.

Four phases take place prior to obtaining the final test result. Even though the virus has been inactivated, the use of protective equipment is mandatory.

STATEMENT BY: ASSISTANT PROFESSOR IGOR DZODZOVSKI, PhD

The first phase takes place in the premises you can see behind me. It is called an extraction room and this is where the extraction takes place, i.e. isolation of the genetic material, or the RNA virus. In conformity with our safety protocol, here in our laboratory within the Faculty of Veterinary Medicine we apply exclusively the procedure of automatic extraction and isolation of the RNA virus. This is for the purpose of highest level of safety for our personnel, but also for the purpose of preventing any possibility for unintentional error or contamination of the samples in our operation.

Following the completion of the process, we have a pure RNA virus, provided that it does exist in the very sample. Consequently, the other phases take place in the premises right next to us. This is the so-called master mix room. The master mix is a mixture with added components that enable a reproduction process for the genetic material. These are certain enzymes, primers that are added. We also have another room where the mixing of these two samples takes place, meaning the extracted RNA and the master mix. Once they are mixed, the reproduction takes place in a third room where the amplification machines are located. This mix, i.e. sample is subject to amplification reaction and ultimately we get the information whether the RNA virus SARS Corona 2 is present in the sample or not.

This is the room where the final results are obtained. The process is automated and there is no room for error. Three target regions are subject to analysis.

STATEMENT BY: ASSISTANT PROFESSOR IGOR DZODZOVSKI, PhD

In the course of the first PCR screening, we resort to targeting, i.e. amplification of two parts of the genome of the COVID-19 virus, the so-called N1 and N2 parts and additionally the beta-actin which is evidence of human DNA in the sample, i.e. we verify whether the sample has been properly taken, i.e. whether we have sufficient human material in the very sample. As you may see, in this very specific sample we have only one curve that goes beyond this threshold line. This is something we can observe if we only choose this parameter. This is the so-called cut-off line and it implies that we have positive amplification of the beta-actin. This implies that the sample was properly taken and that there is human material. In the case of the other targets, both N1 and N2 have a flat line which indicates a negative sample. Hence, there was no virus in this sample and no reproduction either.

As opposed to this sample, and it is possible to analyze the samples individually in terms of all regions, here we can observe successful amplification of the beta-actin. Nonetheless, these are negative samples, and normally everything is negative. In this case, we have a kind of inclining signal and this is something that is to be checked. Now, we have a sample that is clearly positive. We can observe amplification at three curves. Once we proceed with analysis, we may see that in the case of the N1 target we have a positive result, i.e. increase in fluorescence, and the same refers to N2 and beta-actin that are also positive.

TV21: Does this mean that this sample is positive to Coronavirus?

Yes, in this sample we have evidence, i.e. presence of the RNA virus SARS Corona 2.

Nevertheless, the process has not been completed. Despite the initial findings and in order to issue results that the patient is positive to Coronavirus, one more positive reaction is required, i.e. detection of a third part of the genome in the very virus.

STATEMENT BY: ASSISTANT PROFESSOR IGOR DZODZOVSKI, PhD

Provided that in any of these initial two genes, let's say N1 and N2, we have one positive, and one negative finding, we resort to E-gene. If the finding is positive with the E-gene, extraction is performed until we get to three. If it remains inconclusive that we have a clearly positive signal in all three regions, we recommend another sampling and an additional analysis. This may happen because the quantity of the very virus present in the material is minor, which may be because the disease is at its onset or is in its terminal stage, which implies that the quantity of the virus is insufficient or that maybe the sampling procedure was improper. Anything may happen – might be the case with the transport medium or because time has lapsed. In any case, so as to be certain, we are to request another sampling. Only on condition that we have a positive signal in all three regions, we may issue a final positive result.

We took the opportunity to talk to Professor Kiril Krstevski, PhD from the Department of Infectious Diseases and Epidemiology as to whether the virus may be transmitted from people to animals and vice versa. The first case of infected animals with the Coronavirus was observed in two dogs in South Korea, followed by detection of the virus in a domestic cat.

STATEMENT BY: KIRIL KRSTEVSKI – PROFESSOR

The knowledge that we have at present is that there are quite a lot of animal species with big similarities in the receptors that are responsible for the virus penetration and infection. Cats do belong to this category, but there are other categories and other animal species that are not excluded either, primarily cattle and sheep. These preliminary findings do not necessarily imply that these animal species would get infected and diseased. An exception to this is the case of cats for which we already have evidence, but, nevertheless, they are not potentially susceptible or

receptive to the virus. In this context, in the forthcoming period, animal species are expected to be the focal point of the research and experimental studies. Anyhow, let us go back to what is already known, that is the fact that the cats are susceptible to the disease and could get infected, develop a disease, which actually implies that there is a difference between infection and contracting a disease. The disease is a result of the infection. Under experimental conditions in experimental studies it has been proven that cats may transmit the virus to other healthy non-infected cats but to a certain extent only. Nevertheless, it still remains unknown and there is no proof that they are relevant for the spreading of the virus and the epidemiology of this disease among humans. Hence, the recommendations with reference to the confirmed cases would be to limit the contact of infected people with their pets and to treat them as any other member of their family. The reason for this would be because they are receptive and may be infected with the virus.

The professor further adds that the diagnostic approach towards both people and animals remains identical. Serological tests were used in the case of animals with respiratory diseases so as to ascertain whether the Coronavirus was present.

STATEMENT GIVE BY: KIRIL KRSTEVSKI – PROFESSOR

This is what we would like to expand on and not only to focus on testing for presence of COVID but also to consider the role of Coronaviruses and their presence in other animals. Thus, we have conducted several preliminary examinations of materials derived from animals with respiratory diseases and in most cases, and in line with our expectations, we have confirmed presence of bovine Coronavirus. This virus is analogue to the virus among humans causing the common cold. It has been known for a longer period of time and it is manifested in given seasons of the year causing respiratory and digestive problems among cattle.

The head of the Faculty of Veterinary Medicine, Dean Lazo Pendovski stated that since the very onset of the crisis, the Faculty has undertaken all necessary protective measures for its employees.

STATEMENT BY: LAZO PENDOVSKI, PhD – DEAN

Even before the occurrence of the very cases in our country, with the onset of the pandemics, we were aware that it would reach our country as well and consequently in the course of January we undertook steps to procure all necessary reagents and materials, with the focus on animals. Our primary objective is to take care of animals' wellbeing. We were ready in the first months of the year, but then the pandemics occurred. We were among the first laboratories engaged in the testing of COVID-19 among people as well. I would like to highlight that our laboratory has a greater capacity compared to what we have undertaken to perform. Namely, in terms of the arrangement we have made with the Ministry of Health, we are to examine up to 35 samples a day. Our designed capacity is much bigger having in mind our day-to-day examinations of infectious diseases among animals and being at the forefront of preventing the transmission of zoonotic diseases that are spread from animals onto people.

Some people find it strange when veterinarians diagnose the presence of virus among people. Nevertheless, the concept of 'One Health' functions in global terms. The two medicines are to function alongside and be interdisciplinary for the purpose of promoting public health.