Headline

# Emerging viruses

Viruses are rather mysterious things, at least in part because of the debate about their origin, the difficulty of predicting epidemics and their impact on humans.

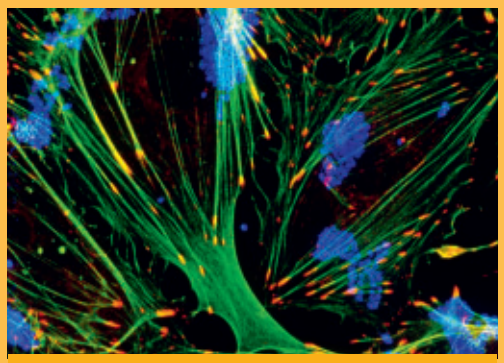
>>> Jacques IZOPET, professor at UPS, hospital doctor and researcher at the Centre de Physiopathologie de Toulouse Purpan (CPTP, joint UPS/Inserm lab) © C. Frésillon/CNRS.

**Viruses have posed a fundamental problem to biologists ever since their discovery. Where do they fit into the living world?** Results from recent studies suggest that viruses, like non-cellular microorganisms, are a second form of life, sharing space with cellular organisms like archebacteria, bacteria and eucaryotes. It is now generally agreed that viruses are much more numerous and diversified than the cells that shelter them. They probably played a major role in the origin and evolution of DNA genomes.

The term "emerging virus" is used to describe an idea that is completely different from that used for the origin of viruses. It comes from epidemiology and describes different contexts: a new host for a known virus, a new geographic region, and occasionally a new virus. The concept of an emerging virus is inherant to the investigation strategies used in response to an event and also to available techniques. We can also speak of re-emerging viruses when an agent that had become rare becomes more abundant. Human activity and environmental factors increase the probability of viruses emerging or re-emerging. Travel, trade,and contact with animals all provide viruses with opportunities to spread into new parts of the planet.

**The outbreak of acute severe resirator syndrome (ASRS) at the beginning of the XXI century was a great shock to humankind.** An animal coronavirus rampaged through south-east China in a few months and threatened health care systems throughout the world. The emergence of a new H1N1 variant of the influenza A virus in 2009 and the uncertainty about its pathogenicity illustrate our present and future challenges.

**Several groups of virologists in Toulouse are focusing on this topic.** These teams have developed projects to study the molecular



>>> Fluorescence microscopy of the Chikungunya virus inside human cells in culture. © Inserm / Institut Pasteur.

mechanisms that enable viruses to cross the species barrier, the pathophysiology of viral infections and the mechanisms by which they survive. The special facilities required for these studies, such as security level P3 laboratories, and A2 and A3 animal facilities, are being developed at the CHU, within the Centre de Physiopathologie de Toulouse-Purpan (CPTP, INSERM U563 lab) and at the Ecole Nationale Vétérinaire de Toulouse (ENVT, joint INRA-ENVT lab). They are supported by the impressive facilities at IFR150 and the translation research carried out in close collaboration with hospital departments dealing with infectious diseases, transplantation, hepatology and neurology.

This report illustrates the contributions of the research groups working on emerging viruses and also those whose research concerns viruses that are pathogens in immunodepressed hosts, like the cytomegalovirus.

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*CPTP: Centre de Physiopathology de Toulouse Purpan/Centre of Physiopathology of Toulouse Purpan*
*ENVT: Ecole National de Vétérinaire de Toulouse/ National Vetenary College of Toulouse*
*IHAP: Interaction Hôte Agent Pathogène/Host Pathogen Agent Interaction*

Headline

# Fighting against the cytomegalovirus

Infection by the cytomegalovirus rarely causes disease in healthy people but poses a severe threat to immunocompromised patients and to fetuses. Elucidating its mode of action should help improve protection strategies.

>>> Christian DAVRINCHE, senior INSERM research scientist at the Centre de Physiopathologie de Toulouse Purpan (CPTP, joint UPS/CNRS lab).

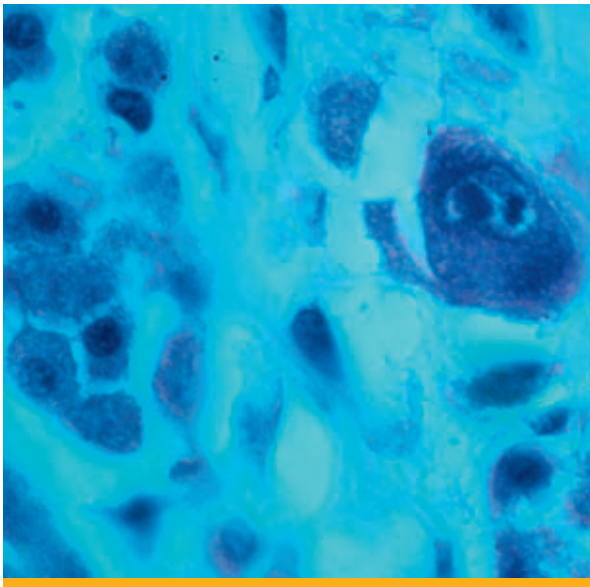
The human cytomegalovirus (HCMV), also known as herpes virus 5 (HHV-5), belongs to the herpes virus family. Following primary infection, the viral genome composed of double-stranded DNA, remains latent in infected cells throughout the patient's life. Whereas infection is usually without symptoms in immunocompetent hosts it may lead to severe pathologies when hosts are unable to control infection, as is the case in fetuses, AIDS and transplanted patients. For these reasons HCMV infection is a major public health concern. Babies born with HCMV infection may show retarded growth and mental and learning disabilities, hearing and vision loss or other disabilities and are usually low birth weight. Analyses of spontaneously aborted fetuses have shown that the placenta was infected, but not the fetus. This implies that the placenta is infected first, and the infection then transmitted to the fetus.

## Vaccine

The aim of our team within the Centre de Physiopathologie de Toulouse Purpan is to develop new prognostic and therapeutic approaches to prevent miscarriages and risks of disability in new-borns. Furthermore, we are interested in characterizing effector mechanisms including the T cell response involved in controlling the spread of the virus in healthy people. Besides benefiting vaccine design against HCMV, these studies could help in monitoring recovery of anti-viral immunity in transplanted patients.

## Targets cells

Several models are available in lab-based in vitro culture of cells isolated from either peripheral blood or from placenta. We assess mechanisms involved in viral escape to T cell response as well as those responsible for disturbance in placental cells. Furthermore these in vitro studies are supported by ex vivo analyses of infected placental explants and blood samples from viremic transplanted patients. An innovative approach using cultures of induced pluripotent stem cells (iPS) is developed to characterize factors responsible for trouble in neuronal differentiation in infected fetuses.



>>> Cytomegalovirus (CMV) (x 800). ©Inserm.

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Headline

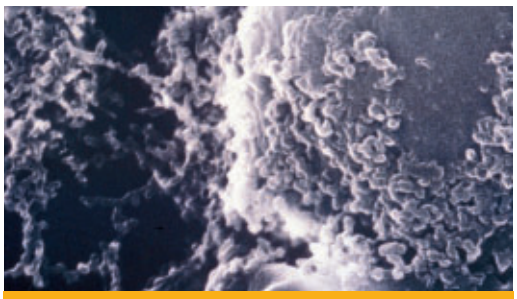
# HIV reservoirs: a major obstacle to virus eradication

The HIV virus is never totally eradicated in AIDS patients despite multiple therapies because cells serve as reservoirs for the virus.

>>> Pierre DELOBEL, MD, PhD., Service des Maladies Infectieuses, CHU Purpan and INSERM U563 (Viral Infections: persistence, host response and pathophysiology; directors J. Izopet & C. Davrinche). © C. Frésillon/CNRS.

Since 1996, highly active antiretroviral treatment (HAART) has effectively controlled replication of type 1 human immunodeficiency virus (HIV-1) in patients and reduced viremia to undetectable levels. This control of the HIV-1 virus substantially restores the immune system and hence results in reduced morbidity and mortality. However, these drug combinations do not actually eradicate the virus because it survives in reservoirs. The major obstacle to completely eradicating HIV-1 is latent infection where latent proviruses are integrated into quiescent long-lived T-CD4 memory lymphocytes. These lymphocytes, which can be induced by cell activation, make up the best known and most important cellular reservoir of latent HIV-1. But other cells types, such as CD4+ naive T cells and tissue macrophages, are also involved in sustaining the virus. The number of cells infected with the latent virus is greatly reduced when HAART is initiated, and then more gradually as treatment continues until the infection reduces to low levels and becomes stable. This apparent stability of the latent HIV-1 reservoir may be due in part to its ongoing rebuilding by residual virus replication that persists despite antiretroviral treatment. Some cell compartments or anatomical sites are in effect more or less inaccessible to immune surveillance and/or antiretroviral drugs. As it is impossible to eradicate HIV-1 using the combinations of antiretroviral drugs presently available, it is essential to identify the mechanisms by which the virus persists and the dynamics of its response to effective antiretroviral treatments. This will allow the development of new therapeutic strategies.

Our group is therefore interested in the dynamics of HIV-1 infection in response to treatment. We have focused on the impact of viruses that use the entry coreceptors CCR5 (R5 virus) and CXCR4 (X4 virus) on T lymphocyte homeostasis in patients taking antiretroviral treatment. Our studies on cell reservoirs in this situation have shown that there is a progressive selection of the X4 virus in the peripheral blood mononuclear cells of these patients. The emergence of the X4 variant is correlated with persistent $CD4^+$ T-cell lymphopenia in certain patients despite undetectable plasma virus load. We also find that the HIV-1



>>> Generation of HIV at the plasma membrane of infected THP-1 cells. Transmission electron microscopy x 20 000, Centre of electron microscopy applied to biology, UPS.

populations are compartmentalized in different circulating blood cells in patients on HAART. We are presently working to characterize the residual replication of HIV-1 in several sanctuary compartments of the body, particularly in the gut-associated lymphoid tissue (GALT). The GALT is a target for HIV-1 infection because it is mostly made up of lymphocytes. It is a major early target for virus replication and a site where there is severe depletion of $CD4^+$ T lymphocytes. This depletion of $CD4^+$ T lymphocytes in the GALT may continue even when the peripheral lymphocyte count has apparently been restored. Residual virus replication in the GALT, together with persistent inflammation and apoptosis, may be responsible for this defect of $CD4^+$ T cell reconstitution.

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Headline

# When viruses target the brain

Viruses and the brain, a complex and often deadly interaction. Psychiatric diseases, a major public health issue, have complex and multiple origins. Besides genetic or environment-related factors, it has long been suspected that viral infections may contribute to the etiology of such disorders.

>>> Daniel DUNIA, CNRS research scientist at the Centre de Physiopathologie de Toulouse-Purpan (CPTP, joint UPS/Inserm)

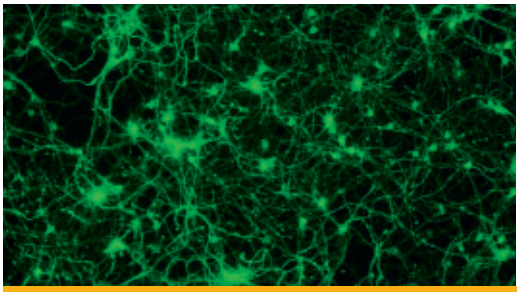
Viruses as a cause of psychiatric disease? A likely hypothesis when considering that the central nervous system can be the target of many persistent viral infections, sometimes leading to behavioral impairment. Borna disease virus (BDV) represents an ideal model to study the impact of persistent viruses on neuronal function and behavior. This neurotropic virus is responsible for neurological diseases in a wide range of mammalian species. BDV is an enveloped virus with a single stranded negatively polarized RNA genome. BDV has unique properties and is characterized by its ability to replicate and transcribe its genome inside the cell nucleus and has developed a complex strategy for regulating its gene expression. Neurological diseases caused by BDV were first described in horses and other farm animals in south-west Germany, near the town of Borna, hence its name. More recent results, however, have shown that BDV prevalence, host range and geographical distribution are much wider than originally estimated.

## Human infections

Serological and molecular evidence suggests that BDV can infect humans and it has been hypothesized that it could be associated with several neuropsychiatric diseases. However, the epidemiology and significance of human BDV infections still remain a highly controversial field to date. In any event, BDV has a remarkably wide host range and serological data combined with BDV detection in human samples point to the possibility that BDV may be a zoonotic agent with yet poorly defined clinical consequences.

## Virus mutants

In this context, our team has developed a research program aimed at analysing the mechanisms whereby BDV impairs neuronal function, including



>>> Primary neuronal culture infected by Borna disease virus. The virus components are revealed using immunofluorescence (green). ©Inserm – D. Dunia.

the situation where there is no cellular destruction or inflammation, and to identify the viral determinants responsible for this outcome. In parallel, we are defining the immune effectors implicated in the development of immunopathological symptoms within the central nervous system using the neuro-inflammatory disease model caused by BDV infection.

Using functional imaging together with electrophysiological techniques, we have been able to demonstrate that BDV selectively interferes with neuronal plasticity processes. We have also performed a global analysis of the impact of BDV infection on the neuronal proteome. This analysis confirmed the selective viral targeting of cellular pathways implicated in neuronal remodeling. More recently, we have used reverse genetics approaches to generate recombinant viruses bearing targeted mutations. With such viruses, we were able to uncover the central role of the viral phosphoprotein and of its interference with protein kinase C-dependent signaling in the physiopathology of BDV infection.

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# Hepatitis E virus: zoonotic transmission and chronic evolution of the infection in immunodepressed patients

The hepatitis E virus was long thought of as an exotic disease. But we are now realizing that the virus is spreading to industrialized countries and need to consider the implications of this for human health.

>>> Jacques IZOPET, Professor at UPS, hospital doctor and researcher at the Centre de physiopathologie de Toulouse Purpan (CPTP, joint UPS/Inserm lab)

The water-borne hepatitis E virus usually causes acute hepatitis that can become epidemic in tropical and subtropical regions. But sporadic cases in people who have never lived in a region where the virus is endemic are being identified with increasing frequency in industrialized countries.

## The hepatitis E virus (HEV)

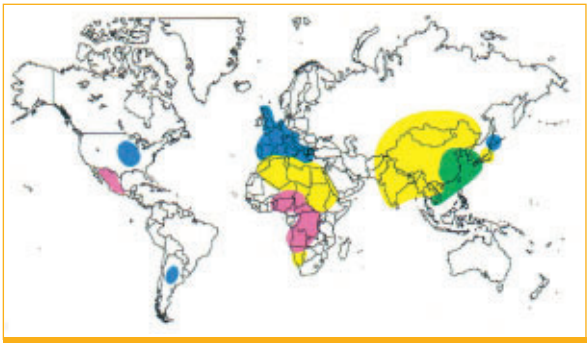
The HEV is a small (about 30 nm diameter), envelope-free virus that has a capsid with icosahedral symmetry. Genotypes 1 and 2 have been isolated only from humans living in non-industrialized countries, while genotypes 3 and 4 have been isolated from both humans and animals (pigs, wild boar and deer). Although genotype 4 is found only in Asia, genotype 3 is widely distributed around the world (see figure). There can also be recombination between animal and human strains of the virus leading to the emergence of more virulent strains or strains better adapted to humans.

## Zoonotic transmission

The vast majority of sporadic cases are of autochthonous origin and all age groups can be infected. The prevalence of anti-HEV antibodies among blood donors in France varies across the country. It is 3.2% in the Paris area and the west of France, but it is 16.6% in the region around Toulouse, where autochtonous cases are frequently reported. The virus can be identified in waste water but this does not prove that the water-borne vector causes the infection. Eating under-cooked or salt-cured uncooked pig meat is a risk factor because many pig farms are contaminated. Direct contact with an infected animal can also lead to infection.

## An infection can become chronic in an immunodepressed patient

It was believed until recently that all HEV infections could dissapear without serious treatment, like a hepatitis A virus infection. But we now know that an acute infection can develop into a chronic one in certain immunodepressed patients.



Geographical distribution of hepatitis E virus genotypes.

Our studies carried out in collaboration with the clinicians of the Organ Transplant unit, and the departments of Hepatology and Internal Medicine at the Toulouse CHU have shown, for the first time, that the virus can persist in the blood of immunodepressed subjects and lead to chronic hepatitis and cirrhosis. We have also shown that interferon-alpha can be used to successfully treat liver transplant patients.

The objectives of our projects are: first to identify the determinants associated with the virus host spectrum and with crossing the species barrier and second, to characterize the virus and host factors that are associated with the persistence of the virus in immunodepressed subjects, and with progression to liver fibrosis in chronically infected individuals.

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Headline

Headline

# How does bird flu become human flu?

The influenza A virus, both in birds and humans, has a great ability to evolve. This allows it to adapt to new hosts.

>>> Jean-Luc GUERIN, head of Intéractions hôte-agents pathogènes lab (IHAP, joint INRA/ENVT lab).

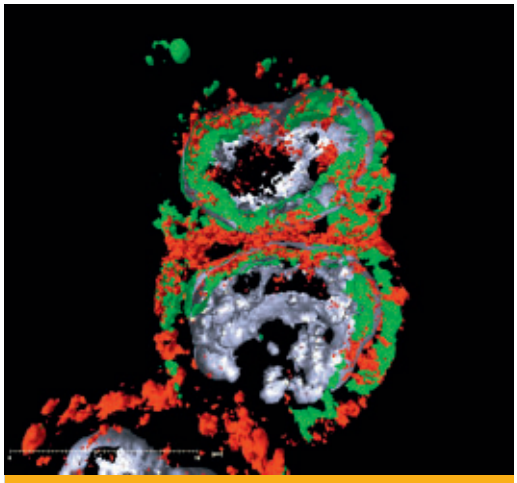
There are several different sub-types of the influenza A virus that are defined by a combination of two surface glycoproteins, hemaglutinin and neuramidase. Viruses that are highly pathogenic to poultry belong to the H5 or H7 sub-types while viruses that infect humans belong to the H1N1 (1918), H2N2 (1957) or H3N2 (1968) sub-types. The genome of these viruses is made of three strands of RNA that can match up in the case of co-infection of a cell targeted by different viral genomes, be they bird, human or swine. These gene exchanges are no doubt at the origin of new pandemics in humans.

The research team hôtes-agents pathogènes (IHAP-Inra-ENVT) at UPS is studying how the influenza A virus infects ducks, and analyzes the antiviral responses developed by these hosts.

## From ducks to chickens

Why ducks? These aquatic birds are the main reservoir for the influenza A virus and it is in these animals that we can isolate the largest variety of viral sub-types. Infections are essentially seen in the digestive tract in ducks, and large quantities of the virus can be found in droppings. Both chicken and ducks show severe symptoms when infected by such highly pathogenic hosts.

The way different hosts react plays a major role in how a virus evolves. When a virus passes from a duck to a chicken, for example, it must decide what to do. If it kills its host, it will have fewer chances of surviving itself and re-infecting another host. To overcome this dilemma, the virus undergoes genetic modifications when it passes between the two animals that give it a certain adaptive advantage as regards the new host. In contrast, the virus often remains genetically stable when it stays in ducks. This stability, together with the absence of outward symptoms, are proof that that there is a special interaction between the influenza virus and its host.



>>> 3D confocal microscopy image of subcellular re-localisation of nucleolar factors in human cells infected by the H5N1 virus. ©Inserm.

## Becoming more powerful

Our team also studies how potent the influenza virus is and how it has developed various strategies for thwarting the host's defence mechanisms. For example, a protein called NS1 can counteract the host's interferon response - an important inherent anti-viral response - by interacting with cellular proteins and blocking how they work. Thanks to a sophisticated ability to evolve, the influenza virus often modifies NS1 protein sequences as it passes from ducks to other hosts. This allows the virus to better multiply and so become more powerful. Identifying such viral "signatures" in specific hosts is crucial for better understanding the risks involved when a virus crosses a species barrier.

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Headline

# Bluetongue in the EU

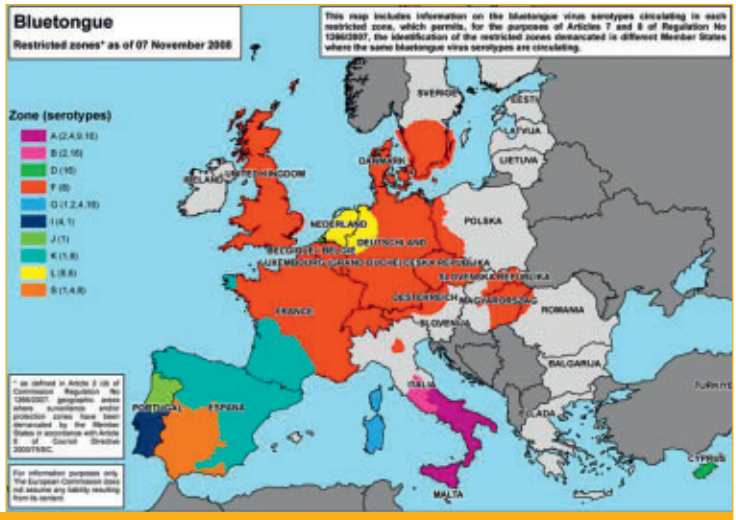
## Bluetongue is spreading throughout Europe but it does not affect all animals in the same way.

>>> Gilles MEYER, assistant professor, and researcher at the laboratoire Interaction hôte-agents pathogènes (IHAP, joint INRA/ Veterinary School of Toulouse).

The bluetongue virus (BTV) is a double-stranded RNA virus of the genus Orbivirus with 24 known serotypes. BTV is responsible for bluetongue, a Culicoides midge-transmitted hemorrhagic disease of ruminants. In 2006, BTV serotype 8, and to a lesser extent serotype 1, have extended their range northwards into areas of Europe never before affected and have persisted in many of these locations causing the greatest epizootic of the disease on record.

### Vaccination against bluetongue is considered to be the best strategy for controlling the disease

The emergence of the new serotypes 8 and 1 of BTV in France have caused heavy economic losses and have justified compulsory vaccination of all domestic ruminant species in France from 2008. Indeed vaccination is considered to be best way to control the disease since there is, to date, no effective measure to limit virus transmission by midges. The current strategy is based on inactivated vaccines, which were efficient for clinical and virological protection. However these vaccines are not able to protect the host against several serotypes and do not allow DIVA (Differentiating Infected from Vaccinated Animals) strategy. All these scientific drawbacks, including the absence of extended knowledge on the immune response induced by BTV infection, restrict the control of the disease. Specifically, absence of cross protection between BTV serotypes implies that vaccination against enzootic serotypes will be inefficient in preventing a new serotype emerging. Consequently, new approaches are being tested to improve vaccination against BTV, including low cost, ability to distinguish between vaccinated and infected animals, broad protective immunity against multiple serotypes and, preferably, with a single dose.




>>> Epizootic in Europe since 2006. Each color indicates a BTV serotype.

### The differences in host sensitivity to bluetongue have not been explained to date

Bluetongue is a hemorrhagic disease that affects both wild and domestic ruminants. Cattle and goats are considered as reservoirs of infection since they essentially develop sub-clinical symptoms while sheep develop a severe disease after being infected. Differences in sensitivity to bluetongue have also been observed between breeds, and specifically bovine breeds. To date we do not know how to explain these differences in sensitivity but they probably include viral factors but also host parameters. Bluetongue is characterized by microvascular injury, including pulmonary edema, disseminated intravascular coagulation (DIC) and vascular thrombosis. Injury to the endothelium of small blood vessels is responsible for the manifestations of disease in BTV-infected sheep. It has been proposed that species-specific differences in endothelial cells (ECs) infection and cytokine production are responsible for the different clinical outcomes of BTV infection of cattle and sheep. However these mechanisms have not been clearly defined. Specifically, it is uncertain whether BTV-mediated injury to the pulmonary vascular endothelium is entirely the result of direct virus-induced cytopathology or also from the activity of inflammatory mediators. Microvascular endothelial cells at the site of inflammation are both active participants in, and regulators of, inflammatory processes and mediators that act on endothelial cells also act on leukocytes and vice versa.

Our scientific project is to determine the mechanisms of host-virus interactions leading to the development of the disease and the variability in the severity of the symptoms among natural hosts. These mechanisms are studied at the host, tissue and cellular levels using two strategies. The first one experimentally assesses in vivo differences in clinical sensitivity to BTV infection. Data will provide clues as to the mechanisms of resistance regarding the viral aspect but also to the host inflammatory and immune response. The second strategy is complementary and studies in vitro the interactions between BTV-8 and the main target cells (endothelial cells and blood cells) potentially involved in differential pathogenesis between ruminants.

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