

A diagnostic test, able to identify a deadly virus believed to be present in up to 10 percent of horses, is now available for the first time.

Isabel Fidalgo Carvalho, a Portuguese vet and equine scientist, is the developer of the first test kit for New Equine Virus (NEV), a lentivirus first identified by Isabel in 2013 which can cause fatal encephalitis in horses.

NEV - the equine equivalent of HIV - is often misdiagnosed or hidden by other diseases that induce similar symptoms, such as anaemia and neurological issues in horses. It's most commonly confused with the Swamp Fever Virus (EIAV) and Equine Herpesviruses (EHV).

Horses thought to have the highly-contagious Swamp Fever can be ordered to be culled by officials in an attempt to stop its spread - a tragedy for the horses, and highly distressing and costly for owners.

By testing a number of horses with anaemia, researchers from Isabel's biotech company, Equigeriminal, first believed they had found the presence of a divergent strain of the Swamp Fever Virus because the horses cross reacted with EIAV, but were negative in the official tests. But subsequent research found they were actually suffering from NEV

NEV, which can lead to severe neurological diseases and may prove fatal, can now be identified through the use of this new diagnostic test. A vet is required to take the horse's blood, which is sent to the Equigeriminal lab where it is tested and then results are returned to the



New test to pinpoint deadly equine virus

owner/vet.

Treatment is currently targeted towards improving the general well-being of the horse, health monitoring, and boosting the animal's immune system. But Isabel says that the next stage is to find a treatment, and ideally, a cure for NEV.

"During my time at University and at Equigeriminal, I noticed unusual anaemia and severe neurological signs in horses which, in my PhD, I wrongly hypothesised to be attributed to Swamp Fever," says Dr Carvalho.

"I then realised, through the samples that this virus was actually closer to equine HIV - New Equine Virus, or NEV."

Isabel launched Equigeriminal in 2011 with fellow equine scientist, inventor and entrepreneur, Alexandre Vieira Pires.

"We have spent the last five years developing a diagnostic test and a potential cure for NEV," says Alexandre. "We now need to raise awareness of the problem and help vets to diagnose this disease correctly."

Developing the NEV diagnostic test

Writing here for NIVT, Isabel Carvalho describes briefly the clinical process which led up to the development by Equigeriminal of the new test for NEV...

The New Equine Virus (NEV) was isolated from horses with anaemia and neurological signs that presented Equine Infectious Anaemia virus (EIAV) - discordant results. We called them EIAV discordant because these horses were positive in sensitive and specific immunoblotting techniques, such as EIAVpV immunoblotting (gp90, gp45 and p26), but negative in EIAV official tests, the Coggins test

(AGID) and commercial ELISAs.

After several attempts, the EIAV genome failed to be amplified from the blood of these horses. Moreover, we've established equine macrophage-like (EML) cell lines obtained from peripheral blood monocytes of 3 EIAV discordant horses.

Cell syncytia formation and cytopathic effects (CPE) appeared after passaging and

viral transfer between infected EML cells. Furthermore, NEV could also be replicated and propagated in Equine Dermal (ED) cells. But, unlike EIAV, NEV is a highly cytopathic virus in ED cells with complete destruction of the cell monolayer after seven to 12 days.

But again, the EIAV genome failed to be amplified in viral RNA and/or proviral DNA obtained from equine PBMCs, infected EML



Isabel is pictured here with the co-founder of Equigeriminal, Alexandre Vieira Pires.

cells and/or viral RNA obtained from gradient purified viral particles.

Proteome analysis showed distinct protein patterns between NEV and EIAV in SDS-PAGE. Moreover, mass spectrometry analysis of NEV viral particles purified and positive for Reverse transcriptase (RT) retrieved more than 76 short peptides (<30 aa). Of these, 62 were blasted against public databases (UNIPROT KB or NCBI), and 14 blasted against Equigeriminal database. Of the 62 peptides, 26 (42 per cent) have sequence identity to part of the HIV-1 proteome, 45 (73 per cent) have identity to retroviridae proteome, but none of these had identity to EIAV proteome.

We've purified NEV viral particles and visualised a lentivirus core morphology by TEM negative staining. TEM positive staining of infected cells also showed that NEV viral budding occurs through the plasma membrane, as with other lentivirus.

The results obtained showed that NEV is a lentivirus different from EIAV that has similarity with the HIV-1 proteome.

Furthermore, we've developed an NEV diagnostic test (a competitive ELISA) based on NEV/HIV-1 homolog peptides as target antigens. Surprisingly, we've found that EIAV discordant sera strongly reacted against

these homolog peptides. To confirm the specificity of peptide-antibody binding, we've generated high affinity and specific mouse monoclonal antibodies (mab) against the NEV/HIV homolog peptides. We've shown that in the presence of discordant sera, the high specific mab couldn't bind and could inhibit the binding to target peptides, confirming therefore the specificity of the peptide - equine sera. Therefore, we've validated the NEV diagnostic test as a valuable tool to determine NEV-infected horses.

The NEV c-ELISA was validated in 700 sera from Portugal, Ireland, France, Brazil and US. NEV seroprevalence was 7-10 per cent. The tested sera were negative for other neurological pathogens (EHV-1, WNV, EPM, VEE, EEV, Hendra, AHV, Rabies), however, some of the NEV seropositives from US, Brazil and France tested positive for EIAV and showed severe clinical signs.

As with EIAV, NEV seropositives could develop asymptomatic/inapparent, chronic or acute disease. Respiratory signs, anaemia, fever, ulcerations and petechial, loss of weight, neonatal dead, and neurological signs, characterise the acute and chronic state.

We're following some of the NEV

seropositives and of these, three have shown severe neurological disease, two have died and one has recovered from a severe neurological episode. NEV lentiviral particles obtained from the sera and cerebrospinal fluid of these three animals were visualised by TEM. More studies will need to be performed to fully characterise NEV disease.

Isabel Carvalho is co-founder of equine biotech firm, Equigeriminal. She formed the company in 2011 following her discovery of New Equine Virus (NEV). The company has completed its research and is now launching a diagnostic test which is available to horse owners and vets in the UK. Carvalho was awarded a PhD in equine virology by the University of Oporto in collaboration with the University of Pittsburgh. She was director of the animal house for Oporto University's associated laboratory - a partnership between the Institute for Molecular and Cell Biology (IBMC) and the Institute for Biomedical Engineering (INEB) - from 2004 until 2010. She was also a researcher in regenerative medicine from 2010 until 2011.