livet

livet scientific white paper

Introduction

Highly contagious diseases such as Influenza and Strangles in horses are of crucial importance in equine health. A timely and precise diagnosis of infectious diseases in a stable can prevent further spreading and limit potentially high costs. However, rapid and reliable diagnosis of veterinary infections, specifically acute infections like respiratory disorders and diarrhea remains challenging (Cummins et al.).

Exact diagnosis of an infection's underlying pathogen takes time since the samples have to be sent to and analyzed in a centralized laboratory. Veterinarians and animal owners will be quick to administer medication rather than wait for the diagnostics to deliver the results – and only then treat the actual infection. A byproduct of untimely delays in diagnosis and unspecific medication is increasing antimicrobial resistance (AMR). AMR was indicated as one of the major global risks at the World Economic Forum in 2013 (WEF).

The current lack of reliable on-site diagnostic tests for acute infections in animals increases this already problematic issue for both the individual and the population. The likelihood of an unspecific therapy failing is increased by AMR (Weese et al.).

Current diagnostic procedures of infectious diseases in animals

Timely diagnosis of infectious diseases is of the utmost importance. It allows for adequate treatment of the affected individual as well as for the limitation of the further spread of the infection to other animals living in the same environment (van de Sande et al.).

Diagnosis in a centralized lab

Centralized laboratories generally use high-precision instruments that are bulky and complex to operate and require trained and experienced personnel for the performance and interpretation to detect pathogens (de Paz et al.). Delays of up to several days in diagnosis and adequate treatment of the patient is caused by the transportation of samples, the preference to wait for sufficient samples (for economical reasons) and is accentuated during the weekend, when laboratory services are just not available. The most common methods for the detection of microorganisms at laboratories are bacterial cultures, enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR), see Table 1.

Table 1: Comparison of standard methods used in centralized laboratories for the detection of pathogens.

Method	Time	Resources	Costs	Sensitivity
Bacterial culture	Several days to weeks	Trained and experienced technicians	medium	+/++
ELISA (antigens/antibodies)	Hours	Trained and experienced technicians	low/medium	+/++
PCR (real-time, end-point)	Hours to a full day	Trained and experienced technicians, special equipment	high	+++

livet ag
Freiburgstrasse 251, 3018 Bern, Switzerland
P: +41 (0) 31 07 17, info@li.vet, www.li.vet

Available point-of-care diagnostics

'Dipstick tests' (lateral flow immunoassays) are currently the most widely used tests for on-site diagnosis of infectious diseases in veterinary medicine (Busin et al.). These tests are often used in small animal clinics and include tests for Borreliosis, Ehrlichiosis, Anaplasmosis or Canine parvovirus. However, for the diagnosis of acute infectious diseases like respiratory infections, lateral flow assays suffer from poor sensitivity due to the initial absence of specific antibodies from the host (Figure 1).

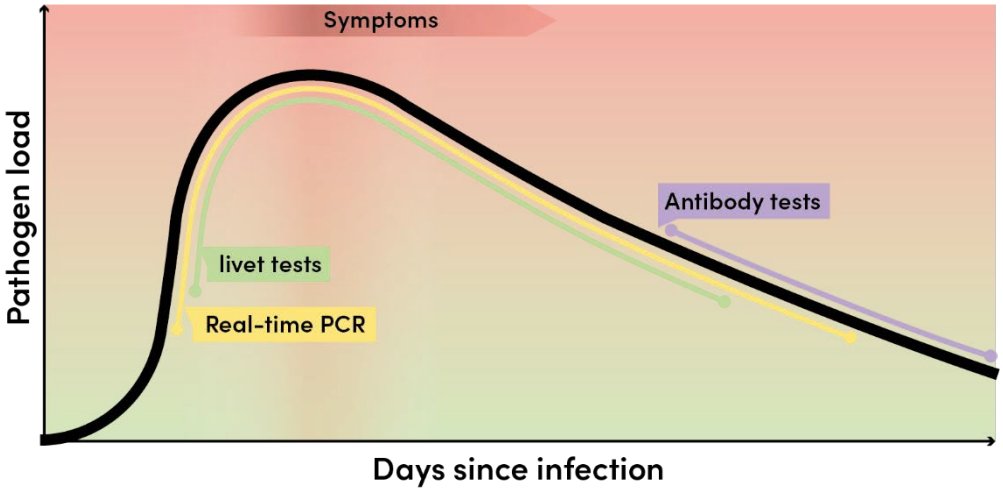

Figure 1: Progression of pathogen load in an infection and respective diagnostic methods over time

Our solution

The founding team of livet has a combined experience of >16 years of working in human microbiology diagnostics at the Institute for Infectious diseases (IFIK), University of Bern in Switzerland; developing and implementing molecular diagnostic tests for the University Hospital of Bern. We have identified the potential of a molecular rapid test for the reliable on-site diagnosis of infectious diseases. With this knowledge we developed a novel isothermal amplification, real-time detection-based method.

A novel method, based on a separate development of this original method, was transferred to livet. livet will develop tests to detect respiratory pathogens in horses such as *Streptococcus equi equi, Equine herpesvirus type 1 and 4* (EHV-1 and -4) and *Equine Influenza*, followed by more tests for other pathogens.

Similar to PCR, the isothermal amplification reaction amplifies a specific nucleic acid sequence with the help of an enzyme and primers. Some examples for isothermal amplification technologies are LAMP (loop mediated isothermal amplification) which was first described by Notomi et al., RPA (recombinase polymerase amplification or SDA (strand displacement amplification).

One of the main issues in isothermal amplification is poor specificity, due to false priming and hence the complexity of primer design. With our experience in isothermal amplification methods we were able to solve these challenges. An internal process control, amplifying in the absence of the target sequence, is standard in our assays for verifying the absence of inhibitors or pipetting errors. The enzyme has helicase activity, hence the denaturation step at 95°C is not required. The reaction takes place at 65°C, resulting in a simpler device the assay is run on.

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Conclusion

We are aware today perhaps more than ever of the need for rapid and reliable diagnostics for infectious diseases. Rapid diagnosis leads to immediate and accurate therapies and as such contribute to the prevention of epidemics. With rapid and effective diagnostics, the handing out of antibiotics on a large and preventative scale can be mitigated and target orientated therapeutics can be administered with the positive side effect of limiting resistance development.
Up until now, laboratory diagnostics of infectious diseases in animals was offered with several days delay, the alternative being unreliable rapid tests that merely detected antibodies. livet offers a rapid and highly reliable molecular method for the point-of-care diagnosis of infectious diseases in animals.

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