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Development and Evaluation of a Duplex Lateral Flow Assay for the Detection and Differentiation between Rabbit Haemorrhagic Disease Virus *Lagovirus europaeus*/GI.1 and /GI.2

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Simple Summary: Rabbit Haemorrhagic Disease is caused by a virus that affects the liver, the spleen and the lungs of rabbits, causing hepatitis, splenomegaly and haemorrhages. A new genotype of the virus was first reported in France in 2010 and has spread globally since then, replacing most of the circulating former viruses in many countries. The detection of the virus and the differentiation of both genotypes is of crucial importance for disease surveillance. In this article, a rapid test for antigen detection is described and evaluated, providing the first description of a quick and easy-to-use test that allows for the simultaneous detection and differentiation of the genotypes. A total of 136 samples, rabbit liver samples and liver exudates (liquid collected after freeze–thawing) classified as infected and non-infected, were analysed, with good results. These data confirm that the developed rapid test can be used as a reliable diagnostic test for disease surveillance, especially in farms and the field.

Abstract: Rabbit Haemorrhagic Disease Virus 2 (RHDV2, recently named *Lagovirus europaeus*/GI.2) was first reported in France in 2010 and has spread globally since then, replacing most of the circulating former RHDV (genotype GI.1) in many countries. The detection and differentiation of both genotypes is of crucial importance for the surveillance of the disease. In this article, a duplex lateral flow assay (LFA) for antigen detection is described and evaluated, providing the first description of a quick and easy-to-use test that allows for the simultaneous detection and differentiation of RHDV genotypes GI.1 and GI.2. A panel of GI.1- or GI.2-infected and non-infected rabbit liver samples and liver exudates (136 samples) was analysed, obtaining a total sensitivity of 94.4% and specificity of 100%. These data confirm that the developed duplex LFA can be used as a reliable diagnostic test for RHD surveillance, especially in farms and the field.

Keywords: Rabbit haemorrhagic disease virus; lateral flow assay; antigen detection

1. Introduction

Rabbit Haemorrhagic Disease Virus (RHDV), a member of the genus *Lagovirus* (family *Caliciviridae*), causes a fatal disease in domestic and wild European rabbits (*Oryctolagus cuniculus*) and was first reported in China in 1984. Since then, it has been reported in over forty countries in the five continents, being endemic in most parts of the world where European rabbits are domesticated. From 70% to 90% of infected rabbits over eight weeks of age die within 12–36 h. The primary target organs of the virus are the liver, the spleen and the lungs. RHD is characterized by acute necrotizing hepatitis, splenomegaly and