Testing of a rapid detection kit for Porcine Epidemic Diarrhea virus (PEDv)

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SUMMARY

Significant economic losses can result from outbreaks of Porcine Epidemic Diarrhea (PED), a serious disease currently circulating in North American swine herds. Accurate and rapid detection of PEDv is essential to expedite implementation of control measures for the disease. A rapid PEDv test kit is needed for this purpose. Comparison of the DNA sequence targeted by the primers included in a current PEDv test kit to the sequences of North American PEDv strains showed potential suitability of these primers for detecting PEDv strains in the Canadian swine herd. This was confirmed in laboratory testing of 20 samples collected from pigs infected with PED, although the test results showed that the visual interpretation of test kit results can be somewhat ambiguous for some types of samples and can be further improved. While definitive results were obtained from most of the test samples, in a few samples the test results showed only slight visual differences between a positive result (indicated by a sky-blue colour) and negative results (indicated by purple or lighter blue colours). To improve the current test kit and to avoid ambiguity in interpreting the results, current work in collaboration with the developer of the original test kit included modifications such as using an alternative dye which would allow better colour differentiation between positive and negative results (yellow vs. pink/red colours). Further revisions such as reformulating reagents to dry format and modifying test sample preparation procedures are also being done. The improved test kit will then be re-tested in the laboratory, where its sensitivity, specificity, and repeatability will also be assessed. Afterwards, the performance of the improved test kit will be validated by field testing in PED-positive pig farms.

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INTRODUCTION

In Canada, significant economic loss attributed to Porcine Epidemic Diarrhea (PED) has been estimated at $125,000 for a 1000-sow herd. While the initial rate of spread has slowed down due to strict biosecurity measures in the industry, new PED outbreaks are still occurring in Canada at present. Currently, real-time reverse transcription polymerase chain reaction (rRT-PCR) is the method of choice for diagnosis of PEDv infection. However, rRT-PCR testing is costly and it requires sending the samples to a central analytical laboratory, which delays the rapid response needed to limit spread and impact of an emerging disease outbreak. Availability of a rapid and economical in-barn test kit would be valuable to deploy appropriate containment actions immediately, while waiting for confirmatory results from rRT-PCR testing.

A test kit originally developed by Domingo and Paraguison-Alili (2015) is based on the Loop-Mediated Isothermal Amplification (LAMP), which can be used as an alternative to rRT-PCR. The test package is technically-simple and user-friendly to implement. More importantly, it has very minimal cost of consumables thus translating to low price point per test, with the original test kit costing only C$10 per test. The original test kit has been developed under university laboratory settings and the developers of the test kit have already conducted extensive validation tests using samples from their local swine industry (in the Philippines). However, further refinement to ensure that the test kit can accurately detect the various PEDv strains present in North America, followed by field validation by applying the test kit on actual Canadian samples, are necessary before the test kit can be adopted as an additional biosecurity tool for the Canadian pig industry. Our current work is aimed to further improve this RT-LAMP-based kit and make it suitable for widespread use in the commercial swine industry in Saskatchewan and other Canadian provinces.

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EXPERIMENTAL PROCEDURES

In silico assessment was performed to ensure that the primers in the original test kit can detect the North American PEDv strains. BLAST – Global alignment of several North American PEDv sequences against the PEDv spike protein (S) gene sequence (GenBank ID KM406181; 4126 bp), which was used by Domingo and Paraguison-Alili (2015) as the target gene sequence, was performed.

Testing and validation of the original rapid PEDv test kit: Twenty samples (5 fecal, 3 jejunal and 12 rectal swab samples) collected from PED-positive pigs at VIDO-Intervac and from pig barns in Saskatchewan and Manitoba, were analyzed at the VIDO-Intervac laboratory for the presence of PEDv through both RT-qPCR and LAMP analyses. RNA was extracted from the samples using QIAGEN RNeasy Plus kit. Then a reverse transcription (RT) step was performed, followed by a qPCR step. The qPCR readings were taken in triplicates and average values were calculated. The LAMP step was performed according to the instructions provided together with the original rapid PEDv test kit and as described by Domingo and Paraguison-Alili (2015).

RESULTS AND DISCUSSION

In silico analysis of the target PEDv sequence: Results of BLAST – Global alignment done against PEDv spike protein (S) gene sequence (GenBank ID KM406181; 4126 bp) indicated that it had minimum 96.8% (in most cases, 99% or higher) similarity to respective sequences of PEDv strains found in Canada. These results indicated that the North American PEDv strains will likely be easily detected using the primers included in the original test kit.

Testing and validation of the rapid PEDv test kit: Results of the testing of the 20 samples to detect PEDv using both RT-qPCR and the application of the RT-LAMP-based test kit showed that the test kit results were mostly comparable to the results from the RT-qPCR testing, indicating very good sensitivity and specificity of the test kit for detecting PEDv. However, for certain samples the interpretation of the LAMP-based kit visual results was not very definitive due to the ambiguity in the resulting color of the LAMP test results. According to the original test kit instructions (Figure 1), a sky-blue colour indicates a positive result, whereas purple or lighter blue colours indicate negative results; Figure 2 shows the actual positive and negative LAMP-PEDv "control" results, which were used as the reference against which the test results from the actual 20 samples were compared to determine whether each sample was positive or negative.

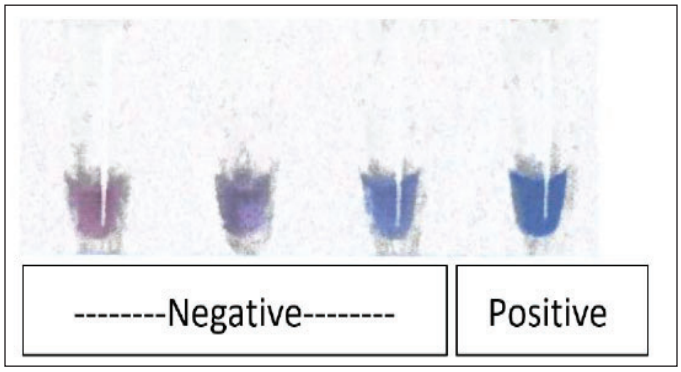

Figure 1. Interpretation of the different colors of the original RT-LAMP-based rapid PEDv detection kit results.

Some of the LAMP test results were difficult to objectively determine visually by comparing with the "positive-control" or "negative-control" tubes. Another confounding factor was the presence of fecal matter in two samples, which rendered the LAMP test tubes difficult to read properly as the resulting colour was outside the provided reference range of colours for comparison (yellow-orange instead of blue-purple). These results indicated the need for further improvement of the test kit, which are currently underway. An alternative dye that would allow better colour differentiation between positive and negative results (yellow vs. pink/red colours) has been explored and is now being used in the new version of the test kit, thereby ensuring better accuracy in objectively interpreting the test results. Additionally, modifications to the test sample preparation procedure, i.e., pre-filtration or centrifugation of certain samples with fecal material, are being investigated to ensure that the LAMP test tubes consistently show colors that can be interpreted properly against the reference color scale. Additional optimization modifications in the formulation of the reagents for better handling and storage (i.e., dry format, instead of the current liquid reagents which were found to be susceptible to leakage and evaporation) are also being done with the new version of the test kit. Once these modifications are completed, the new modified test kit will be subjected again to laboratory testing to confirm its efficacy and to assess performance parameters such as sensitivity, specificity, and repeatability. Finally, the revised test kit will be validated through field testing in actual PED-positive barns.

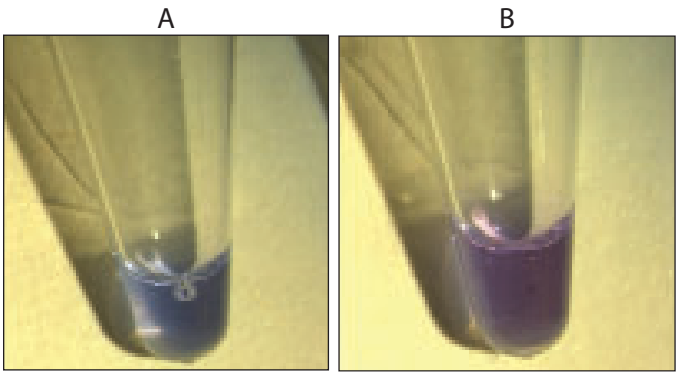

Figure 2. Visible RT-LAMP "PEDv control" results: (A) positive control and (B) negative control.

IMPLICATIONS

The primers included in the original rapid RT-LAMP-based PEDv test kit are suitable for detecting North American PEDv strains. However, modifications to the test kit are needed to allow more visual differentiation between positive and negative results, and to improve test sample preparation procedures. Once the improved test kit has been validated through field testing, it will be valuable in deploying appropriate containment actions immediately, which will help keep PEDv out of other swine production facilities.

ACKNOWLEDGEMENTS

Financial support for this research project was provided by the Saskatchewan Agriculture Development Fund. The authors would also like to acknowledge the strategic program funding to Prairie Swine Centre provided by Sask Pork, Alberta Pork, Ontario Pork, the Manitoba Pork Council and the Saskatchewan Agriculture Development Fund.