

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Development of a Rapid, Sensitive and Specific Probe-based Real-Time PCR for the Detection of Chronic bee paralysis virus, Sacbrood virus and Chalkbrood in honey bees

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COOPERATORS: OSU College of Veterinary Medicine, OSU Veterinary Molecular diagnostic lab, OSU Honey bee lab, Oregon Beekeepers and Oregon State Beekeepers Association

EXECUTIVE SUMMARY: The honeybee pollination of crops is critical to Oregon's agricultural economy. There has been much concern over the steep decline in populations of honeybees due to Colony collapse disorder and other infectious diseases that abruptly wiped out entire hives of honeybees across the United States, exacerbating the already dire situation for honeybees. The most important causative agents of some of the most destructive honeybee diseases are Deformed wing virus (DWV), *Paenibacillus larvae*, Acute Bee Paralysis Virus (ABPV) and microsporidian parasites *Nosema ceranae* and *Nosema apis*. We have received funds to work on DWV and *Paenibacillus larvae* and therefore, we proposed to add Chronic bee paralysis virus, Sacbrood virus and Chalkbrood (a fungal disease) for this grant submission. The current diagnostic methods for these pathogens, such as culture and conventional PCR, are not only time-consuming, but their efficacy is hindered by low sensitivity and specificity. Hence there is an urgent need for rapid, highly sensitive and reliable diagnostic tests to detect the above pathogens. Our goal is to develop a rapid, selective, sensitive, and quantitative real time probe-based PCR assay to detect Chronic bee paralysis virus, Sacbrood virus and Chalkbrood. We have successfully developed a real-time PCR for Chronic bee paralysis virus, Sacbrood virus and Chalkbrood *in vitro* in spiked samples and validation of the tests in field samples have been completed. Out of 50 field samples obtained, only two samples had Chronic bee paralysis virus, one sample had Sacbrood virus and 4 samples had Chalkbrood. In conclusion, the above viruses and the fungal disease are not very much prevalent to affect the overall health of the bee colonies. The Information obtained from this study will enable the Oregon Veterinary Diagnostic lab and the OSU Honey Bee Lab to serve the stakeholders (growers and beekeepers) by providing timely diagnosis to mitigate risks to bee colonies, potentially strengthening the economic sustainability of both beekeepers and producers.

OBJECTIVES:

1. Develop a real-time PCR assay for the detection of Chronic bee paralysis virus, Sacbrood virus and Chalkbrood *in spiked samples of honey bee in vitro*.
2. Validate the real-time PCR assay for the detection of Chronic bee paralysis virus, Sacbrood virus and Chalkbrood *from honeybee samples collected from colonies of*

select Oregon beekeepers.

PROCEDURES:

For objective 1, pools of 5 honeybees was collected alive from the brood nests of each colony and immediately frozen on dry ice and stored at -80°C until used. For total DNA/RNA extraction, bees were first homogenized in 2 ml of Tris-NaCl buffer (Tris 10 mM; NaCl 400 mM; pH 7.5). An aliquot of 100 µl of the homogenate was used for DNA/RNA extraction with the 5X MagMAX-96 Pathogen DNA/RNA Isolation kit (Life Technologies) following the recommendations of the supplier. Serially diluted stocks of pathogens (obtained from Dr. Sagili's lab at OSU) were spiked into honeybee homogenates and RNA/DNA extracted as described above. The primers and probes were designed from the conserved genes of Chronic bee paralysis virus, Sacbrood virus and Chalkbrood. One-step RT-PCR was performed according to standard protocols using the Path-ID™ Multiplex One-Step RT-PCR Kit (Life Technologies). The following thermocycle program was used: 10 min at 48°C, 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Reactions without template DNA was run as negative control. AB7500 Fast Real-Time PCR system was used for amplification and data acquisition.

In objective 2, the diagnostic tool developed in this proposal was used to validate the assays and evaluated the prevalence of Chronic bee paralysis virus, Sacbrood virus and Chalkbrood in apiaries in Oregon. We collected 50 samples from apiaries. Samples were taken directly from both strong and weak colonies, with or without specific symptoms. The tests were conducted as described in Objective 1.

SIGNIFICANT ACCOMPLISHMENTS:

We have successfully developed a real-time PCR for Chronic bee paralysis virus, Sacbrood virus and Chalkbrood and validation of the tests in field samples were completed. We obtained Chronic bee paralysis virus, Sacbrood virus and Chalkbrood infected honeybees from Dr. Sagili's honeybee lab at OSU. The bees were first homogenized in 2 ml of Tris-NaCl buffer (Tris 10 mM; NaCl 400 mM; pH 7.5). An aliquot of 100 µl of the homogenate was used for RNA extraction with the 5X MagMAX-96 Pathogen DNA/RNA Isolation kit (Life Technologies) following the recommendations of the supplier. Serially diluted stock (nucleic acid) was spiked into uninfected honeybee homogenates and RNA/DNA extracted as described above. The primers and probes were designed from the conserved genes of Chronic bee paralysis virus, Sacbrood virus and Chalkbrood. One-step RT-PCR was performed according to standard protocols using the Path-ID™ Multiplex One-Step RT-PCR Kit (Life Technologies). The following thermocycle program was used: 10 min at 48°C, 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Reactions without template DNA was run as negative control. AB7500 Fast Real-Time PCR system was used for amplification and data acquisition.

The dilution at 1:50 resulted in a Ct value of 26 and was used as a positive control in the field specimen testing. Validation of the diagnostic test for Chronic bee paralysis virus, Sacbrood

virus and Chalkbrood *in vitro* has been completed. Further, we have obtained honeybees samples from different beekeepers around Oregon. and validation of the tests in field samples have also been completed. Out of 50 field samples obtained, only two samples had detectable Chronic bee paralysis virus, one sample had detectable Sacbrood virus and 4 samples had detectable Chalkbrood. In conclusion, the above viruses and the fungal disease are not very much prevalent to affect the overall health of the bee colonies. We hope the diagnostic tests will greatly benefit clients from honeybee and bumble bee industry by providing more options for rapid, accurate and affordable testing.

One undergraduate and a graduate student have been trained in the above procedure. The Molecular Veterinary Diagnostic laboratory supervisor, Donna Mulrooney is also training the students in extraction procedure and PCR protocols as recommended by National Animal Health Laboratory Network (NAHLN).

BENEFITS & IMPACT: The Information obtained from this study will enable the Oregon Veterinary Diagnostic lab and the OSU Honey Bee Lab to serve the stakeholders (growers and beekeepers) by providing timely diagnosis to mitigate risks to bee colonies, potentially strengthening the economic sustainability of both beekeepers and producers. We hope the diagnostic tests will greatly benefit clients from honeybee and bumble bee industry by providing more options for rapid, accurate and affordable testing.

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FUTURE FUNDING POSSIBILITIES: College of Veterinary Medicine, OSU; USDA; Honeybee keepers association and other stakeholders.