AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Detection of Multiple-Viruses in Bovine Respiratory Specimens by Real-Time Polymerase Chain Reaction (RT-PCR): Validation of bovine respiratory panel RT-PCR test in Clinical samples

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SUMMARY: Bovine Respiratory disease (BRD) is underdiagnosed because of subclinical signs in many animals. Respiratory samples from animals with BRD often fail to show viral/bacterial growth in conventional culture. This is a major problem in subclinical BRD but also common in clinical BRD, and is a concern for all diagnostic laboratories, veterinarians, and dairy/beef producers. Our RT-PCR panel is highly accurate and efficient in detecting multiple viruses in subclinical cases. The Bovine Respiratory PCR Panel includes IBR/BVD multiplex assay, BRSV/CoV multiplex assay, Mycoplasma bovis singleplex assay and PI-3 singleplex assay. Validation for the multiplex and singleplex assays comprising the Bovine Respiratory PCR Panel was performed using clinical specimens previously tested by other PCR assays, as well as use of dilutions of reference positive virus pools and bacterial isolates to determine the sensitivity and specificity of each assay. For each assay, the sensitivity and specificity were 100% and 95%-98.3% respectively, when compared to results from previously used assays. Since implementation of the Bovine Respiratory PCR Panel (May 2014 – January 2015), 85 clinical specimens have been tested.

OBJECTIVES: Our objective in this proposal was to validate bovine respiratory panel RT-PCR to detect multiple respiratory viruses genome in clinical samples at Oregon Veterinary Molecular Diagnostic Laboratory.

PROCEDURES: The Bovine Respiratory PCR Panel protocol was obtained from Dr. Kathy Toohey-Kurth's laboratory at Wisconsin Veterinary Diagnostic Laboratory. This PCR Panel includes detection of BRSV, BVDV, CoV, IBR, Mycoplasma bovis and PI-3. Four separate PCR assays are performed; BVDV/IBR multiplex, BRSV/CoV multiplex, M. bovis singleplex and PI-3 singleplex. The assays may also be performed individually. Each assay in the panel was verified by comparing it to assays previously validated in this laboratory, or by using well characterized, known positive specimens or virus isolates. The Mycoplasma bovis PCR protocol was developed in house and previously validated.

SIGNIFICANT ACCOMPLISHMENTS: We have accomplished the validation for the multiplex and singleplex assays comprising the Bovine Respiratory PCR Panel using clinical specimens previously tested by other PCR assays, as well as use of dilutions of reference positive virus pools and bacterial isolates to determine the sensitivity and specificity of each assay. For each assay, the sensitivity and specificity were 100% and 95%-98.3% respectively, when compared to results from previously used assays. The following clinical specimens that were tested previously using current validated PCR assays were tested using the described multiplex and singleplex PCR assays.

	BRSV	BVD	CoV	IBR	M. bovis	PI-3
POSITIVE	17	14	23	13	27	8
NEGATIVE	12	11	10	7	10	7

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BENEFITS & IMPACT: The validation of multiplex-bovine respiratory disease RT-PCR test panel has helped our veterinary diagnostic laboratory to serve our diary/beef industry clients and veterinarians to identify animals with BRD and to treat or prevent the condition. Since implementation of the Bovine Respiratory PCR Panel (May 2014 – January 2015), 85 clinical specimens have been tested.
Validating a RT-PCR test for the detection of multiple respiratory viruses is critical for the eradication of this economically significant disease in Oregon.

ADDITIONAL FUNDING RECEIVED: None

FUTURE FUNDING: None required.