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Association of *Bovine Herpes virus 4* (BHV4) with clinical metritis among postpartum dairy cows in California: Epidemiologic and molecular genetic studies

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ovine herpesvirus-4 (BHV-4), is a widely-distributed virus and for decades, has been considered as "a virus looking for a disease". It $m{D}$ has been isolated from both healthy and diseased cattle, with a variety of illnesses, including abortion, metritis, vaginitis, enteritis, and pneumonia. However, there is insufficient evidence to ascertain its role as etiologic agent of a specific disease entity. The aims of the study were: To determine incidence and association of BHV-4 with metritis, investigate potential role of viral co-factors namely Bovine herpesvirus-2, BHV-2, Bovine viral diarrhea virus, BVDV, and Parapox virus, PPV), and determine the predominant strains of BVH-4 in our study area. Uterine swabs samples were collected from a total of 88 dairy cows (n=69 from Davis and n=19 from Tulare) and tested for BHV-4 using real-time PCR. Concurrently, the uterine samples were tested for presence of BHV-2, BVDV, and PPV. Descriptive statistics and logistic regression model to predict BHV4 infection based on recorded variables (metritis, season, geographic location, number and duration of lactation) were performed. The BHV-4 isolated from uterine samples were subjected to genotypic and phylogenetic analysis using 4 genes. The study revealed strong association of BHV-4 with metritis (Odds ratio=8.0 [95% CI:8.1-117]. Significantly higher BHV-4 infected cases were reported in Tulare than Davis (OR=1.99[95% CI:1.4-2.8]. Molecular genetic studies revealed that the thymidine kinase gene and the glycoprotein H sequences of all field strains were phylogenetically related to the American strain, but clustered in defined subgroups when compared with the European and the American reference strain. Approximately, 30% of the glycoprotein B sequences were phylogenetically related to the European reference strain and the remaining 70% were closely related to the American reference strain. Parapox virus, BHV-2 and BVDV were not significantly associated to have a co-infection role in metritis associated with BHV-4. This is the first preliminary study documenting association of BHV-4 with clinical metritis in California and the predominant strains of the virus circulating among dairy population in the study area.

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