Investigation of Malignant Catarrhal Fever in cattle comparing PCR and ELISA methods for diagnosis

S.A. Orono¹,², J.P. Mpatswenumugabo¹,³, M. Chepkwony², C. Ndinda², E. Okoth², G.C. Russell⁴, G.C. Gitao¹, V. Nene², E.A.J. Cook#²

¹University of Nairobi ²International Livestock Research Institute, ³University of Rwanda, ⁴Moredun Institute
INTRODUCTION

• MCF is a widespread often lethal virus illness especially of cows and other ruminants;
• Caused by gammaherpesviruses of the genus *Macavirus*, sub family *Gammaherpesvirinae*.
• AlHV-1 and OvHV-2 are the two important viruses.
• Blue and black wildebeest exist as the natural reservoirs of AlHV-1 while sheep are the natural host for OvHV-2.
• The disease is fatal only to susceptible species that are poorly adapted to the viruses.
• Wildebeest associated MCF (WA-MCF) occurs when cattle get infected with AlHV-1.
**INTRODUCTION**

- WA-MCF occurs (April to June) in the South Western region of Kenya
- There is no effective vaccine nor treatment for the WA-MCF
- Separation of carrier and susceptible species is the only control method
- The disease has great economic impacts on both small scale and large scale farmers

**AIM:** To compare nested PCR to ELISA in diagnosing clinical WA-MCF cases at Kapiti Ranch, Kenya
METHODOLOGY

- 33800 acre ranch
- 40 km east of Nairobi
- Extensive open grassland.
- Cattle and sheep are the main livestock species
- Other wild herbivores
METHODOLOGY

Clinical diagnosis
• Between 2014 to 2016, 326 cases of WA-MCF
• Diagnosis on clinical signs
  – pyrexia, bilateral corneal opacity, serosanguinous nasal discharge

Sampling
• Opportunistic samples from 123 cases of clinically suspected WA-MCF
• Blood samples collected by jugular venipuncture into EDTA vacutainer tubes
**METHODOLOGY**

**Serology**
- Antibodies to AlHV-1 were screened by indirect ELISA

**DNA extractions**
- Qiagen Flexigene kit was used to extract DNA

**Nested PCR (Traul et al, 2005)**
- The initial round PCR used outer forward primer and outer reverse primer (C500-1 and C500-2)
- The second amplification used the first round product (2 µl), by inner forward primer and inner reverse primer (C500-3 and C500-4).
**Statistical analysis**

- The Cohen kappa statistic was used to assess agreement between the indirect ELISA and the nested PCR.
- Sensitivity and specificity of indirect ELISA calculated by comparing to PCR.
- The Bayesian Agreement Index (B.A.I) framework was performed in the R software package to determine the level of agreement between these two assays.
RESULTS

- Of the 123 samples 77 tested positive (62.6%) by indirect ELISA and 116 (94.3%) by PCR.
- The ELISA sensitivity was 63.8% (95% C.I. 55.8-72.9) while specificity was 57.1% (95% C.I. 25.2-84.5) when compared to PCR.
- The kappa statistic between the two assays was \( k = 0.05 \).
- B.A.I revealed agreement for the two assays to be 76.7% (95% BCI 70-83%) in the positive direction and 15.1% (95% BCI 4.4-30.6%) in the negative direction.
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DISCUSSION

• This study revealed indirect ELISA is a poor tool for diagnosing acute WA-MCF
• The reason for the low sensitivity of the ELISA might be that animals died without seroconverting
• Nested PCR proved to be a more appropriate tool
• However it is expensive and required well trained personnel to use specialized equipment
• Confirms reports of other studies of PCR being a more effective tool for diagnosing WA-MCF
• The future of diagnosing WA-MCF thus should focus on development of cheap penside techniques to better manage WA-MCF outbreaks in the field
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