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Epidemiologic and phylogenetic characteristics of Crimean-Congo haemorrhagic fever in South Africa, 1981-2013

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Background: Crimean-Congo haemorrhagic fever (CCHF) is a viral tick-borne zoonosis that is widespread in Asia, Africa and Eastern Europe. CCHF was first recognized in South Africa in 1981 and is since then sporadically detected through passive laboratory surveillance.

Methods & Materials: A retrospective epidemiological analysis including counts, outcomes, place and type of exposure were conducted for laboratory confirmed human CCHF cases in SA for the past 32 years (1981-2013). In order to investigate the genetic diversity of CCHF viruses isolated from human cases, conventional Sanger sequencing for a 536 base pair region of the S segment was performed for isolates collected from 2003 to 2013. For similiar sequences for CCHF reported from other countries and human cases SA prior to 2002, GENBANK derived sequences were included in the analysis.MEGA software was used to perform phylogenetic analysis of sequences using the neighbour-joining distance method and sequence divergence determined by calculating p distances between sequences.

Results: A total of 193 human cases were laboratory confirmed for South Africa for the period 1981-2013. Most cases were reported from the semi-arid regions occupying the Northern Cape (n=44) and Free State (n=21) Provinces. Nearly two-thirds of cases were associated with tick exposures. The fatality rate over the 32 year period was 24% (n=46). Phylogenetic inference of CCHF virus isolates revealed six phylogenetic groups that partially related to the geographic origin of the viruses. Partial S segment sequencing of human isolates from South Africa collected over a three decade period revealed pair-wise differences ranging from 0.4-18% for nucleotide and 0-7% for deduced amino acids. All South African sequences clustered in one of three distinct phylogroups.

Conclusion: Despite indication from previous studies of high-seroprevalence in livestock in certian regions of South Africa, CCHF remains rarely reported in humans.Partial S segment sequencing revealed high level of sequence heterogeneity and phylogenetic inference indicated co-circulation of viruses belonging to three phylogroups. The grouping did not correlate with clinical severity of outcome.

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A slide-SAB method for the detection of the antibody of *Lawsonia intracellularis*

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Background: L*awsonia intracellularis* is the pathogen in porcine proliferative enteropathy (PPE) and equine proliferative enteropathy (EPE). PPE causes a big economic loss through malgrowth. EPE causes fever, peripheral oedema, diarrhoea, colic and weight loss. The diagnosis during the lifetime is the detection of the antibody of *L. intracellularis* in serum and/or the detection of the *L. intracellularis* gene by the PCR. The sero-diagnosis requres either the indirect fluorescent antibody method (IFA), immunoperoxidase monolayer assay (IPMA) or ELISA method. However, an expensive fluorescent microscope is necessary to perform the IFA method, *L. intracellularis* culture technique is necessary for IPMA, and an expensive ELISA kit is required to perform it. In this study, we developed an inexpensive streptavidin biotin method (s-SAB) on a glass slide.

Methods & Materials: The Antigens were used as the *L. intracellularis* cell (Ags from Dr. S. McOrist provided and *L. intracellularis* vaccine from Enterisol®Ileitis(Boehringer Ingelheim)). The Ag was dropped into 12 spots on a glass slide. The test serum was dropped onto the slide and reacted with the Ag at 37 □ for 30 minutes. Biotinylation secondary antibody IgG was reacted at 37 □ for 30 minutes. Peroxidase-labeled streptavidin was reacted at room temperature for 20 minutes. Enzyme substrate AEC was developed at room temperature for 5 minutes. After enclosure, the slide was observed by an light microscope. The red-brown color of the comma-formed small bacillus on the slide proved the serum to be positive. In order to apply these results to sero-diagnosis, a reproducible evaluation and sensitivity and a specific evaluation were carried out. The results of these evaluations were compared with the results of either the IFA or the ELISA.

Results: Strong-colored *L. intracellularis* cells were observed in the positive serum with both antigens. In the reproducible evaluation, the stainability was constant with all sera. The IFA&ELISA and s-SAB judgement result completely accorded with the sensitivity in the specific evaluation.

Conclusion: It was suggested that the sensitivity and the specificity of the s-SAB were equal to the IFA and ELISA. Therefore, the s-SAB method is effective as a sero-diagnosis of both PPE and the EPE.

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