18.020

Genetic Characterisation of Rabies Viruses from Nigeria

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In Nigeria, rabies still poses the greatest public and veterinary health risks, similar to other countries in Africa and Asia. The disease remains one of the most neglected endemic zoonosis despite the availability of biologicals for its control. There is no active surveillance system for the disease; in addition, underreporting and misdiagnosis are common. A study was therefore undertaken to elucidate the molecular epidemiology of rabies in Nigeria. A cohort of 100 rabies viruses all recovered from dogs between 1989 and 2007 were included in this investigation. The samples were confirmed to be rabies using the direct fluorescent antibody test (FAT). Reverse transcription and amplification were performed on a partial region of the glycoprotein gene of each of viral RNAs of rabies isolates analysed. The PCR products were sequenced and a 592-nucleotide sequence encompassing the cytoplasmic domain of the glycoprotein and the G-L intergenic region using the Big Dye Terminator V3.1 cycle sequencing kit (Applied Biosystem). The phylogenetic analysis demonstrated that the panel of Nigerian rabies viruses were closely related with 93.2 - 100% sequence identity. However, despite this close genetic relationships, the viruses could be differentiated into one major group of viruses recovered from dogs from the North Central states of Nigeria, and the other two from the from the North East and South West regions respectively. The implication of the data on control of rabies in Nigeria will be discussed.

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Bacterial Causes of Ovine Abortion and Neonatal Mortality in Iran

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Background: Abortion and prenatal lamb mortality is the major cause in lowering of productivity of sheep. The objectives of the present study were to explore the problem in lambs in Iran and to identify the causal bacteria.

Methods: During a one year survey a total of 45 aborted fetuses and 47 neonatal deaths from different farms were examined to determine the bacterial causes. Tissues were collected aseptically for microbiologic examination. Routine examination included aerobic and micro-aerobic culturing of lung, liver and stomach content. New diagnostic assays such as PCR was added to standard protocol for confirming Salmonella isolates by detecting invA gene which are specific for Salmonella.

Results: Bacterial agents were diagnosed on the basis of isolating an organism from several fetal tissues in pure or nearly pure culture. Bacterial agents associated with inflammatory reaction were detected in 16 cases of aborted fetus (35.56%) and 17 cases of neonatal death (36.17%). Bacteria responsible for abortion were: Salmonella spp. (9%), Brucella melitensis (4.5%), Campylobacter fetus subsp fetus (4.5%), Moraxella spp.(4.5%), Escherichia coli (4.5%), Corynebacterium spp.(4.5%). Bacteria responsible for neonatal mortality were: Salmonella spp(8.5%)., Escherichia coli (8.5%), Pasteurella multocida (6.4%), Mannheimia haemolytica (6.4%), Campylobacter fetus subsp fetus (4.25%) and Brucella melitensis (2.12%).

Conclusion: Salmonella spp. was the most frequent bacterial species identified as cause of abortion and neonatal mortality in lambs in Khorasan province of Iran. We found other organisms in pure culture with low virulence related to abortion and neonatal deaths. Because of suppression of immune reaction at the junction of the fetal and maternal placentas, any infectious agent that is able to reach the junction of the maternal and fetal placentas could be free to multiply and cause lesions unhindered by immune reaction. Under these immunologic conditions, bacteria of low virulence may cause abortion and neonatal deaths.

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Prevalence of Feline Coronavirus Antibodies in Cats in Bursa (Turkey) by An Enzyme-Linked Immunosorbent Assay

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Since feline coronavirus (FCoVs) are ubiquitous pathogens, the seroprevalence was studied to monitor infection in Turkey. One hundred sera, collected from cats belonging to catteries or community shelters and to households, were tested. The VN test was performed and compared with ELISA. Each sample was heat-inactivated and serial two-fold dilutions starting from 1/2 were mixed with 100TCID50/50 μl of FCoV type II strain 25/92. Subsequently, freshly trypsinized CRFK cells were added and the plates were incubated at 37 °C for 96 h. The VN antibody titer was expressed as the reciprocal of the highest serum dilution that completely inhibited viral cytopathic effect. Microtiter NUNC-polysorp immunoplates were coated with 25 μg/ml of FCoV type II antigen (purified whole virus) and incubated overnight at 4 °C with shaking. Each serum, diluted 1/50, was added and the plates were incubated for 90 min at 37 °C. Rabbit anti-cat IgG was added and then freshly prepared substrates were used. The OD were determined at 405 nm. The results were compared using the Cohen's kappa test for agreement and repeatability.

The VN revealed 79 sera as negative and 21 as positive. The negative sera were subsequently examined by ELISA which confirmed 74 as negative while 5 resulted positive. These 74 negative sera were also found to be free of FCoVs specific antibodies by western blotting. Using the VN test as the gold standard test, ELISA had a sensitivity of 100% and

a specificity of 93.6%, with an overall agreement of 95%. Kappa test indicated high association between the two test (0.86). The positive predicted value (PPV) was 0.8, and the negative predicted value (NPV) was 0.93.

ELISA proved to be a sensitive test. Considering the cross-reactivity between the two serotypes, ELISA was able to detect antibodies against both, allowing the use of the assay as a reference test for sera screening.

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Seroprevalence of avian influenza A/H5N1 among poultry farmers in rural Indonesia

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Background: Since 2003, about a third (>100 cases) of human cases of highly pathogenic avian influenza (HPAI) A/H5N1 worldwide are reported from Indonesia. There is concern for viral reassortment and a pandemic. Seroprevalence studies that may reveal unrecognized cases are lacking. We aimed to measure H5N1 seroprevalence among poultry farmers in rural Indonesia.

Methods: The present cross-sectional study is an extension of an agricultural HPAI project by Dutch and Indonesian Ministries, who recruited 12 farms in a postulated endemic area in West-Java. In 2007, poultry workers and farm residents were interviewed about possible risk factors. H5N1 serostatus was determined by hemagglutination inhibition tests by NIHRD Jakarta and neutralization tests by NIID Tokyo.

Results: In the 12 farms, 495 of 622 (80%) farmers participated. Of these, 95% lived on the farm, 71% were male and median age was 29 years (interquartile range 23—36 years). In the previous six months, confirmation of H5N1 in poultry was available for one farm. Masks were never worn by 54% of participants. Eighty-six percent were afraid to become infected. Fever, cough and shortness of breath were reported as not being a symptom by 23%, 31% and 33%. Validation of serological tests is pending.

Conclusions: Validation of serological tests is pending. These results influence the rationale for concern for frequent mild infections in poultry farmers. Incomplete evidence for H5N1 in the poultry sheds doubt on the H5N1 exposure. In light of the severity of human HPAI infection and the risk of a pandemic, we recommend to sustain ongoing efforts to educate poultry farmers about HPAI prevention and symptoms.

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Seroresponse Against Avian Influenza A/H5N1 Among Poultry Workers in Jakarta

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Background: Since 2005, Indonesia has reported more than 100 human cases of highly pathogenic avian influenza (HPAI) A/H5N1. Cases were typically associated with poultry exposure and 20% were from Jakarta. Human infections raise concern for viral adaptation to humans and a pandemic. Seroprevalence studies that may reveal additional unrecognised mild infections are lacking. We aimed to measure H5N1 seroresponse and identify associated determinants among poultry workers in Jakarta.

Methods: This cross-sectional study was an extension of an agricultural HPAI project by Dutch and Indonesian Ministries. We approached 40 collector houses in Jakarta, where poultry is channelled from the countryside to markets. Collector house poultry workers contributed blood for H5N1 serological testing and were interviewed about possible determinants. H5N1 seroresponse was measured by hemagglutination inhibition tests at NIHRD Jakarta and neutralization tests at NIID Tokyo.

Results: In 34 of 40 collector houses, 218 of 276 (79%) workers participated. Of these, 206 of 218 (94%) lived in or next doors to the collector houses, 206 of 218 (94%) were male and the median age was 29 years (interquartile range 24—37 years). Validation of serological tests is pending.

Conclusions: Validation of serological tests is pending. These results influence the rationale for concern for frequent mild infections in poultry farmers. If seroresponse is confirmed, this finding highlights the importance of ongoing HPAI education efforts among poultry workers.

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