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CONFERENCE PAPER · OCTOBER 2015

DOI: 10.5176/2382-5685_VETSCI15.24

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Screening for Nipah Virus Infections in Pigs of Kathmandu Valley and Chitwan District of Nepal

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Abstract-Nipah Virus infection (NiV) is an emerging infectious disease of public health importance in South East Asia affecting pigs and people and bat acts as the reservoir host of the virus. In Nepal, the status of NiV infection is unknown. Hence, this study aims to screen for Nipah virus antibody in sera of pigs A descriptive cross sectional study was carried out in pigs of Kathmandu valley and Chitwan district where bats of the genus Pteropus sps (fruit bats) are also present. Blood samples of pigs from ear vein were collected and serum was separated and stored in deep freeze at -80^{0}C. These sera samples were dispatched to Animal Health Veterinary Laboratory Agency (AHVLA), UK and tested using Luminex binding multiplexed assay. Out of 120 pig sera, only 3 sera samples were found doubtful to Hendra virus and Nipah virus by Luminex binding assay. Those three samples did show levels of binding >500MFI and can be regarded as suspect positives. Though the results from duplicates are not identical, this is commonly seen with pig sera probably exhibiting a lot of non-specific binding from other proteins or immunoglobulins in the sera. It was tried to reduce by using a higher dilution factor but there could still be non-specific binding present, rather than a "positive". Therefore, confirmatory analysis should be carried out using a virus neutralisation test. This study is a preliminary study against Nipah virus infections in pigs of Nepal using Luminex binding multiplexed assay. Bats of genus pteropus sps are reservoir hosts of Henipa virus and are present in Nepal. Hence continous surveillance study on bats and pigs is very important for early detection, prevention and control of disease.

Keywords-Nipah virus, pigs, bats, Luminex binding multiplexed assay

Introduction

Nipah virus infection is an emerging zoonotic disease caused by Nipah virus (NiV), a highly pathogenic paramyxovirus which belongs to the genus Henipavirus. Nipah viruses are enveloped, single-stranded, negative sense RNA viruses [1] and are closely related to Hendra viruses [2]. Virological studies of Nipah virus revealed that it had ultrastructural, antigenic, serologic, and molecular characteristics similar to Hendra virus [3]. Molecular studies confirmed that Nipah virus was closely related to Hendra virus, with specific genes sharing 70% to 88% nucleotide homologies and 67% to 92% amino acid homologies, and with identical intergenic regions and nearly identical gene start-and-stop sequences [4].

Zoonotically, this virus is very important as it causes severe febrile encephalitis in humans [3]. Nipah is the name of the village near Kuala Lumpur where the virus struck first, in Malaysia [5]. This virus affects pigs and people and sometimes causes fatal encephalitis. Bats of the genus Pteropus (fruit bats or flying foxes) are the main reservoir host of this virus [2].These fruit bats are distributed in Australia, Cambodia, Indonesia, Malaysia, Maldives, China, Pakistan, Bangladesh, India, Nepal Srilanka and other south East region [5]. Pig acts as the amplifying host [2]. Infected bats shed virus in their excretion and secretion such as saliva, urine, semen and excreta but they are symptomless carrier [5]. Infective viruses has been detected in partially eaten fruit by wild Pteropus bats [6]. Despite high sero-prevalence rates, very few bats in the colony may shed the virus at given time, and excretion from the colony may be sporadic [2]. The route of transmission from bats to domesticated animals is uncertain, however, pigs are found infected by eating contaminated fruits with bat saliva or urine, by drinking contaminated water or by eating aborted bat fetuses or birth products [2]. The NiV is highly contagious among pigs, spread by coughing [5].The virus can be transmitted from pigs to humans via direct contact with sick pigs through animal's blood, urine, bronchial secretion and amniotic fluid and other body fluids. In addition, humans may be infected due to exposure to infected fruit bats or materials contaminated by infected bats [2] or direct human to human transmission is also possible [7-8].

The incubation period for Nipah virus infection in pigs is estimated to be 7 to 14 days, but may be as short as four days. In experimentally infected cats,

3rd Annual International Conference on Veterinary Science Research (VETSCI 2015)

ISSN 2382-5685
doi: 10.5176/2382-5685_VETSCI15.24

incubation periods of six to eight days have been reported [2]. The incubation period in humans is usually 4 to 20 days; however, incubation periods as short as two days or as long as a month has been reported. Some people may remain asymptomatic during the initial infection, but develop serious neurological disease up to four years later [2].

Nipah virus infection appears in two forms: encephalitic and respiratory. In animals the respiratory type is more common [9], while in humans, neurological disorder is more common [10]. Clinically the infected pigs present the symptoms of fever, nasal discharge, open mouth breathing, rapid and labored respiration and a loud barking cough. Generally, mortality of infected pigs is low but morbidity is very high. Humans infected with Nipah virus shows initial symptoms of flu-like, high fever, headache and myalgia. In case of encephalitis, the symptoms may include drowsiness, disorientation, convulsions, and coma. Less often, patients develop respiratory signs, which may include acute respiratory distress syndrome.

In the context of Nepal, Pteropus sps (reservoir hosts) of bats are recorded from central and eastern Nepal [5]. In a survey conducted by Puspa Raj Acharya in Kathmandu valley in 2008, discovered the three Ptero –camps of Indian flying foxes. They are Keshar Mahal Ptero-camp, Sallahghari Ptero-camp and Gokarna Ptero camp. Regarding pigs, the intermediate host, pig farming has been increasing in Nepal due to high Feed Conversion ratio, highly palatable for local ethnic group (Limbu). The total population of pigs for 2010/2011 is recorded to be 11,08,465 [11]. In Kathmandu, pig population is found to be 10163 and in Bhaktapur, it is found to be 3205 [11]. Since, there is a higher population of both reservoir and intermediate hosts of Nipah virus, presence of Nipah virus in Nepal cannot be neglected. There have been no any such studies conducted yet to determine the prevalence of Nipah virus in bats and animals. Hence this serological study will be an attempt to find out antibodies against Nipah Virus infection and will help to provide baseline data for the Nipah virus infection.

Methods and Methodology:

A descriptive cross sectional study was conducted to find out the prevalence of Nipah virus in pigs through serological screening test. The study was conducted in Kathmandu and Chitwan district of Nepal as these areas are the roosting areas of fruit bats. In Kathmandu, Keshar Mahal and Sallaghari areas are selected for study of fruit bats as Pteropus bats are found in these areas [12]. Therefore, there is a possibility that density reared pigs in those areas may be infected with nipah virus. Pig serum samples were collected from Jaruwarasi, (Lalitpur) Pig farm of Khumaltar, (Lalitpur), Goldhunga pig farm (Balaju), Dharmasthali pig farm, Jagatpur (Chitwan), Gita Nagar (Chitwan). During sample collection, existence of bat around the area was also asked. Collected pig sera were stored in Defreeze until tested at -40 degree Celsius.

Samples were tested in Animal Health Veterinary Laboratory Agency (AHVLA), UK according to Luminex binding multiplexed assay protocol previously used by Peel et al.2012. Luminex binding multiplexed assay look for antibodies binding to both HeV and NiV recombinant soluble G glycoprotein (sG) [13]. Briefly, recombinant HeV and NiV glycoproteins were conjugated to internally coloured and distinguishable microspheres, allowing multiplexing. Antibody binding to each microsphere was detected after conjugation of bound antibodies with biotinylated Protein A and fluorescent streptavidin-R-phycoerythrin. Binding results are given as median fluorescence intensities (MFI) of at least 100 microspheres for each virus type." (Peel et al. 2012)

Sera was diluted 1:200 (1:50 is the usual dilution) prior to testing, because preliminary assay results showed a high level of background fluorescence at 1:50, which was reduced by diluting 1:200.Samples, standards and no-sera controls were run in duplicate. Positive controls were run as single wells.

The recombinant sG proteins were generated using a mammalian expression system in a soluble form by removing the transmembrane domain (Bossart KN et al, 2005). In this method, test sera is added to a mixture of sG_{Niv} and sG_{HeV} coupled microsphere subsets and then incubated so that antibodies bind to analyte of interest. It was further incubated with biotynilated protein A/G and streptavidin-phycoerythrin for detection. Antibodies which bound to the sG_{Niv} or sG_{HeV} coated beads are spectrally distinct which are quantified by the fluorescence emitted by phycoerythrin. This is read as the median fluorescence intensity (M.F.I).

Results:

Of the 121 sera tested, 3 sera were found doubtful to Hendra virus by Luminex binding assay. Thses sera should be further confirmed by Serum neutralization test.

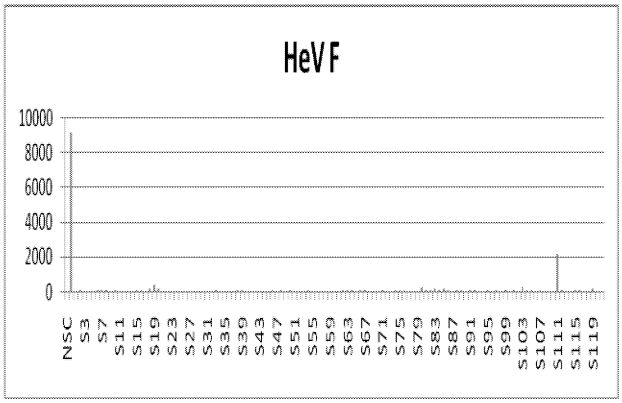

Fig 1

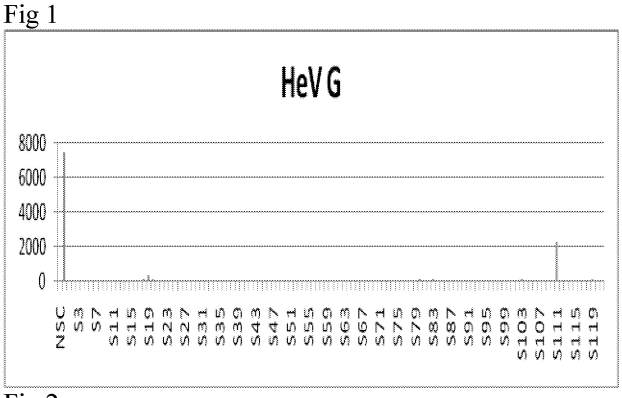

Fig 2

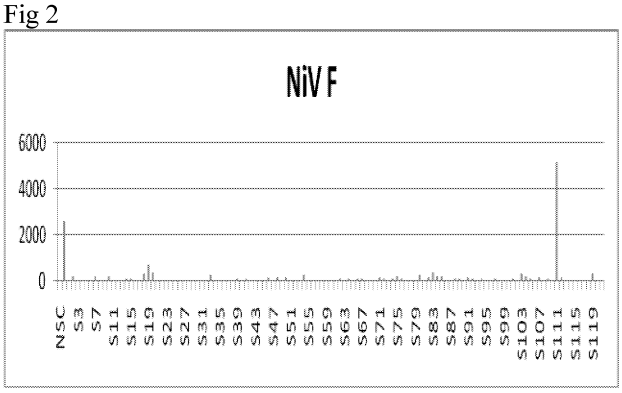

Fig 3

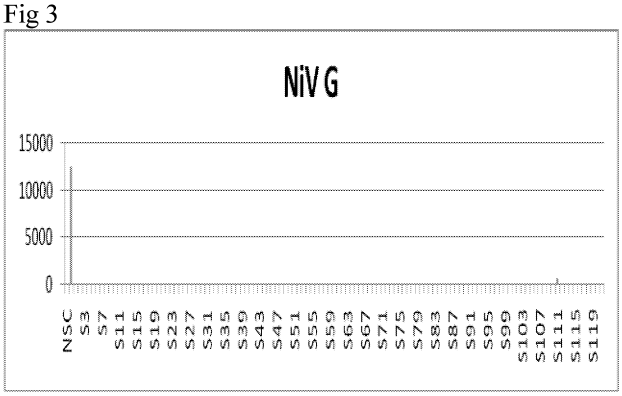

Fig 4

Fig 1-4. Detection of Nipah virus and Hendra virus antibodies in Luminex binding multiplexed assay

In figure 1,2 and 3; samples S19, S20 and S111 did show the level of binding against the nuclear proteins HeVF, HeVG, and NiV F. Fig 4 shows that only sample S111 shows low level of binding against NiVG in comparison to the positive control sera.

Discussion:

The aim of this study was to assess the presence of Nipah virus and hendra virus antibodies in pigs of Nepal. This study suspected 3 sera samples positivity for Nipah and hendra virus antibodies by luminex binding multiplexed assay. Those three samples were regarded as suspect positives as it showed levels of binding >500MFI. Though the results from duplicates are not identical, this is commonly seen with pig sera probably exhibiting a lot of non-specific binding from other proteins or immunoglobulins in the sera. It was tried to reduce by using a higher dilution factor but there could still be non-specific binding present, rather than a "positive". Therefore, confirmatory analysis should be carried out using a virus neutralisation test.

Conclusion and Recommendation:

This study is a preliminary study against Nipah virus infections in pigs of Nepal using Luminex binding multiplexed assay. Since bats of genus pteropus sps are reservoir hosts of Henipa virus, bat should also be studied. Insufficient lab facilities are the major problem in conducting the study against this deadly virus. Therefore government should provide facilities and establish BSL 4 lab for conducting test against such virus. Surveillance of this virus should be carried out regularly for early prevention of diseases.

Acknowledgement:

The author would like to thank Mahesh K.C (B.V.Sc &A.H), V.C Jha (Chief, CVL) , Susil Poudyal (B.V.Sc & A.H) for their support in accomplishing the project objectives and Feed the future innovation lab, collaborative research (Adapting livestock systems to climate change) for funding, their necessary guidance, and empowerment. Last but not the least; I am indebted to my mother for her support and encouragement.

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