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Short Communication

AGGLUTINATION OF RED BLOOD CELLS BY CANINE DISTEMPER VIRUS

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INTRODUCTION

Canine distemper is a disease of dogs and other carnivores. However Gordon *et al.* (1991) has reported association of canine distemper virus in cases of Pagets disease of humans. This suggests that the disease is of zoonotic importance. Canine distemper disease is caused by a morbilivirus which possesses the haemagglutin antigen (Gibbs *et al.*, 1979). Despite this, haemagglutination test has not been developed for diagnosis of canine distemper. Current methods of diagnosing canine distemper disease include, the use of clinical signs and gross lesions (Hagan and Brunner, 1961), histopathological examination of tissues (Frisk *et al.*, 1999), virus isolation in cell cultures or in embryonated chicken eggs (Evans *et al.*, 1991, Kai *et al.*, 1993), serum neutralization (Frjolic *et al.*, 2000), Enzyme Linked Immunosorbent Assay (ELISA) (Welsh *et al.*, 1992) and Florescent Antibody Technique (FAT) (Yoshida *et al.*, 1999). Use of clinical signs and gross lesions alone are not reliable because there are other diseases which show similar manifestations (Horst, 1975). Histopathology is reliable only when both cytoplasmic and intranuclear inclusion bodies are found (Pare *et al.*, 1999). In addition, this method is time consuming. ELISA and FAT require sophisticated and expensive equipment often not available in developing countries. Virus isolation in cell culture and in embryonated chicken eggs take days before results can be obtained. Haemagglutionation and the corresponding haemagglutination - inhibition tests are very simple, valid, cheap and rapid techniques (Johnson, 1971). Haemagglutination of red blood cells by morbilliviruses such as measles virus and peste des petits ruminants (PPR) virus has already been reported (Wosu, 1985, Ramarchandran *et al.*, 1993). This work reports a successful attempt to demonstrate haemagglutination technique for diagnosis of canine distemper.

KEY WORDS: Canine Distemper, Haemagglutination test, Dogs, Body fluids, Organs

MATERIALS AND METHODS

Sample Collection

Tissues of Seven 12-week old puppies infected with 0.1 ml of chorioallantoic membrane (CAM) of chicken eggs in which *Canine distemper* virus was isolated were used for the test. The virus was isolated from clinical cases got from an outbreak in Nsukka in South - East Nigeria. The 0.1ml of the CAM had fifty percent egg infectivity dose (EID_{50}) of 10^5.

Samples collected from the infected dogs include cerebrospinal fluid (CSF), bile and extracts of liver, spleen, lungs, kidney, and lymphnodes. The samples were confirmed positive for *canine distemper* via histopathology by demonstration of both intranuclear and intracytoplasmic inclusion bodies in different cell types (Frisk *et al.*, 1999) and by Agar gel precipitation test using measles antiserum prepared in rats (Mori *et al.*, 1994).

Haemagglutination (HA) Test

HA test was done for each of the cerebrospinal fluid, bile and extracts of the infected dog organs with chicken red blood cells (RBC), pig RBC, human group "O" RBC and goat RBC by the method described for PPR by Wosu (1985). The HA test was done using 0.05ml of PBS (pH 6.8) deposited in each of the wells in a microtitre plate. Then 0.05ml of the *canine distemper* antigen was added to the first well in a row and double diluted serially over the wells. Following 0.05ml of the 0.6% RBC was added to each well. For RBC control, only 0.05ml of RBC was added to the 0.05ml of PBS. For the virus control, in a row containing the distemper virus diluted in PBS as described above, 0.05ml of measles positive serum was added to each well and incubated for 45 minutes before 0.05ml of the RBC was added. The set up was incubated at room temperature (25°C) for 30 minutes when the RBC in the RBC control wells settled. Haemagglutination titre of the tested samples was read as reciprocals of the highest dilutions which gave a complete haemagglutination.

RESULTS AND DISCUSSION

Results of HA test with extracts of brain and Cerebrospinal fluid (CSF) of three of the seven dogs were HA positive with titres of 32 to 4096 and 32 to 2048 respectively. Liver, lymph nodes and spleen were HA negative. Lungs and kidney extracts were consistently positive but had low titre of between 8 and 64. The HA results of the extracts are shown on Table I. In each case, the RBC control was good. Also the measles positive serum inhibited agglutination of red blood cells by the fluids and tissue extracts.

TABLE 1: Distribution of *Canine distemper* haemagglutinin (HA positive) in infected dog tissues

CSF/Organ Extract	HA Titre 1	2	3	4	5	6	7
CFS	2048	1024	32	–	–	–	–
Brain	4096	1024	32	–	–	–	–
Liver	–	–	–	–	–	–	–
Kidney	32	32	16	64	64	8	8
Lung	64	16	16	32	32	8	64
Lymph node	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	–

With CSF as a source of antigen and PBS of pH 6.8, HA titre varied among the species red blood cells used in the study. Human group "O" RBC was the most sensitive followed by chicken RBC (Table II). Haemagglutination by the fluids and extracts of organs from the dogs which was inhibited by known measles positive serum shows that the haemagglutination was due to *Canine distemper* virus because antigenic relationship exists between the morbilliviruses (Johnson *et al.*, 1968; Hamdy *et al.*, 1976). Also cross protection between measles and *canine distemper* has been recorded (Horst, 1975). Vaccines made from Rinderpest H and F antigens were able to protect Ferrets against lethal *Canine distemper* virus (Jones *et al.*, 1997). Sixt *et al.* (1998), have reported that protection by measles vaccines against *Canine distemper* is due to the presence of the anti-H, and anti-F and or anti-N antibodies in the sera of the vaccinated dogs. This information on protection by measles vaccine justifies use of measles serum in place of *Canine distemper* specific serum for this work.

TABLE II: Sensitivity of different RBCS to *Canine distemper* haemagglutinin

Rbc	HA Titre
Man "O"	2048
Chicken	1024
Porcine	512
Dog	zero

Measles nonspecific vaccine is available even in the remote places of the developing countries. So measles serum can be prepared by small laboratories for use in confirming Canine distemper by the HA technique. On the other hand, Canine distemper vaccines usually come in combination with other vaccines. So use of measles serum for this work appears to have some advantage over use of Canine distemper specific serum.

Wosu (1985) who demonstrated for the first time that PPR virus could cause haemagglutination further observed that cultured PPR viruses obtained from reference laboratories could not produce agglutination of any RBC. Durojaiye et al. (1983) had earlier reported that cultured PPR viruses obtained from a reference laboratory (National Veterinary Research institute, Vom, Nigeria) were unable to produce clinical PPR disease in experimentally infected sheep. So, in this work homogenate antigens were used for the attempt to develop a haemagglutination test for Canine distemper.

The clinical signs and gross lesions observed in the dogs used in this work agree with the clinical disease described for Canine distemper by Horst (1975). The histopathologic lesions observed in the infected dogs are also similar to those described for Canine distemper by Frisk et al. (1999). In addition, production of line of precipitation between the antigens and the measles serum in agar gel precipitation test is a further confirmation of our diagnosis of Canine distemper.

Results of this experiment suggest that HA technique has been developed for confirmation of diagnosis of Canine distemper. The specimens to use for diagnosis of Canine distemper by HA technique appear to be the kidney and the lungs which were consistently positive in the seven dogs. The cerebrospinal fluid and the brain can also be tested. These findings appear to be supported by report of Alldinger et al. (2000) who reported that inflammatory processes clear Canine distemper virus from the blood and other organs but not from the brain. In the

presence of humoral immunity Canine distemper virus often gets to the brain by migrating through the cells without getting into the blood or other body fluids (Zurbriggen et al., 1995). This migration to the brain through the cells may be responsible for the high HA titre obtained from the brain extracts and from the closely associating cerebrospinal fluid in three of the cases examined. It is possible that the other four dogs where the brain and CSF were HA negative the virus did not get to the brain.

The neutralizing antibodies against Canine distemper virus include anti-haemagglutinin (Hirayama et al., 1991). So failure in this work to get haemagglutination with extracts of the liver, spleen and lymph nodes in the dogs tested may be due to neutralization of the Canine distemper haemagglutinins in these organs of the dogs used. The low HA titre recorded with kidneys and lungs may be due to partial clearance of the haemagglutinin antigens from these organs in the dogs.

Demonstration of a haemagglutination test for Canine distemper virus will facilitate laboratory confirmation of the disease in the developing countries where hitherto diagnosis was based on only clinical signs due to lack of facilities for the other sophisticated and expensive techniques.

Use of 0.6% RBC concentration at 25o and acidic pH of 6.8 to produce positive HA result with Canine distemper is similar to the conditions used for PPR virus by Wosu (1985). Wosu (1985) had reported that PPR homogenate virus was able to agglutinate only piglet RBC. However, a conference on Morbilliviruses in Edinburgh confirmed that PPR virus agglutinates pig RBC and also other red blood cells including those of human group "O", monkeys and chickens (Ramachadran et al., 1993). The result of this work also shows that human RBC is the most sensitive to canine distemper haemagglutinins. Chicken RBC follows the human RBC in sensitivity to canine distemper haemagglutinins.

CONCLUSION

It has therefore been concluded that haemagglutination test can be adopted for confirmation of diagnosis of canine distemper by using PBS at pH of 6.8 and with either human group "O" RBC or chicken RBC.

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