ISOLATION AND TYPING OF CANINE PARVOVIRUS FROM DOGS FECAL IN SOME AREA IN INDONESIA

E. Raharjo Jusa [1], G. Mudiarto[2], M. Aeny Rochman Noor[2], S. Bahri Siregar[2], and M. Partadiredja[2].

SUMMARY

Canine parvovirus (CPV) has supopsedly been in Indonesia since 1981, but the isolation of aetiological agent has never been reported. In the present study, isolation and typing of CPV in Indonesia is presented. Out of 180 fecal samples collected from unvaccinated dogs were detected 12 isolated virus by using haemagglutination test after growing the virus in CRFK cell line. Typing the isolates were done with monoclonal antibodies. The results showed that all of the isolates are of "new type".

INTRODUCTION

The outbreaks of CPV infection, which the main clinical signs are diarrhea, vomiting, depression and anorexia has supposedly been in Indonesia since 1981 (Directorate of Animals Health, 1981).

Canine parvovirus appears to be transmitted from one dog to another through the feces as the primary source of infection (Kramer *et al.*, 1980). Laboratory diagnosis of CPV could be confirmed by virus isolation from feces in tissue culture and detection of viral particles by electron microscopy (Carmichael *et al.*, 1980 and Senda *et al.*, 1987). Typing of CPV from feces infected dogs had been reported (Parrish *et al.*, 1982, Parrish *et al.*, 1988 and Senda *et al.*, 1988).

The present study deals with the first isolation and typing of CPV from field cases with a total of 180 fecal samples collected from unvaccinated dogs in Indonesia.

MATERIALS NAD METHODS

Materials

Fecal samples : A total of 180 samples, comprising of 165 samples from healthy dogs (Table. 1) and 15 samples from clinically ill dogs with enteric disease (Table. 2)

1. Veterinary Drug Assay Laboratory, Gunungsindur, Bogor 16340.
2. Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor.

were collected between October and December 1989.

Cell, Reference virus and Monoclonal antibodies (MAbs) : Crandell felline Kidney (CRFK) cell line, reference virus of new type (CPV-Y-1) and old type were supplied by National veterinary Assay Laboratory, Tokyo, and Tokyo University, Japan respectivelly. Monoclonal antibodies used were supplied by Dr. Parrish (Cornell University, USA), where MAbs CIDI reacts with the new type, MAbs B3B11 with the old type, and A2FB reacts commonly with CPV of both types (Parrish *et al.*, 1982, Parrish *et al.*, 1985 and Parrish *et al.*, 1988).

Methods

The fecal samples were centrifuged at 10.000 rpm for 15 minutes in 4°C condition. The supernatan of each samples were colleted and kept in deep freezer (−80°C) before testing. The suspension of CRFK cell (1 x 10^5 cells/ml) was grown in Eagle MEM suplemented with 10% fetal calf serum, 0,295% tryptose phosphate broth, 0.08% $NaHCO_3$ and antibiotics. The supernatan of each samples were inoculated 0.1 ml onto CRFK cells of 80% confluency and incubated at 37°C under 5% CO_2. One day after inoculation, the growth medium was changed by maintenant medium (2–5% of fetal calf serum).The culture fluids harvested from the second passage were tested by haemagglutination (HA) and haemagglutination inhibition (HI) for detecting of virus.

HA – HI test. These tests were performed as decrribed previously (Senda *et al.*, 1986), using borate buffered saline (BBS) and virus adjusting buffer (VAD) as diluent for virus and erythrocytes respectivelly.

HA test. Serial two-fold dilution of each isolates in cell free supernatan were prepared in 96 well plastic v-plates. An equal volume of 0.5% pig red blood cell (RBC) suspension was added to each well. The results were scored after incubation at 4°C and 37°C overnight, temperature dependence was determined from relative value of HA titer at 4°C to that at 37°C (Senda *et al.*, 1988). The HA titer was expressed as the reciprocal of the cell-free supernatant fluids showing complete heamagglutination.

RESULTS

The HA test finding from the total of 180 samples after the second passege in the CRFK cell are as follows : In the HA test from a total of 165 healthy dog samples, two samples were positive, one from Bandung and another one from Tasikmalaya area (Table. 1). The age of the reactors were oneyear and threemonth old respectivelly. Ten out of 15 samples from the clinically

ill dogs were positive (Table. 2). The twelve isolated CPV which have had positive reactions in the HA test did not show temperature dependent HA, due to the fact that HA titer with these isolates remained constant at 4°C and 37°C. All isolates reacted with positive conventional sera, MAbs CIDI and MAbs A2F8 (Table. 3).

DISCUSSION

The present study reporting the first isolation and confirmation of CPV infection in Indonesia. The result presented on table 2 shows that 10 of 15 clinically ill dogs with enteric disease were positive with CPV. This data indicates a high frequency of CPV infection in this area. In contras only 2 samples were positive from 165 healthy dogs. They were perhaps due to asymtomatic infection or these dog had just been affected with parvovirus.

Serological analysis of isolates carried out between 1978 and 1984 (Parrish *et al.*, 1985 and Parrish *et al.*, 1988) in 7 areas of United State, Denmark, France, Belgium, and Australia by monoclonal antibodies clearly distinguished two strain of virus, each was specific for either the old type and new type respectivdely. The old type was detected up to 1982 and since then was replaced by the new type virus. Other report (Senda., 1989) in Japan suggested that strains isolated since 1983 were all classified as the new type with the HA activity showing temperature-independent HA. All isolates prensented here revealing the new type canine parvovirus. It suggested that the replacement of CPV strains has also been found in Indonesia.

ACKNOWLEDGEMENTS

We wish to thank Dr. Megumi Senda and Dr. Tadashi Sugimori for donating of CRFK cell line and refernce of virus. Support was received from Japan International Cooperation Agency (JICA), Shinjuku-ku, Tokyo, 163 Japan.

Table 1. Detection of CPV from fecal material in healthy dogs.

Area	No. of fecal test	No. of positive
Jakarta	57	–
Bogor	10	–
Bandung	21	1
Tasikmalaya	20	1
Madiun-Nganjuk	19	–
Toraja	10	–
Medan	28	–
Total	165	2

Table 2. Detection of CPV from fecal material in clinically ill dogs in Jakarta area.

No.	Code of test	Age	HA activity (2nd passage)	HA activity in feces
1.	58*	3 months	+	+
2.	26	6 months	+	+
3.	27	4 months	+	+
4.	F	4 months	+	ND**
5.	1	4 months	+	ND**
6.	2	1 year	–	–
7.	3	10 months	–	–
8.	4	3 years	–	–
9.	5	3 years	+	+
10.	6	2 years	–	–
11.	7	1 year	+	+
12.	8	15 years	–	–
13.	9	4 months	+	+
14.	10	4 months	+	ND**
15.	11	2 months	+	ND**

* Fecal materials and intestines (dog was died)

** ND, not determined.

Table 3. Typing of CPV isolation by temperature dependence of HA reaction pattern with conventional sera and MAbs.

Code of CPV isolates	Temperature dependence	HI test: Conpentional sera positive	HI test: Conpentional sera negative	HI test: MAbs CIDI	HI test: MAbs A2F8	HI test: MAbs B3B11
94	−	+	−	+	+	−
113	−	+	−	+	+	−
58	−	+	−	+	+	−
26	−	+	−	+	+	−
27	−	+	−	+	+	−
F	−	+	−	+	+	−
1	−	+	−	+	+	−
5	−	+	−	+	+	−
7	−	+	−	+	+	−
9	−	+	−	+	+	−
10	−	+	−	+	+	−
11	−	+	−	+	+	−
Reference virus						
Cp82031 (old type)	+	+	−	−	+	+
Cp Y−1 (new type)	−	+	−	+	+	−

REFERENCES

Carmichael L.E., Joubert J.C., and Pollock R.V.H. (1980). Hemagglutination by Canine Parvovirus : Serologic Studies and Diagnosis Applications. *Am. J. Vet. Res.* **41**, 784–791.

Directorate of Animals Health, (1981). Pedoman Pengendalian Penyakit Hewan Menular. Dir. Gen. of Liv. Ser. Dep. of Agr. III, 59–61.

Kramer J.M., Meumer P.C., and Pollock V.H, (1980). Canine parvovirus Up date. Vet. Med. Small Animal Clinicians, 1542–1547.

Parrish C.R., Carmichael L.E., and Antczak, (1982). Antigenic Relationships Between Canine Parvovirus Type 2, Feline Panleukopenia Virus and Mink Enteritis Virus Using Conventional antisera and Monoclonal antibodies. *Archives of Virology* **72**, 267–278.

Parrish C.R., O'Connell P.N., Evermann J.P., and Carmichael L.E. (1985). Natural Vermation of Canine Parvovirus. Science 230, 1046–1048.

Parrish C.R., Have. P., Forcyt W.J., Evermann J.F., Senda M., and Carmichael L.E., (1988). The Global Spread and Replacement of Canine Parvoviruses strains. *J. Gen. Virol* **69**, 1111–1116.

Senda M., Hirayama N., Yamamoto., and Kurata K. (1986). An Improved Hemagglunation Test for Study of Canine Parvovirus. *Vet. Micr.* **12**, 1–6.

Senda M., Ohishi K., Hirayama N., and Yamamoto H. (1987). Detection of Parvovirus in Cell Culture. *Ann. Rep. Natl. Vet. Assay Lab.* **24**, 11–14.

Senda M., Hirayama N., Itoh O., and Yamamoto H. (1988). Canine Parvovirus : Strain Difference in Haemagglutination Activity and Antigenicity. *J.Gen. Vir.* **69**, 349–354.

Senda M., (1989). Studies on Canine Parvovirus Haemagglutination. Abstract of the thesis. *Nat. Vet. Assay Lab. Kokubunji, Tokyo* **185** : 4–6.