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Departments of Virology, The National Veterinary Institute,
and The Faculty of Veterinary Medicine,
The Swedish University of Agricultural Sciences, Biomedicum,
Uppsala, Sweden

Diagnostic Experience from an Epidemic of Canine Parvoviral Enteritis

By

B. KLINGEBORN and J. MORENO-LÓPEZ

With 2 figures and one table

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Introduction

In 1978 a new disease of dogs — canine parvoviral enteritis — emerged in some countries with first exhaustive descriptions from the United States (1, 4, 7). Even if, at the beginning, a coronavirus has been found in feces (1), the predominant virus was a parvovirus, serologically different from the "minute virus of canines (MCV)" (6), also a parvovirus known since 1970 (3).

The new disease of dogs has similarities to feline panleukopenia (1, 7), and the canine parvovirus (CPV) involved was found to be serologically closely related to feline panleukopenia and mink enteritis virus (2, 4). It is anticipated that the CPV is a mutant of feline panleukopenia virus (6), but a comparative analysis of these viruses has yet to be made.

In the winter months of 1979/80 a huge epidemic of canine enteritis occurred in Sweden in connection with a dog exhibition, attacking mainly young dogs. Thousands of dogs were taken ill. The epidemic was suspected to be canine parvoviral enteritis and therefore feces and serum samples were submitted to virological examination. The rapid diagnosis was favored following CARMICHAEL's procedure of detecting the CPV hemagglutinin in feces and the antihemagglutinin in serum (6). To this we added electron microscopy and immune electron microscopy of particles in fecal samples, immunodiffusion and counterimmunoelectrophoresis. The present report is a summary of experience using these procedures.

Material and Methods

(Some abbreviations: HA, hemagglutination; HI, hemagglutination inhibition; EM, electron microscopy; ID, immunodiffusion)

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Dogs

All the dogs examined were hospitalized in two animal clinics. Most of these dogs showed signs of severe gastroenteritis, i. e., vomiting, anorexia, diarrhea and rapid dehydration. On the basis of these signs and lymphocytopenia the disease was suspected to be canine parvoviral enteritis.

Feces and serum

The feces and/or serum samples were collected during the acute stage of illness. In the tests described below, the fecal samples were used as supernatants of 10 to 20 % suspensions in PBS-D (see below) after centrifugation at 16,300 x g. and 4 °C. If necessary, the supernatants were kept at − 70 °C until tested.

HA- and HI-tests

For these, the original procedure (6) was modified as follows. The erythrocytes were from newborn or 3 week-old piglets instead of from adult pigs. These latter erythrocytes usually showed spontaneous agglutination. The diluent was Dulbecco's PBS devoid of $CaCl_2$ and $MgCl_2$ (PBS-D). Using this solution the results of HA- and HI-tests became more clearcut in a shorter time (1 to 2 h at 2 to 4 °C). The HA by fecal samples diluted 1 : 64 or higher proved to be specific; at lower dilutions the HA was mostly non-specific. In the HI-tests the routine antigen was a fecal sample with an HA titer of 1 : 2,048. The HI titers were specific first at a dilution of 1 : 160; in a few serum samples non-specific inhibitors of HA were present up to the dilution of 1 : 80. The specificity of reactions was checked with a reference CPV strain (Pearl) and a reference antiserum, both kindly supplied by Dr. L. E. CARMICHAEL.

EM and immune EM

Supernatants of fecal suspensions (see above) were examined in a Philips EM 300 electron microscope by negative staining with 2 % neutralized phosphotungstic acid (PTA). For immune EM, 50 μl. of the reference antiserum diluted 1 : 10 or 1 : 50 in phosphate buffer, pH 7.0, was mixed with 50 μl. of a supernatant with an appropriate number of virus particles. The mixture was incubated for 1 h at 37 °C. A drop of this mixture was then mounted on carbon formvar grids, the grids gently rinsed with double-distilled water, stained with 2 % PTA and examined.

ID-tests

The original method of double diffusion in Petri dishes as well as WADSWORTH's modification (12) were used. To prepare the gel, Special agar-Noble (Difco) was dissolved in TEN buffer as described elsewhere (8). Counterimmunoelectrophoresis was carried out in the Millipore System.

Results

Fecal samples from a total of 58 hospitalized dogs were examined in HA-tests and 15 (25.9 %) showed titers between 1 : 64 and 1 : 2.048 (Table 1).

Table 1

Diagnosis of canine parvoviral enteritis by tests on fecal samples from diseased, hospitalized dogs

Animal hospital	CPV or its antigen in feces and number of dogs			
	HA titer		EM	
	≥1 : 64	<1 : 64	+	−
A 23 dogs	8	15	10	13
B 35 dogs	7	28	18	17
Total 58 dogs	15	43	28	30

HA, hemagglutination; EM, electron microscopy.

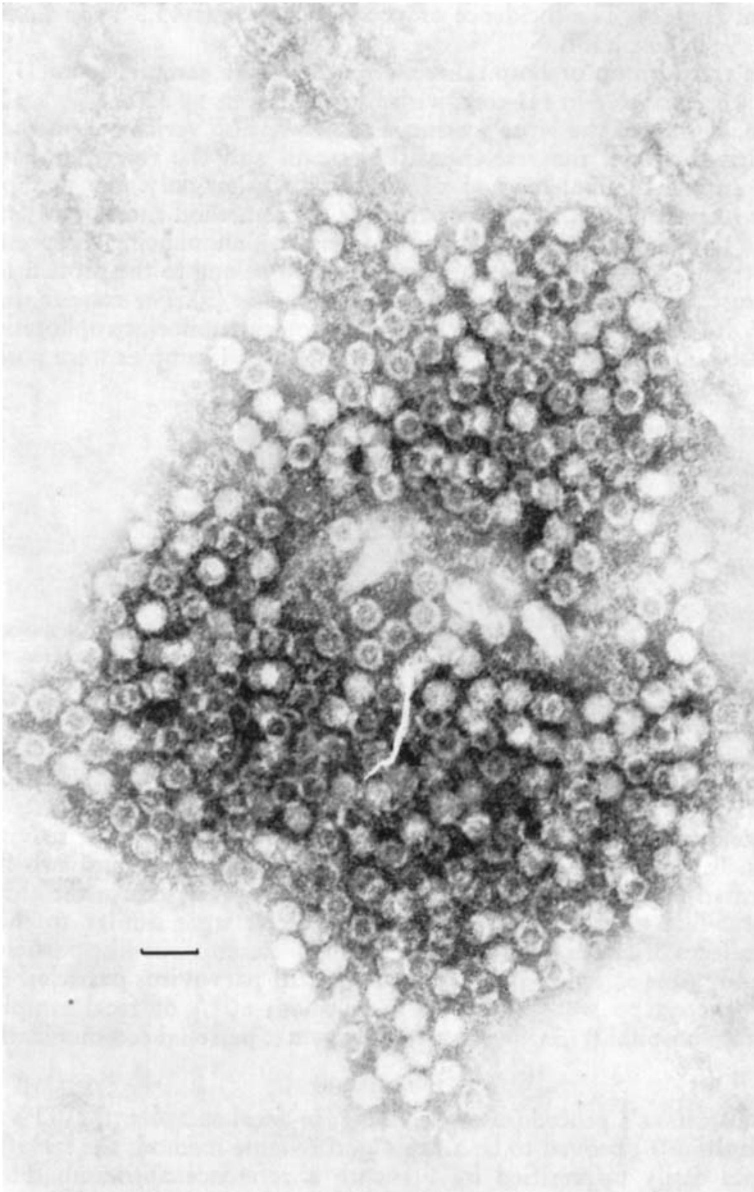

Fig. 1. Immune electron microscopy. Aggregated particles in a mixture with reference antiserum. Magnification × 194,000. Bar = 37 nm

The specificity of HA was confirmed with reference antiserum in HI-tests. The incidence of positive dogs was 34.8 % in hospital A and 20 % in hospital B.

The same fecal samples as above were also examined by EM and 28 (48.3 %) were positive (Tab. 1). The positive samples showed the presence of either several or numerous particles of parvoviral size, some of them being empty. For immune EM, six positive samples were chosen. The particles present therein were found to be aggregated after addition of reference

antiserum (Fig. 1). The incidence of positive dogs was 43.5 % in hospital A and 51.4 % in hospital B.

In a third group of hospitalized dogs the serum samples from 17 out of 19 dogs were positive in HI-tests with titers between 1 : 320 to ≧ 1 : 20.480.

The identity of the virus present in feces was also verified by ID using an HA-positive sample, the reference CPV strain and the reference antiserum (Fig. 2). In the original method of double diffusion only one precipitation line was formed (Fig. 2). In WADSWORTH's micromethod there were one very faint and two strong lines of identity (not shown), announcing the presence of three antigens. Three polypeptides are known to belong to the protein of some parvoviruses, e. g. the virus of feline panleukopenia (9). For routine diagnosis of CPV in the feces, the ID-test and counterimmunoelectrophoresis were found to be insensitive, i. e., only five of a total of 54 samples were positive in both these tests (data not shown).

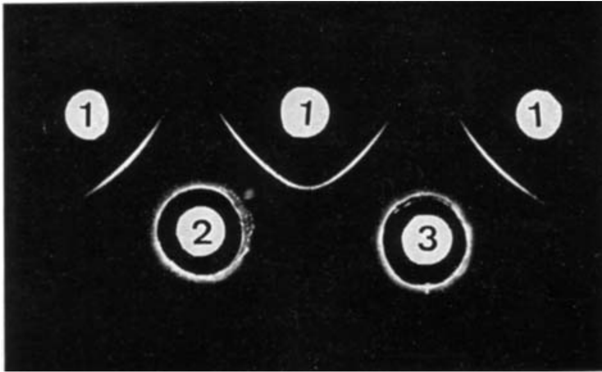

Fig. 2. Immunodiffusion test. The reference antiserum (1) was tested against the reference CPV strain (2) and an HA-positive fecal sample (3). A single line of identity was formed

In one of the positive fecal samples we also found, by EM, reovirus-like particles. In three further samples, one from a healthy bitch and two from its two diseased puppies, so-called "fringed" particles were seen in the absence of parvovirus-like particles. The "fringed" particles were similar to those observed in feces of calves with diarrhea (10, 11). Enterovirus-like particles were also seen in some samples; they were larger than parvovirus particles. Finally, *Campylobacter* spp were cultivated from about 60 % of fecal samples examined from hospital B (SANDSTEDT and HURVELL, personal communication).

Discussion

CARMICHAEL's procedure of detecting, in fecal samples, the CPV via its hemagglutinin (6) proved to be a rapid and reliable method; the specificity of HA could easily be verified by HI with a reference antiserum. However, using this procedure the percentage of positive dogs was low when compared with that detected by EM, i. e., 25.9 versus 48.3 %. On the other hand, in several samples the number of viral particles might have been too small to give HA or the particles to be identified as CPV by immunoaggregation. The presence of another parvovirus of dogs, the MCV (3) instead of CPV, is conceivable. Besides, in many routine laboratories an electron microscope is not available. The procedure of cultivating the CPV from feces in cell cultures proved to be time-consuming and unreliable (data not shown).

The infection of dogs with CPV is followed by development of an antibody response reaching comparatively high levels during the acute stage of illness. This could easily be demonstrated in HI-tests against a reference CPV strain.

For the HA- and HI-tests the erythrocytes from piglets were found to be much more suitable than those from older pigs whose erythrocytes tend to agglutinate spontaneously. The reason for this is unknown to us, but a changed charge in the membrane of erythrocytes from older pigs might be responsible.

It is not known to what extent the severity of enteritis was influenced by concomitant infections with *Campylobacter* spp. These bacteria are known to occur in dogs which can be the source of infection and enteritis in man (5). Exceptionally reovirus-like and "fringed" particles were seen in the feces; their possible role as disease agents is unknown.

Summary

Hemagglutination (HA) test and electron microscopy (EM) were used to diagnose canine parvoviral enteritis on fecal samples from 58 hospitalized dogs of a huge epidemic. By HA-tests the presence of the canine parvovirus (CPV) involved was shown in samples from 15 dogs (~ 26 %). A reference antiserum was used to identify the virus of each sample by hemagglutination inhibition (HI). By EM parvovirus-like particles were seen in samples from 28 dogs (~ 48 %). Particles in six samples were identified as CPV by immunoaggregation. Sera from 17 out of 19 dogs examined showed specific HI titers during the acute stage of illness. A high incidence of concomitant infection with *Campylobacter* spp was found by the bacteriologists.

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Zusammenfassung

Diagnostische Erfahrungen während einer Epidemie von Parvovirus-Enteritis der Hunde

Während einer grossen Epidemie von Parvovirus-Enteritis der Hunde (in Schweden) wurde der Nachweis von Virus in Kotproben mit Hilfe der Hämagglutination (HA) und Elektronenmikroskopie (EM) versucht; die Kotproben stammten von 58 hospitalisierten Hunden. Mit dem HA-Test ließ sich das ursächliche Parvovirus in den Proben von 15 Hunden nachweisen (= ~ 26 %). In jeder von diesen Proben wurde das Virus durch Hämagglutination-Hemmung (HAH) mit einem Referenzserum identifiziert. Mit der EM waren Parvovirus-ähnliche Partikel in den Proben von 28 Hunden zu finden (= ~ 48 %). In 6 von diesen Proben wurden die Partikel mittels der Immunaggregation als das ursächlichen Parvovirus identifiziert. In 17 Serumproben von 19 untersuchten Hunden wurden spezifische HAH-Titer während der akuten Krankheitsphase festgestellt. Die Bakteriologen wiesen ein gehäuftes Vorkommen von gleichzeitiger Infektion mit *Campylobacter* spp nach.

Résumé

Expériences de diagnostic durant une épidémie d'entérite à Parvovirus chez des chiens

On a recherché la mise en évidence du virus dans des matières fécales à l'aide de l'hémagglutination (HA) et de la microscopie électronique (EM) durant une forte épidémie d'entérite à Parvovirus chez des chiens en Suède. Les échantillons d'excréments provenaient de 58 chiens hospitalisés. Le Parvovirus a été mis en évidence par test HA dans les échantillons de 15 chiens

(~ 26 %). Le virus a été identifié dans chacun de ces échantillons au moyen de l'inhibition de l'hémagglutination (HAH) avec un sérum de référence. On a trouvé des particules identiques au microscope électronique dans les échantillons de 28 chiens (~ 48 %). Les particules dans 6 de ces prélèvements furent identifiées comme Parvovirus au moyen de l'immunoaggrégation. Un titre HAH spécifique a été établi durant la phase aiguë de la maladie dans 17 échantillons sérologiques sur 19 chiens examinés. La bactériologie a montré la présence fréquente d'une infection simultanée à *Campylobacter* spp.

Resumen

Experiencias en el diagnóstico de una epidemia de parvovirus-enteritis en caninos

Mediante pruebas de hemaglutinación y microscopía electrónica se diagnosticó parvovirus-enteritis en caninos en muestras fecales de 58 caninos hospitalizados a raiz de una extensa epidemia. Con la prueba de hemaglutinación se detectó la presencia de virus en muestras fecales de 15 caninos (~ 26 %). Un antisuero de referencia se utilizó para la identificación del virus (prueba de inhibición de la hemaglutinación). Con microscopía electrónica se identificó parvovirus en muestras fecales de 28 caninos (~ 48 %). El virus fué identificado en seis muestras fecales utilizando la prueba de inmunoagregación. Sueros de 17 de 19 caninos examinados demostraron títulos específicos de inhibición de la hemaglutinación durante la etapa aguda de la enfermedad. Se demostró también una alta incidencia de infección concomitante con *Campylobacter* spp.

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Address of authors: Dr. BERNDT KLINGEBORN, Dept. of Virology, Biomedicum, P. O. Box 585, S-75123 Uppsala/Sweden.