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The use of enzyme-linked immunosorbent assay systems for serology and antigen detection in parvovirus, coronavirus and rotavirus infections in dogs in The Netherlands

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ABSTRACT

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Complex trapping blocking (CTB) enzyme-linked immunosorbent assays (ELISAs) and indirect ELISAs for the detection of antibodies to canine parvovirus (CPV), canine coronavirus (CCV) and rotavirus in sera of dogs were established. Double antibody sandwich ELISAs for the detection of CPV-, CCV- and rotavirus antigens in fecal samples were also developed. Both the serological and antigen-detection ELISAs were used to screen samples from dogs in The Netherlands, with or without a history of acute diarrhea. It was shown that the results of the respective serological ELISAs correlated well and that CPV was the major cause of virus-induced acute diarrhea in dogs in The Netherlands.

INTRODUCTION

Three different viruses, canine parvovirus (CPV), canine coronavirus (CCV) and rotavirus, have been shown to be associated with acute gastrointestinal disease and diarrhea in the dog (Keenan et al., 1976; Appel et al., 1978; Appel et al., 1979a,b; Eugster and Sidwa, 1979; England and Poston, 1980; Evermann et al., 1980; McNulty et al., 1980; Osterhaus et al., 1980b; Vandeberghe et al., 1980; Williams, 1980; Fulton et al., 1981; Johnson et al., 1983). The relative role of each of these viruses in causing gastrointestinal disease in this species has so far attracted limited attention (Osterhaus et al.,

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1980a). This is partly due to the lack of fast and reliable serological and virological assay systems for the screening of large number of serum and fecal samples.

The methods developed for the quantitation of serum antibodies against these viruses, such as the virus neutralization (VN), immunofluorescence (IF), haemagglutination inhibition (HI) and complement fixation (CF) assays, are laborious and unsuitable for inclusion in automated screening procedures. This also holds true for the tests used in the detection of these viruses or their antigens in fecal samples, such as virus isolation, haemagglutination (HA) and electron microscope (EM) procedures.

In the present paper we describe the comparison of newly developed complex trapping blocking (CTB) enzyme-linked immunosorbent assays (ELISA), that have been applied successfully for the detection of antibodies to bovine leukemia virus and hantaanvirus (De Boer et al., 1987; Groen et al., 1989), and indirect ELISA systems for the detection of serum antibodies against CPV, CCV and rotavirus, and the use of double antibody sandwich ELISA (DAS-ELISA) systems for the detection of viral antigens in fecal samples from dogs. A selected panel of serum and fecal samples from different dog populations in The Netherlands, with or without a history of acute diarrhea, was therefore assayed in the respective ELISA systems. Results obtained in the two serological ELISAs, which are based on different principles, showed a good correlation. These data and the results obtained in the antigen ELISAs provide evidence that CPV is the major cause of viral gastrointestinal disease in dogs in The Netherlands. CCV infections were also shown to occur more frequently in dogs with a history of gastroenteritis and diarrhea. No indications that rotavirus infections are important in this respect were found.

MATERIALS AND METHODS

Serum samples of dogs

Serum samples from 105 dogs in kennels with recurrent problems of acute gastrointestinal disease and diarrhea in The Netherlands in 1987 were provided by Dr. K. Weijer, EVL B.V., Amsterdam. Serum samples from 98 dogs in the open dog population of The Netherlands, without apparent gastrointestinal disease or diarrhea, and serum samples of 48 specific pathogen-free (SPF) dogs (Harlan/Olac Centraal Proefdier Bedrijf, Zeist, The Netherlands), free from all known canine viruses (unpublished observation), were also collected in 1987. The CPV vaccination status of the dogs in kennels with recurrent acute diarrhea is assumed to be similar to that of dogs in the open dog population, since a general vaccination strategy is advised in The Netherlands. This consists of a first vaccination between 8 and 12 weeks of age, a second vaccination between three and four months, and annual revaccination.

Fecal samples of dogs

Fecal samples or rectal swabs ($n=223$) were collected from dogs with apparently infectious acute diarrheal disease. These samples had been submitted to the Regional Animal Health Service Centre, Zwolle, The Netherlands. Fecal samples were also collected from dogs in the open dog population without problems of diarrhea ($n=75$), and from SPF dogs ($n=48$).

Antigen preparations

CPV (strain C780916, Carmichael et al., 1981) was propagated in A-72 cells as described (Rimmelzwaan et al., 1987). Supernatant of infected A-72 cells showing complete cytopathic changes (HA titre ≥ 512) was used as the antigen preparation in the respective ELISA systems.

For the preparation of CCV antigen used in the indirect ELISA (see below), a canine coronavirus strain initially described by Binn et al. (1975) was used. Feline whole fetus cells (FCWF) (Pedersen et al., 1981) were infected, and when cytopathic changes were almost complete the culture medium was decanted, and the cells were washed with PBS and harvested with a rubber policeman. The cells were subsequently washed and pelleted by low-speed centrifugation. The resulting pellet was resuspended in PBS and disrupted by ultrasonic treatment. The suspension was clarified by low-speed centrifugation and the supernatant was used as antigen in the indirect ELISA.

In the CTB-ELISA a closely related coronavirus, transmissible gastroenteritis virus (TGEV) (Horzinek et al., 1982) was used as antigen (titer in antigen ELISA = 320). The antigen was prepared by infection of PK-15 cells essentially as described for the preparation of CCV antigen.

Rotavirus antigen (titer in antigen ELISA = 640) used in the indirect ELISA was prepared essentially as described by Ghose et al. (1978), using rotavirus strain SA-11 (ATCC VR 899).

For the CTB-ELISA, newborn calf diarrhea rotavirus (NCDV strain) was used after passage in fetal bovine kidney cells cultured in Hanks' medium containing 0.5% lactalbumin, 10% fetal calf serum (FCS), 100 IU penicillin/ml, 100 μg streptomycin/ml and 0.05% sodium carbonate. Seven days after the first cytopathic changes were observed, culture flasks (150 cm^2) were frozen and thawed. The suspension was pelleted by low-speed centrifugation, and the pellet was resuspended in culture medium (4 ml per culture flask) and disrupted by ultrasonic treatment for 1 min. Cell debris was pelleted by low-speed centrifugation. The supernatant was heat-inactivated for 30 min at 65°C, and used as the antigen source. The antigen (titer in antigen ELISA = 640) was lyophilized and stored at 4°C until use.

ELISA techniques for the detection of virus-specific antibodies

CTB-ELISA for the detection of CPV-specific antibodies. Microtiter plates (Titertek, Flow Laboratories) were coated with two monoclonal antibodies (mAbs) (H-1 and H-2), purified by protein A-Sepharose chromatography (Pharmacia, Uppsala, Sweden); these mAbs recognize two different epitopes of CPV (Rimmelzwaan et al., 1987). Volumes of 0.1 ml containing 250 ng of each mAb in carbonate buffer, pH 9.6, were incubated in microtiter plates for 16 h at 4°C. 50 μl of serial twofold dilutions of dog serum samples, starting at 1:5, were incubated simultanously with 50 μl of CPV-containing culture supernatant (HA titer = 1024) of infected A-72 cells at an optimal dilution for 16 h at 20°C. Remaining binding of CPV was detected by incubation with horseradish peroxidase (HRP)-conjugated mAbs H-1 and H-2. All dilutions were made in PBS containing 0.05% Tween 80, 1% BSA and 1 M NaCl. The plates were washed twice after each incubation. All optimal dilutions of the reagents used in all the ELISA systems were determined by checkerboard titration. 0.1 ml substrate solution (0.1 mg/ml tetramethylbenzidine (TMB) (Sigma, St. Louis, U.S.A.) and 0.003% H_2O_2 in 0.1 M NaAc buffer, pH 5.5) was added to each well. After 10 min incubation at room temperature, 0.1 ml of 2 M H_2SO_4 was added to stop the color reaction. Absorbance at 450 nm was read in a Titertek Multiscan (Flow Laboratories). The test samples were considered to be positive if a reduction of absorbance at 450 nm of 50% or more was observed.

Indirect ELISA for the detection of CPV-specific antibodies. This has been described previously (Rimmelzwaan et al., 1990). Briefly, plates were coated with the CPV-specific monoclonal antibodies H-1 and H-2 as described above. CPV from culture supernatant of infected A-72 cells was allowed to bind to the mAbs. Serial twofold dilutions of dog serum samples were incubated, and CPV-binding immunoglobulins (Ig) were detected with a goat anti-dog IgG and IgM antibody preparation conjugated to HRP.

CTB-ELISA for the detection of coronavirus-specific antibodies. The CTB-ELISA for the detection of CCV-specific antibodies was essentially performed as described for the detection of CPV-specific antibodies. The Ig fraction was protein A-purified from sera of cats with high antibody titers against feline infectious peritonitis virus (FIP), another closely related coronavirus (Horzinek et al., 1982). These sera were shown to be negative for antibodies to rotavirus and CPV. A part of this Ig preparation was conjugated to biotin by incubating the antibody solution, which was dialysed against 0.1 M $NaCO_3$, pH 8.3, (1 mg protein/ml), with N-hydroxysuccinimido biotin (Sigma) sol-

ubilized in dimethyl sulfoxide (1 mg/ml) at a biotin:protein ratio of 1:8 (*w/w*) for 4 h at room temperature. The conjugates were dialysed against PBS and stored at −20°C.

Microtiter plates (Titertek) were coated with the anti-FIP antibody preparation in 0.1 M carbonate buffer, pH 9.6, for 16 h at 20°C in 0.1-ml volumes. Then 50-μl volumes of twofold serial dilutions of dog serum samples, starting at 1:5, were added simultaneously with 50 μl of a preparation of TGEV. After incubation for 16 h at 20°C, the remaining binding of TGEV was detected, using a cat anti-FIP virus antibody preparation conjugated to biotin. After incubation for 2 h, HRP-bound streptavidin (Amersham International, Amersham, Great Britain) was allowed to bind to biotin for 30 min at 37°C. Plates were developed as described, using TMB as a substrate.

Indirect ELISA for the detection of coronavirus-specific antibodies. Microtiter plates were coated for 16 h at 20°C with 50-μl volumes of CCV antigen diluted in 0.1 M carbonate buffer, pH 9.6. Plates were blocked with PBS containing 0.05% Tween, 1% BSA and 1 M NaCl for 1 h at 37°C. 50-μl volumes of twofold serial dilutions of dog serum samples, starting at 1:50, were added and incubated for 1 h at 37°C. Goat anti-dog IgG (Cappel, Cooper Biomedical, U.S.A.) and goat anti-dog IgM (Kirkegaard and Perry, U.S.A.) HRP conjugates were used to detect CCV-bound Ig. The plates were washed twice after each incubation and developed as described above.

CTB-ELISA for the detection of rotavirus-specific antibodies. This was essentially performed as described for the detection of CPV-specific antibodies. Briefly, microtiter plates (Titertek) were coated with protein A-purified Ig from a rotavirus-specific rabbit antiserum in 0.1 M carbonate buffer, pH 9.6, for 16 h at 20°C in 0.1-ml volumes. 50-μl volumes of twofold serial dilutions of dog serum samples, starting at 1:5, were added together with 50 μl of a preparation of a rotavirus antigen, and incubated for 16 h at 20°C. Remaining binding of antigen was detected by purified Ig from a monospecific rabbit anti-rotavirus antiserum conjugated to biotin. After incubation for 1 h at 37°C, HRP-bound streptavidin (Amersham International) was allowed to bind to biotin for 30 min at 37°C. The plates were developed as described above. Test samples were considered to be positive if a reduction of 50% or more was observed.

Indirect ELISA for the detection of rotavirus-specific antibodies. Microtiter plates (Titertek) were coated for 16 h at 20°C with 50-μl volumes of rotavirus antigen or with control antigen, diluted equally in 0.1 M carbonate buffer, pH 9.6. After blocking the plates with PBS containing 1% BSA, 0.05% Tween and 1 M NaCl, 50-μl volumes of twofold serial dilutions of serum samples were added in blocking buffer, starting at 1:50. Antibodies binding to rota-

virus were detected using a combination of a goat anti-dog IgG (Cappel) and a goat anti-dog IgM preparation (Kirkegaard and Perry) conjugated to HRP. After each incubation the plates were washed twice with demineralized water containing 0.05% Tween. Plates were developed as described above. Dilutions giving absorbance values at 450 nm at least three times higher than background values obtained with control antigen-coated plates were considered to be positive. Endpoint titers were determined by using a dilution giving a reduction in optical density at 450 nm of at least 0.1.

DAS-ELISAs for the detection of viral antigens in feces

Plates were coated with anti-CPV monoclonal antibodies, cat anti-FIP virus or rabbit anti-rotavirus antibodies, as described above for the detection of CPV, CCV and rotavirus in feces respectively. For the detection of CPV antigen, 50-μl volumes of a mixture of two HRP-conjugated anti-CPV mAbs was used as described (Rimmelzwaan et al., 1987). For the detection of CCV or rotavirus antigens, 50-μl volumes of fecal samples diluted 1 : 10 were incubated for 1 h in the antibody-coated plates. Rotavirus was detected with a rabbit anti-rotavirus antibody preparation conjugated to biotin. Coronavirus was detected using a biotinylated cat anti-FIP virus antibody preparation. All antibody preparations were used at dilutions as determined for use in the CTB-ELISAs. Plates were washed twice after each incubation and developed as described. To confirm positive reactions, a CTB-ELISA was performed with these samples using monoclonal antibodies (CPV) or monospecific antisera to these viruses (CCV or rotavirus) and the fecal samples as the source of antigen.

RESULTS

Detection of CPV-specific serum antibodies

The 105 serum samples from the dogs in kennels with recurrent acute gastrointestinal disease and diarrhea, the 98 serum samples from dogs in an open dog population without apparent diarrhoea and the 48 serum samples from the SPF dogs were assayed for their levels of CPV-specific antibody in the CTB-ELISA and in the indirect ELISA (Fig. 1). In the CTB-ELISA, 95 (91%) of the samples from dogs in kennels with problems of gastrointestinal disease and diarrhea scored positive, with titers ranging from 10 to ≥ 1280; most were > 320. Although there was a correlation between the results of this ELISA and the indirect ELISA, in which titers ranged from 50 to $\geq 110\,000$, the indirect test, proved more sensitive overall, since it generally gave 10–20 times higher titers and detected seven low-titer positive samples (7%) that were negative in the CTB-ELISA (Fig. 1a). When 98 samples of sera from dogs without problems of diarrhea were tested (Fig. 1b), 77 of the samples (79%) scored

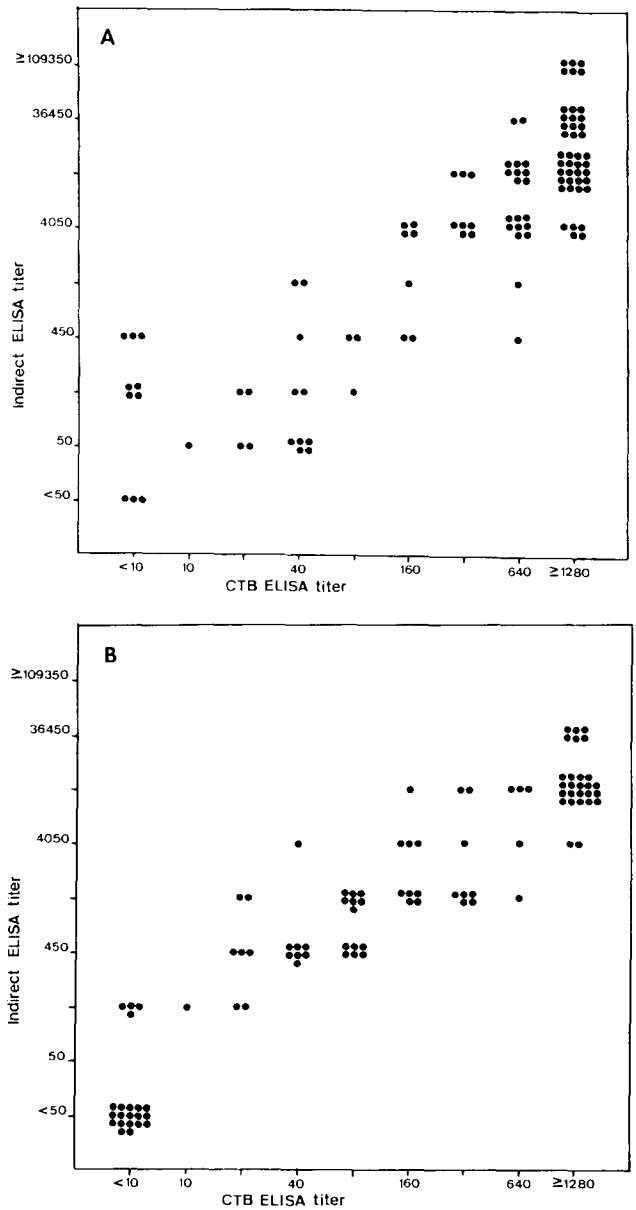

Fig. 1. CPV-specific antibody titers in sera from dogs in kennels with recurrent acute gastrointestinal disease and diarrhea (A) and dogs in an open population without diarrhea (B), measured by CTB-ELISA and by indirect ELISA.

positive in the CTB-ELISA, with titers ranging from 10 to > 1280 (most were > 320). Again a correlation was obtained between results of this ELISA and the indirect ELISA. The latter detected four more low-titer samples (4%). In both ELISA systems the serum samples from all 48 SPF dogs proved to be negative (not shown).

The overall correlation between positive versus negative results obtained in both assays was 96%. Also, the titers obtained in the two ELISA systems correlated well (correlation coefficient = 0.91). The titers obtained in the indirect ELISA were approximately 20 times higher than those obtained in the CTB-ELISA.

Detection of coronavirus-specific serum antibodies

The serum samples tested for the presence of CPV-specific antibodies were also tested for the presence of CCV-specific antibodies by CTB-ELISA and by indirect ELISA. In the CTB-ELISA, 64 (61%) of the samples from the problem kennels scored positive, with titers ranging from 10 to > 1280 (Fig. 2a). There was a correlation between this test and the indirect ELISA, with only a small number of discrepant serum samples showing a relatively low titer in one of the assays: five CTB-ELISA-negative serum samples displayed a titer of 50 in the indirect ELISA, and two indirect ELISA negative samples showed a titer of 10 or 20 in the CTB-ELISA. When 98 sera from dogs of the open dog population without problems of gastrointestinal disease or diarrhea were tested, 44 (45%) scored positive in the CTB-ELISA, with titers ranging from 10 to 320 (Fig. 2b). The serum samples of the 48 SPF dogs all scored negative in both ELISA systems (not shown). The overall correlation between positive versus negative results obtained in both assay was 92%. The titers obtained in the two ELISAs also correlated well (correlation coefficient = 0.90). The titers obtained in the indirect ELISA were approximately four times higher than those obtained in the CTB-ELISA.

Detection of rotavirus-specific serum antibodies

When tested for the presence of rotavirus-specific antibodies in the CTB-ELISA, 96 (91%) of the 105 serum samples from the dogs in the problem kennels scored positive, with titers ranging from 10 to > 1280. Also in this case there proved to be a correlation with the results obtained with the indirect ELISA (Fig. 3a). Only six samples that were positive in the CTB-ELISA, most of them showing low titers, scored negative in the indirect ELISA system. Of the 98 serum samples from the dogs in kennels without diarrhea, 82 (84%) proved to contain antibodies to rotavirus when tested in the CTB-ELISA (Fig. 3b); 79 (81%) of these samples also scored positive in the indirect ELISA.

All the serum samples of the 48 SPF dogs were also negative in both these ELISA systems (not shown). The overall correlation between positive versus negative results obtained in both ELISAs was 96%. Again, the titers obtained

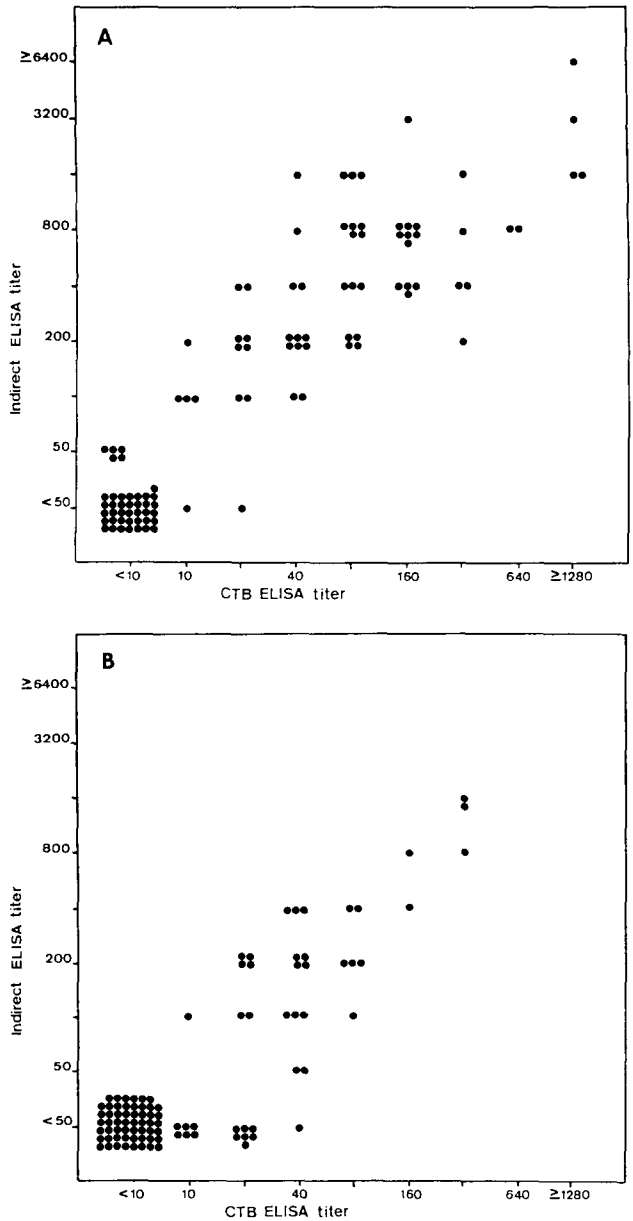

Fig. 2. CCV-specific antibody titers in sera from dogs in kennels with recurrent acute gastrointestinal disease and diarrhea (A) and dogs in an open population without diarrhea (B), measured by CTB-ELISA and by indirect ELISA.

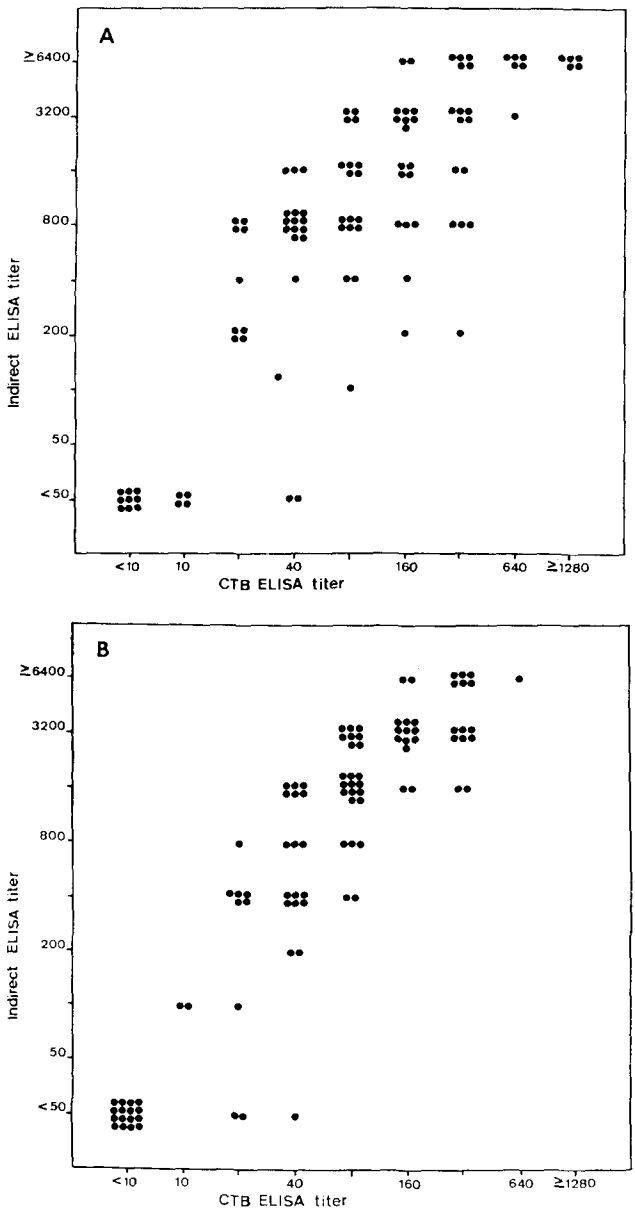

Fig. 3. Rotavirus-specific antibody titers in sera from dogs in kennels with recurrent gastrointestinal disease and diarrhea (A) and dogs in the open population without diarrhea (B), measured by CTB-ELISA and by indirect ELISA.

in both ELISAs also correlated well (correlation coefficient=0.86). The titers obtained in the indirect ELISA were approximately ten times higher than those obtained in the CTB-ELISA.

Detection of viral antigens in fecal samples

The fecal samples from SPF dogs and from dogs in the open population with and without problems of diarrhoea were tested in the antigen ELISA systems for the detection of CPV, CCV and rotavirus antigens respectively (Fig. 4). In the CPV and CCV antigen ELISAs, samples scored positive when at least 12 or 20 $TCID_{50}$ of the respective viruses proved to be present. The detection level of rotavirus antigen in the ELISA was equivalent to about five complement-fixing units, as measured in the complement fixation assay. Of the 223 fecal samples from dogs with acute gastrointestinal symptoms or diarrhea, 82 were positive in the CPV antigen ELISA (37% of dogs with diarrhea). Similarly, 24 fecal samples from dogs with diarrhea were positive in the CCV antigen ELISA (11% of dogs with diarrhea). Sixteen samples scored positive for the presence of both CPV and CCV antigen (7%). In none of the samples could rotavirus antigen be detected in the ELISA. All the fecal samples from the SPF dogs scored negative in the three antigen ELISAs (Fig. 4). When 75 fecal samples from dogs in the open dog population without diarrhea were tested, all samples also scored negative in the antigen ELISAs for the detection of CPV and rotavirus antigen. However, five (7%) of these fecal samples were positive for CCV antigen ELISA. The specificity of all positive samples was confirmed in CTB-ELISAs, using specific antibody preparations against the respective viruses.

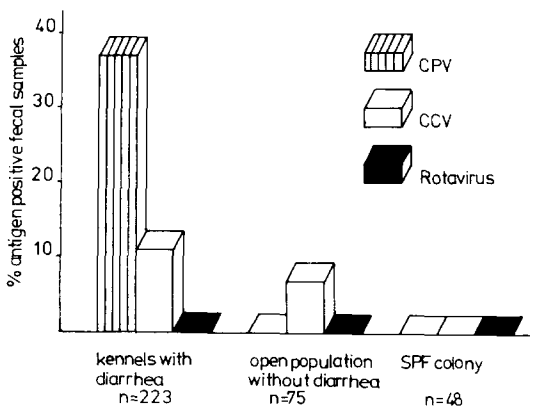

Fig. 4. Detection of CPV antigen (hatched bars), CCV antigen (open bars) and rotavirus antigen (solid bars) in fecal samples from dogs in kennels with problems of acute gastrointestinal disease and diarrhea ($n=223$), an open dog population without apparent diarrhea ($n=75$) and an SPF dog colony ($n=48$).

DISCUSSION

In the present paper we describe a comparison of two ELISA systems for the determination of serum antibodies to CPV, CCV or rotavirus, and an ELISA system for the demonstration of CPV, CCV or rotavirus antigens in the feces of dogs. It should be realized that both serological ELISA systems detect group-specific antiviral antibodies against the respective viruses. We took advantage of the broad antigenic cross-reactivities which have been demonstrated between FIP virus, TGE virus and CCV on the one hand (Osterhaus et al., 1977; Pedersen et al., 1978; Pensaert et al., 1981; Horzinek et al., 1982) and between simian and canine rotaviruses on the other hand (Gaul et al., 1982) to incorporate heterologous antigens in the assays, which were chosen on practical grounds.

As has also been documented previously, the sensitivity of indirect ELISA systems for the detection of serum antibodies to CPV, CCV and rotavirus was about 2–10 times higher than conventional methods such as HI, VN and CF (Ghose et al., 1978; Osterhaus et al., 1980a; Fiscus et al., 1985; Rimmelzwaan et al., 1990). Results obtained with the same sera in the CTB-ELISA system, which is based on a different principle, correlated well with those obtained in the indirect ELISA system. This is a clear indication of the specificities of both systems for antibodies to the respective viruses. The specificities of these ELISA systems were also confirmed by the fact that all sera from the SPF dogs tested were negative in all the ELISAs. The sensitivity of the indirect ELISA system proved to be generally higher than that of the CTB-ELISA system. The indirect ELISA system for the detection of antibodies to CPV, in which monoclonal antibodies were used as capture antibodies, proved to be very sensitive.

A major advantage of both ELISA systems over the conventional assays mentioned is that they are easy and rapid to perform, and can be incorporated in automated routine screening procedures. The CTB-ELISA system, although slightly less sensitive, has the advantage that one less washing step is needed, which makes it even more suitable for large-scale screening. Another advantage of this system is that serum samples from other animal species can also be tested. Cats and mink, for example, which may be infected with mink enteritis virus or feline panleukopenia virus – two viruses closely related to CPV – can be screened. The indirect ELISA system, however, has the advantage that, if the appropriate anti-Ig reagents are used, antiviral antibodies of the IgM class can also be detected. This is of diagnostic relevance since it may be indicative of recent infections, as has been shown for CPV infections (Florent, 1986).

Using the serological ELISA systems, we have screened selected panels of sera from dogs for the presence of antibodies against the respective viruses. In the Dutch open dog population, the majority of the animals tested (79%)

proved to have CPV-specific serum antibodies; this is probably due to both natural infections and the high vaccination level. The percentage of CPV-seropositive dogs in kennels with a history of acute infectious diarrhea was considerably higher (91%), and antibody titers were generally higher, in this group (Fig. 1): 62% of the samples from these kennels had CPV antibody titers ≥ 640 in the indirect ELISA, whereas only 34% of the dogs in the open population had titers ≥ 640. The most likely explanation for these high percentages may be the active circulation of natural CPV infections in these kennels, since no clear differences in vaccination policy between the two groups tested could be expected to occur, because a general vaccination strategy is advised in The Netherlands. Similarly, a difference was found between the incidence and titers of antibodies against CCV. In problem kennels 61% of the dogs were seropositive for CCV (22% with titers ≥ 160), whereas 45% of the dogs in the open population were seropositive (5% with titers ≥ 160). Since vaccination against CCV infection was not carried out in either group at that time, these differences must be considered a reflection of differences in circulating natural CCV infections. There proved to be little difference in the percentages of rotavirus-seropositive dogs between the two groups (91% and 89% respectively). Since neither group had been vaccinated against this virus infection, it can be concluded that most of the dogs tested had acquired natural rotavirus infections. It cannot be concluded from these data that this infection is a major cause of diarrhea in adult dogs. They rather suggest that rotavirus infection, as has been described for other mammalian species, mainly causes problems in young animals (Ghose et al., 1978).

Apart from the application of the serological ELISA systems for epizootiological and diagnostic purposes, they may also be used for determining the optimal time for the vaccination of pups. Especially in kennels with recurrent acute infectious diarrhea, the quantitation of levels of maternal antibodies against CPV, which interfere with the induction of protective immunity by vaccination, may be helpful in vaccination strategies against CPV infections.

The ELISA system used for the detection of viral antigens in the feces of dogs has been used previously for the detection of CPV antigen, and proved to correlate well with results obtained in the HA assay. This confirmed both the specificity and the sensitivity of this ELISA (Mildbrand et al., 1984; Teramoto et al., 1984; Rimmelzwaan et al., 1990). The specificities of the antigen ELISAs were further confirmed by using the appropriate specific antiviral antibodies in CTB-ELISAs with all the positive fecal samples, and by showing that all the samples from SPF dogs were negative. The sensitivities of the assays were estimated by relating positivity to minimal titers of infectious virus (for CPV and CCV) or to the number of complement-fixing units (for rotavirus) in the positive fecal samples. The results obtained with these ELISAs showed that CPV was present in none of the dogs of the open population and in 37% of the dogs in kennels with recurrent diarrhea. It may there-

fore be concluded that CPV is a major cause of acute infectious diarrhea in kennels. CCV was found in 11% of the feces samples from dogs with diarrhea in kennels with recurrent problems, in 7% of the feces samples from dogs in the open population without diarrhea, and double infections with CPV and CCV were found in 7% of the dogs with diarrhea. The role of CCV in the problems in the kennels was therefore less clear. The fact that no rotavirus antigen was found in the feces of any of the dogs tested is consistent with the serological findings, which showed no clear difference between the open population and the kennels, and suggests that rotavirus infections are not a major cause of the recurrent problems in the kennels.

In summary, we conclude that the ELISA systems described for serological purposes and for the detection of CPV, CCV and rotavirus antigens in the feces are useful and reliable tools for epidemiological and diagnostic purposes, and may help to combat infections with these viruses in kennels. On the basis of the serological and antigen detection data obtained with these assay systems, it is concluded that CPV is the major cause of recurrent problems with acute virus-induced gastrointestinal disease in kennels in The Netherlands.

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