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Antiviral Antibodies in Dogs in the Netherlands

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With 4 figures and 3 tables

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Introduction

Besides the classic canine viruses causing distemper, infectious hepatitis (canine adenovirus type 1; CAV), and rabies a number of viruses have been isolated from dogs (5). Some of these were known to occur in other species, like parainfluenza type 2 (PI2; SV), mumps virus, REOvirus type 1, ECHO-virus type 6, Coxsackie B viruses (types 1, 3 and 5), eastern and western equine encephalitis virus and pseudorabies virus, whereas others like canine herpes-virus (CHV), canine adenovirus type 2 (CAV2) and canine parvovirus appear to be specific for dogs. An even wider range of viruses has been found to induce antibodies in the dog e.g. the human influenza viruses, the parainfluenza viruses and the REOviruses with their respective serotypes (1, 8). The role played by the dog in the epizootiology of viruses occurring in other species is still unknown. Serologic surveys have been carried out in several countries in order to estimate the incidence of different virus infections in the dog (2, 6, 10, 11, 14, 16, 20). As in other countries infectious laryngotracheitis (kennel cough) and neonatal mortality (NM) represent unresolved problems in the Netherlands, too. Viruses which have been connected with kennel cough are CAV1, CAV2, CHV, REO, PI2 and influenza A2/Hongkong virus (1, 3, 4, 15, 23); viruses known to play a role in NM of dogs are CAV1 and CHV (17, 21).

In this paper a serologic survey is presented of antibodies against viruses in open and closed dog populations in the Netherlands. A comparison is made between the data from different groups and the results are compared with similar studies carried out in other countries.

Material and Methods

Sera

Sera were collected from dogs in open populations (OP), closed populations (CP) and in kennels with a history of NM.
a) OP (458 sera): Small Animal Clinic, Veterinary Faculty, State University, Utrecht; Central Laboratory for Experimental Medicine, Free University, Amsterdam; Private kennels.

b) CP (174 sera): Closed breeding kennel, Central Institute for the Breeding of Laboratory Animals, T. N. O. Zeist; closed kennel of the Clinic of Obstetrics and Gynaecology, Veterinary Faculty, State University Utrecht.

c) NM kennels (115 sera): private kennels with a high incidence of NM. In addition six sera from animal technicians (TNO, Zeist) were tested.

Serum pretreatment

All sera were kept in storage at $-20^\circ$C; before use they were heat inactivated for 30 minutes at $56^\circ$C. For hemagglutination-inhibition (HAI) tests, sera were pretreated with kaolin (18), trypsin and periodate (19) or CaCl$_2$-dextran sulfate (13) in order to remove nonspecific inhibitors (see Table 1).

Viruses and antigen preparations

**Canine adenoviruses (CAV$_1$ and CAV$_2$)**

Infectious canine hepatitis virus (CAV$_1$ and CAV$_2$) (strain Manhattan), with specific antisera, were obtained from Dr. PEEREBOOM (Philips Duphar, Weesp). The viruses were propagated in Madin Darby canine kidney (MDCK) cells. Supernatants of infected cultures showing complete cytopathic effect (CPE) were used as antigen in HAI tests after three cycles of freezing and thawing, followed by low speed centrifugation.

**Canine herpesvirus (CHV)**

The strain F 205 of CHV was obtained from Dr. WRIGHT (Department of Veterinary Pathology, University of Glasgow, Scotland) and specific antiserum from Dr. BINN (Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, U. S. A.). The virus was propagated in MDCK cells. Two days p. i. when CPE was still incomplete, culture fluid was harvested and kept in liquid nitrogen until use.

**Polyoma virus**

Polyoma virus (strain LID-1) together with specific antiserum was obtained from Dr. VAN DER VEEN (Medical Faculty, Medical Microbiology, Catholic University, Nijmegen). The virus was propagated in primary mouse embryo cells. Culture supernatants twelve days p. i. were used as antigen for HAI tests, after three cycles of freezing and thawing, followed by low speed centrifugation. For neutralization tests the same preparations were used as virus stocks.

**REOviruses**

REOvirus type 1 (strain Lang), type 2 (strain Jones) and type 3 (strain Abney) with specific antisera were obtained from Dr. KAPSENBERG (R. I. V. Bilthoven).

REOvirus types 1 and 2 were propagated in Vero cells. Cultures showing complete CPE were used as antigen in HAI tests after three cycles of freezing and thawing followed by low speed centrifugation. REOvirus type 3 was propagated in mouse L-cells. Cells from monolayers showing incomplete CPE (two days p. i.) were scraped into a small volume of medium, frozen and thawed three times and disrupted by ultrasonic treatment. The preparation
was then centrifuged at low speed and the supernatant was used immediately. If antigen preparations had to be stored, ultrasonic treatment was performed just before use.

**Parainfluenza viruses (Pl, P2, and P3)**

Pl (strain Sendai), P2 (strain Greer) and P3 (strain Chanock) with specific antisera were obtained from Dr. KAFSEBERG (R. I. V. Bilthoven). PI was propagated in the allantoic cavity of eleven day old embryonated chicken eggs, the Pl and P3 viruses were propagated in Vero cells. Antigens for HAI tests were prepared from infected cell cultures 14 days p. i. by three cycles of freezing and thawing, followed by low speed centrifugation. The supernatants and allantoic fluids (Pl) were used as antigens after Tween 80/ether treatment (12).

**Influenza viruses**

Influenza A equi 1 (strain Praha) and influenza A equi 2 (strain Miami) viral antigens for HAI were available as native allantoic fluids containing merthiolate. Together with specific antisera they were obtained from Dr. MASUREL (Institute of Virology, Medical Faculty, Erasmus University, Rotterdam).

**Coronavirus**

Vomiting and wasting disease virus (VW; hemagglutinating encephalitis virus) of pigs, with specific antisera, was obtained from Dr. PENSARAT (Veterinary Faculty, Laboratory of Virology, Gent, Belgium). The virus was propagated in PK-15 cells. Eight days p. i. culture fluids were used as antigen in HAI tests, after three cycles of freezing and thawing, followed by low speed centrifugation.

**Serologic Tests**

**HAI tests**

HAI tests were carried out in a Takátsy type microtiter system (Cooke Engineering Co.) employing U-type trays. Four hemagglutinating units (HAU) of the respective antigen preparations in 25 μl. were added to equal volumes of serial two-fold dilutions of the pretreated sera filled with 50 μl.

**Table 1**

<table>
<thead>
<tr>
<th>Antigen-antibody reaction</th>
<th>Red blood cell Sedimentation</th>
<th>Diluent</th>
<th>Source Erythrocytes</th>
<th>Pretreatment inactivated sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAV1, CAV2</td>
<td>1 hr, 20°C</td>
<td>2 hr, 20°C</td>
<td>VGB **</td>
<td>Human ‘O’ kaolin</td>
</tr>
<tr>
<td></td>
<td>1 hr, 20°C</td>
<td>2 hr, 20°C</td>
<td>VGB</td>
<td>Human ‘O’ kaolin / CaCl2-dextr. sulf.</td>
</tr>
<tr>
<td>REO1, REO2</td>
<td>1 hr, 20°C</td>
<td>2 hr, 20°C</td>
<td>VGB + 0.15 % BSA***</td>
<td>kaolin</td>
</tr>
<tr>
<td>REO3</td>
<td>1 hr, 4°C</td>
<td>4 hr, 4°C</td>
<td>VGB + 0.1 % BSA</td>
<td>Guinea pig trypsin and periodate</td>
</tr>
<tr>
<td>Pl1</td>
<td>1 hr, 20°C</td>
<td>2 hr, 4°C</td>
<td>VGB + 0.2 % BSA</td>
<td>Guinea pig kaolin</td>
</tr>
<tr>
<td>Pl2</td>
<td>1 hr, 20°C</td>
<td>75 min, 37°C</td>
<td>VGB + 0.2 % BSA</td>
<td>Guinea pig trypsin and periodate</td>
</tr>
<tr>
<td>Pl3</td>
<td>1 hr, 20°C</td>
<td>75 min, 37°C</td>
<td>VGB + 0.4 % BSA</td>
<td>Chicken** periodate</td>
</tr>
<tr>
<td>VW</td>
<td>1 hr, 20°C</td>
<td>1 hr, 20°C</td>
<td>VGB</td>
<td>Guinea pig kaolin</td>
</tr>
<tr>
<td>Infl. A / equi 1</td>
<td>1 hr, 20°C</td>
<td>2 hr, 24°C</td>
<td>VGB</td>
<td>Guinea pig kaolin</td>
</tr>
<tr>
<td>Infl. A / equi 2</td>
<td>1 hr, 20°C</td>
<td>1 hr, 4°C</td>
<td>VGB</td>
<td>Guinea pig kaolin</td>
</tr>
<tr>
<td>Polyoma</td>
<td>1 hr, 4°C</td>
<td>1 hr, 4°C</td>
<td>VGB</td>
<td>Guinea pig kaolin</td>
</tr>
</tbody>
</table>

* variation in hemagglutinability between batches of different animals.
** VGB = veronal gelatine buffer (pH 7.4).
*** BSA = bovine serum albumine.
volumes/cup of a photometrically standardized suspension of erythrocytes in a buffered diluent. The trays were again shaken, tape-sealed and the erythrocytes were permitted to settle; Table 1 shows the conditions used for the different tests. Titers $< 20$ were not included in the evaluation.

**Complement dependent neutralization test for CHV**

This test was carried out in microtiter system [L]-type trays: 50 $\mu$l of CHV-containing culture medium containing 50—200 TCID$_{50}$ were incubated with equal volumes of serial two-fold serum dilutions supplemented with 50 $\mu$l of a 1 : 10 dilution of unheated guinea pig serum (7) for one hour at 20 $^\circ$C. Subsequently the mixtures were pipetted onto MDCK monolayers and incubated at 37 $^\circ$C in a humidified CO$_2$ atmosphere. The tests could be read microscopically after two days. Titers $< 4$ were disregarded.

**Neutralization test for polyoma virus**

Neutralization indexes were determined from selected HAI positive and negative sera in the following way: 50 $\mu$l of 1 : 10 diluted sera were incubated with serial ten-fold dilutions of polyoma virus (50 $\mu$l) for one hour at 20 $^\circ$C. Subsequently the mixtures were pipetted into tissue culture microtrays, together with 100 $\mu$l of mouse embryo cell suspensions containing $4 \times 10^5$ cells/ml. The trays were incubated for twelve days at 37 $^\circ$C in a humidified CO$_2$ atmosphere. Subsequently culture fluids of each virus-serum mixture were tested for HA activity. Neutralization indexes were calculated by comparing the HA results of this test with the TCID$_{50}$ of the virus-stock determined in the same way.

The results described below have been obtained by at least two independent and unbiased tests.

**Fig. 1 A**

**Fig. 1 B**

- **Fig. 1 A.** Distribution of HAI antibodies to canine adenoviruses in open and closed populations. The percentages of seropositive dogs are given in parenthesis
- **Fig. 1 B.** Percentage of sera with antibodies to one or both canine adenovirus serotypes in open and closed populations
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Results

Canine adenoviruses

The OP and CP dog sera were assayed for HA1 antibodies to CAV₁ and CAV₂. In the OP sera a higher prevalence of titers against CAV₁ was noted as compared with the closed collectives; also titer values exceeded those in the CP and antibodies to CAV₂ were absent in the CP (see Fig. 1 A). Fig. 1 B shows the percentages of OP and CP sera with antibodies to one or both adenovirus types.

Canine herpesvirus

From ten kennels with a history of NM 115 sera were tested for the prevalence of complement dependent neutralizing antibodies to CHV. As a reference, 107 randomly selected OP sera were included in the tests. As can be seen from Fig. 2, seropositive dogs are more frequently encountered in problem kennels; however, only half of the kennels account for this difference (Table 2). The seropositive dogs in the problem kennels were randomly distributed over male and female animals of different age.

Table 2

Incidence of neutralizing antibodies against canine herpesvirus in sera from kennels with frequent neonatal mortality

<table>
<thead>
<tr>
<th>kennel</th>
<th>number of positive sera / number of sera tested</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6 / 9</td>
<td>67</td>
</tr>
<tr>
<td>B</td>
<td>3 / 6</td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>3 / 10</td>
<td>30</td>
</tr>
<tr>
<td>D</td>
<td>1 / 12</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>1 / 16</td>
<td>6</td>
</tr>
<tr>
<td>F</td>
<td>0 / 4</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0 / 11</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0 / 13</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0 / 16</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>0 / 19</td>
<td>0</td>
</tr>
</tbody>
</table>
Polyoma virus

From the OP 340 sera were tested for HAI antibodies to polyoma virus. Five HAI positive sera were found, with titers ranging from 20 to 160. These positive sera were assayed in the neutralization test, together with 5 randomly selected HAI-negative OP sera. One serum with a HAI titer of 20 neutralized $10^4$ infectious doses of polyoma virus at a dilution of 1:10.

REOviruses

All OP and CP sera were tested for HAI antibodies to REOvirus types 1 and 2. As indicated in Fig. 3 A, titers to REOvirus type 1 occurred in 14% of the sera from the OP and only in 0.6% in the CP. In contrast to this observation antibodies to REOvirus type 2 were seen in equal amounts and levels in OP and CP sera (14%). In both populations 34% of the sera tested were found to contain antibodies to REOvirus type 3 with about the same levels. In Fig. 3 B an evaluation is made between OP and CP sera containing antibodies against one single, two and three REOvirus serotypes, respectively.

Parainfluenza viruses

Screening was performed on our OP and CP sera for the prevalence of HAI antibodies to PI₁, PI₂ and PI₃. As shown in Fig. 4 A, antibodies to PI₁ and PI₃ could be demonstrated only in the OP and in very low amounts, whereas no significant difference in PI₂ antibodies could be demonstrated between the OP and CP sera. Fig. 4 B shows how many sera were positive to one or more of the three serotypes.
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Fig. 4 A

Fig. 4 B

Fig. 4 A. Distribution of HAI antibodies to parainfluenza virus serotypes in open and closed populations

Fig. 4 B. Percentages of sera with antibodies to one or more parainfluenza virus serotypes in open and closed populations

Equine influenza viruses

From the OP 131 sera and from the CP 119 sera were tested for the prevalence of HAI antibodies to the two serotypes of equine influenza viruses A. While no antibodies could be found in the latter, three dogs from the OP

Table 3

Distribution of antibodies in sera from six animal attendants, labelled A—F, from the closed breeding colony at Zeist

<table>
<thead>
<tr>
<th>Antibodies to</th>
<th>in person</th>
</tr>
</thead>
<tbody>
<tr>
<td>REO1, REO3, PI1, PI2, PI3, VW</td>
<td>A</td>
</tr>
<tr>
<td>CAV1, REO1, REO2, PI1, PI3</td>
<td>B</td>
</tr>
<tr>
<td>REO2, REO3, PI3</td>
<td>C</td>
</tr>
<tr>
<td>REO1, PI2, PI3</td>
<td>D</td>
</tr>
<tr>
<td>PI1, VW</td>
<td>E</td>
</tr>
<tr>
<td>PI1</td>
<td>F</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CAV1</th>
<th>CAV2</th>
<th>REO1</th>
<th>REO2</th>
<th>REO3</th>
<th>PI1</th>
<th>PI2</th>
<th>PI3</th>
<th>VW</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>B</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>A</td>
<td>A</td>
<td>AE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td></td>
<td>F</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>B</td>
<td>ABD</td>
<td>BC</td>
<td>AC</td>
<td>AEF</td>
<td>ABD</td>
<td>ABCD</td>
<td>AE</td>
</tr>
</tbody>
</table>
were seropositive, two of them for type 1 (titers 20 and 40, respectively) and one for type 2 (titer 80).

**Coronavirus**

From the OP 355 sera and from the CP 157 sera were tested for the prevalence of antibodies to VW virus of the pig. In the sera from the CP no antibodies were found, but two of the sera from the OP showed titers (20 and 40, respectively).

**Antibodies in humans**

Sera from six animal attendants from a CP kennel, comprising a total of at least 170 dogs, were tested for the prevalence of HAI antibodies to CAV1, CAV2, REOvirus types 1, 2 and 3, parainfluenzavirus types 1, 2 and 3 and VW virus. The results are shown in Table 3.

**Discussion**

Serological surveys are usually performed with the intention to show the incidence of selected infectious agents in a geographically defined host population. For canine viruses, surveys have been performed in several countries (2, 6, 10, 11, 14, 16, 20). The present study is the first of this kind for the Netherlands, with an estimated dog population of one million animals.

In our collection of sera sampled from dogs, antibodies to CAV1 are found more frequently and with higher levels in sera from the OP. Considering that most dogs from both populations had been vaccinated against CAV1, and that their age distributions are comparable, this might mean that hepatitis virus is continuing to spread in the OP and that these dogs undergo a booster infection from time to time. Especially with regard to NM and kennel cough problems, this might have its implications: as has been shown by Wright et al., dogs solidly immune to intravenous challenge with CAV1 virus are still susceptible to virus aerosols, resulting in respiratory disease (22). In contrast to results obtained by workers in North-America (6, 11), CAV2 HAI antibodies were found in only 3% of the OP sera; indications for its role in the etiology of kennel cough — a condition observed annually by clinicians in this country — cannot be derived from our data. CAV2 HAI antibodies were absent in the CP.

Neutralizing antibodies to CHV in the OP occur with frequencies comparable to those found by workers in North-America (6, 11) and in West-Germany (1). The possible role played by CHV for NM in “problem”-kennels in the Netherlands is indicated by the number of titers found. However, some of the kennels have high percentages of seropositive animals, whereas others fail to show any. The observations that CHV is poorly immunogenic and that neutralizing antibodies are difficult to detect (7) suggest, that in kennels with many seropositive dogs, this virus probably has played a role in NM.

REO-virus type 1 is considered to be an agent responsible for canine respiratory disease. As can be seen from our results and from similar data from other countries (1, 8), antibodies to the other serotypes are found as well. No differences in the frequency of antibodies to REOVirus types 2 and 3 were noted between both populations; antibodies to serotype 1, however, were practically lacking in the CP observed. Rodents are not likely to constitute a major source of infection since most animals were kept on mesh wire floors; the role of man is suggested from Table 3.
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Antibodies to parainfluenza viruses were rare in our collection of canine sera, especially in the CP (Fig. 4). Parainfluenza virus type 2, which has been connected with respiratory diseases in the dog (9) has caused titers in about 3% of the animals which is in accordance with data from workers in North-America (6) and West-Germany (1). The lack of difference in number of PI2 titers between the OP and CP can be interpreted in different ways: In the first place a possible role of humans in transmission of parainfluenza virus is suggested in Table 3. Secondly the incidence of animal-to-animal-transmission will be higher in the more crowded CP.

In addition sera were screened for the prevalence of HAI antibodies to viruses of other species which are not known to play a pathogenic role in the dog, like mouse polyoma virus, influenza virus A equi 1 and 2 and VW virus of the pig. From our data it appears that these animal species only incidentally transmit viruses to dogs. This is in contrast to man, where antiviral antibodies in sera from animal technicians show that they represent a potential hazard for SPF dog breeding kennels.

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Summary

Antiviral antibodies in dogs in the Netherlands

A collection of more than 700 canine sera, coming from open and closed populations and from kennels with frequent neonatal mortality, were screened for the prevalence of antibodies to canine adenoviruses, canine herpesvirus, polyoma virus, REOViruses, parainfluenza viruses, equine influenza viruses and vomiting and wasting disease virus of pigs. The data from the different groups were compared and related to the results of similar studies carried out in other countries.

Zusammenfassung

Virus-Antikörper bei Hunden in den Niederlanden


Résumé

Anticorps antiviraux chez des chiens aux Pays-Bas

On a recherché des anticorps contre les virus suivants sur plus que 700 sérum de chiens pris dans la population ouverte, dans des collectivités isolées et dans des chenils avec forte mortalité des chiots: Adeno- et Herpesvirus canins, Polyomavirus, REOVirus, Parainfluenzavirus, Influenzavirus du cheval et le virus du «vomiting ans wasting disease» du porc. Les valeurs obtenues
dans les différentes collectives ont été comparées entre elles et également avec les résultats de recherches semblables faites à l'étranger.

Resumen
Anticuerpos antivirales en perros de los Países Bajos
Se examinaron más de 700 sueros sanguíneos de perros de la población abierta, colectivos aislados y perreras con mortalidad elevada de cachorros en cuanto a la presencia de anticuerpos contra los virus siguientes: adenov y herpes caninos, poliomavirus, REO, parainfluenza, influenza equina y de la enfermedad del vómito y extenuación en porcinos. Se compararon entre sí los valores obtenidos en los diversos colectivos, así como también con los resultados de pesquisas semejantes llevadas a cabo en el extranjero.

References

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