Presence of Canine Parainfluenza Virus (CPIV) in the Icelandic dog population

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Summary

In Iceland, the release of vaccines against infectious agents not native to the animal population in the country is prohibited. It was therefore necessary to establish the presence of Canine Parainfluenza Virus (CPIV) in the Icelandic dog population before the release of a combined *Bordetella bronchiseptica*, Canine Adenovirus -2 (CAV-2) and CPIV vaccine. To maximize detection of CPIV, blood samples were taken from dogs with symptoms of kennel cough, a contagious respiratory disease that can be caused by CPIV. Furthermore, dogs, which were living in a kennel were tested, as it has been shown that if the virus is present it will spread rapidly under such conditions. By haemagglutination inhibition antibody test, antibodies were measured against the virus. The results showed that 16 of 57 (28%) dogs tested were positive regarding antibodies towards CPIV. It should therefore from a legal point of view in Iceland, be possible to introduce a new vaccine containing CPIV.

Introduction

Until December 1992 there were no vaccines for dogs allowed in Iceland, due to the fact that there were no fatal contagious diseases in the dog population. Canine Distemper Virus (CDV) destroyed over 75% of dogs in Iceland in the late 19th century, leading to a ban in import of dogs (Pálsson, 1999). Today, import of dogs to Iceland is allowed, with a special permit from the Icelandic Ministry of Agriculture. In order to keep the Icelandic population of dogs free of disease, imported dogs are kept in isolation for at least four weeks. In 1992, Canine Parvovirus was first detected in two Icelandic dogs that died after a short period of illness. Both dogs were housed in the same boarding kennel. Furthermore, 20-30 dogs became sick of parvovirus that year and the authorities gave permission for a vaccination with Candur P, an inactivated canine parvovirus type 2 (CPV-2) vaccine (Gunnarsson, 1993). Around that time it also became clear that Canine Adenovirus-1 (CAV-1) causing Hepatitis Contagiosa Canis (HCC) was spreading among the dog
population in Iceland. In 1996 a new vaccine was introduced, Canlan-2, which contained inactivated CPV-2 and inactivated CAV-1. Vaccines against both viruses are rated as “core” or recommended vaccines by the American Animal Hospital Association (AAHA) Canine Vaccine Task Force, as published in Canine Vaccine Guidelines from 2003. The argument is that the diseases caused by CAV-1 and CPV-2, have significant morbidity and mortality and are widely distributed (Paul et al., 2003). In 2003 the production of Canlan-2 was stopped and consequently the vaccination of dogs in Iceland against HCC. Today the only vaccine available in Iceland is a monovalent live CPV-2 vaccine (Parvodog ® from Merial and Nobivac® from Intervet). Today, more and more dogs have been reported sick of HCC in Iceland often with fatal outcome specially when there are puppies involved. Thus the pressure on the authorities has increased to release a new vaccine against the virus, CAV-1. According to the Canine Vaccine Guidelines from 2003, the CAV-1 vaccine has been associated with an unacceptable rate of serious adverse events, such as interstitial nephritis and anterior uveitis and should not be administrated. Instead it is recommended to use the close related virus, canine adenovirus-2 (CAV-2), which can cause respiratory disease in some infected dogs but produces an immune response that cross-protects against CAV-1 (Paul et al., 2003). Almost all available CAV-2 vaccines are modified live vaccines including the CDV (Paul et al., 2003). In Iceland it is prohibited to release vaccines with viruses that are not present in the country and CDV has not been reported in Iceland since 1967 (Pálsson 1999). Introduction of a modified live vaccine against CDV in a dog population free of the disease could result in an outbreak of the disease. Viremia could occur in vaccinated animals with possible excretion of the virus and infection of non-vaccinated animals (Pálsson, 1999). At this point in time, there is only one vaccine that could possible meet the requirements set by the Icelandic veterinary authority and that is a modified live vaccine against Bordetella bronchiseptica, CPIV and CAV-2. But prior to the release of this vaccine, the presence of CPIV in the Icelandic dog population has to be established.
**Virus properties**

CPIV belongs to the parainfluenza Type 5 group within the family Paramyxoviridae. The virus is composed of a single-stranded RNA genome and is surrounded by a lipid envelope (Appel & Binn 1987, Buonavoglia & Martella 2007) A close antigenic relationship is between CPIV and Simian Virus 5, which beside monkeys and dogs, is known to infect guinea pigs, mice, hamsters and cats. A closely related paramyxovirus can infect humans but cross infection between dogs and humans has never been established (Crandell et al., 1968; Appel & Binn, 1987, Buonavoglia & Martella 2007).

**Pathogenesis and disease signs**

Infectious tracheobronchitis (ITB) or kennel cough, is an acute, contagious respiratory infection occurring in dogs which are usually housed in groups, for example in re-homing centres, boarding kennels or veterinary hospitals (Binn et al., 1968; Appel & Percy, 1970; Appel & Binn, 1987; Ueland, 1990). Aerosol and contact exposure produces clinical signs restricted to the respiratory tract (Appel & Percy, 1970; Erles et al., 2004). CPIV is one of the main causes of ITB. The incubation period for CPIV has been found to range from 1-8 days (Wagener et al., 1984; Thrusfield et al., 1991). After exposure the virus can be found in oro-nasal swabs 1-9 days after infection. The virus is not found in blood, as systemic infection does not occur. Virus neutralizing antibodies are found in serum from day 10, post infection and they increase in amount up to 3 or 4 weeks, declining thereafter and are usually not present 3 or 4 months after exposure (Appel & Percy, 1970; McCandlish et al., 1978; Ajiki et al., 1982; Wagener et al., 1984). The disease is characterized by a dry non-productive hacking cough and other symptoms can be nasal discharge and slight fever, which in most cases is cleared within a short time (Binn et al., 1968; Appel & Percy, 1970; Wagener et al., 1984; Appel & Binn, 1987; Thrusfield et al., 1991). However, studies confirm that the etiology can be complex and that CPIV can act as a trigger for bacterial or mycoplasma infections, which can lead to a severe bronchopneumonia or death (Binn et al., 1968; Appel & Percy, 1970; Ajiki et al., 1975; Azetaka et al., 1988; Ueland, 1990; Erles et al., 2004). A variety of other viruses have been reported as causative agents of ITB or kennel cough,
such as Canine Herpes Virus (CHV), CAV-2, Canine Respiratory Coronavirus (CRCoV), and reovirus. The pathogens involved can act alone or in a combination and they may not be the same in every outbreak (Appel & Percy, 1970; Binn et al., 1970; Cornwell et al., 1976; Azetaka et al., 1988; Erles et al., 2004; Damián et al., 2005; Erles & Brownlie, 2005).

**Epidemiology**

In 1967, CPIV was isolated from sentry dogs with respiratory disease at an air force base in Texas, USA (Appel & Percy, 1970). In USA, in 1968, 59 of 176 dogs (34%), developed signs of disease, a non-productive cough during a military training in USA. Results of serum neutralizations tests indicated that CPIV was spreading rapidly among the dogs (Binn et al., 1968). In 1970, serum was collected from dogs entering veterinary hospitals in several states of the United States. The dogs varied in age from 4 month to 14 years. Viruses antibodies occurred in a number of areas of the country but there was no correlation with the presence of antibody and the incidence of respiratory signs, nor did there seem to be any relationship to the occurrence of antibody with the respect to age (Bittle & Emery, 1970). In a serological survey of dogs in the Netherlands in 1976, parainfluenza virus caused titres in about 3% of the animals (Osterhaus et al., 1976). Seroepizootiological studies on the importance of parainfluenza infection in Germany 1975 showed that 30% of 456 canine sera had antibodies against CPIV (Bibrack & Benary, 1975). In a serologic survey of random street dogs in two Japanese districts, antibody against CPIV was first detected in sera collected in 1976 (5,9%) and in 1977 (22,7%) (Ajiki et al., 1982). In an outbreak of ITB in Zaria, Nigeria in 1980, several different organisms were isolated but viral isolation was unsuccessful (Tedek et al., 1982).

In an outbreak of kennel cough in Japan 1985, CPIV was isolated in two out of 33 dogs tested, or 6% (Azetaka et al., 1988). In 1985 the virus was first isolated in Australia (Moloney et al., 1985) and in 1989, a seroepidemiological survey was done prior to the release of a CPIV vaccine. The survey reported 63 antibody positive dogs out of 192 tested, 33% (McGavin et al., 1989). In an outbreak of kennel cough in Norway 1988, at least 79% of the affected dogs showed a rise in CPIV antibody titre and CPIV was therefore was considered
to be the causative agent. The dogs involved had no history of kennel boarding or participation in dog shows or similar crowding activities (Ueland 1990). In a seroepidemiological survey of 302 healthy pet dogs in Sweden 2003, CPIV had a seroprevalence of 28%. The dog ages, gender or breed did not correlate with the seroprevalence (Engelund et al., 2003). In Mexico 2004, CPIV was identified in 28 of 35 (51%) cases of dogs that died from acute or subacute pneumonia. Two cases (6%) were positive only for CPIV, the rest were mixed virus infections (Damián et al., 2005). In 2005, an investigation into the cause of kennel cough was started at a dog-training centre in England. Several outbreaks of ITB occurred during the year and the antibodies detected were against CRCoV and CHV (Erles et al., 2005).

The above-cited research/surveys clearly indicate that CPIV can cause kennel cough in dogs but on the other hand kennel cough is not always caused by CPIV (Appel & Percy, 1970). CPIV has an undoubtedly worldwide distribution, however CPIV's presence in the Icelandic dog population has not been established yet. The main purpose of this survey is to test Icelandic dogs with symptoms of ITB and dogs in kennels that are likely to have been in contact with CIPV in an attempt to detect antibodies against the virus.

Materials and Methods

Dogs with clinical symptoms of kennel cough were specifically chosen for sampling in order to maximize detection of CPIV. The dog population in Iceland is less than 10,000 and the expected prevalence of antibody positive dogs was set to be 5%. A sample size of 59 dogs was required to be 95% certain of detecting at least one sero-positive dog (Pfeiffer, 2002).

During the survey 35 dogs with respiratory symptoms were tested. All had either a history of being boarded at a kennel and becoming sick afterwards or being in close contact with such a dog and then becoming sick themselves. To reach the required sample size of 59, dogs living at a large boarding kennel were tested, the same which some of the sick dogs in the survey had
been boarded at. Dogs owned by the staff of a Veterinary Hospital and dogs, which came regularly to that hospital for check-ups were also in the sample group. Dogs imported to Iceland were excluded from the survey as they could have been vaccinated against CPIV.

Blood was collected from a superficial vein in a serum separator tube and centrifuged, the serum was transferred to a plastic tube and stored until shipment at -20 °C. Serum samples were sent frozen to National Veterinary Institute of Sweden* and tested for antibodies against CPIV in haemagglutination inhibition test (HI). Briefly, non-specific agglutination was eliminated by absorption with guinea pig erythrocytes. To eliminate non-specific inhibitors of haemagglutination, the serum samples were pre-treated with trypsin and potassium periodate. For each test, 0.2 ml of dog serum was prediluted to 1:8 and two-fold dilutions were then made in microwells (first well = the 1:8 diluted serum) and tested with 4 haemagglutinating (HA) units of parainfluenza-2 virus/simian virus 5 (SV-5) and guinea pig erythrocytes. Titres of less than 1:8 were considered negative, and titres of 1:8 or above were considered positive (Engelund et al., 2003).

Results

Results came from 57 dogs, of them, 16 tested positive for CPIV antibodies or 28%. The results show that out of 35 dogs with signs of respiratory disease, seven tested positive for CPIV. Out of 14 dogs, staying regularly at the veterinary hospital, nine had antibodies to CPIV. None of the dogs living at the large kennel had antibodies against CPIV (table 2).

Table 2

* Sveriges lantbruksuniversitet 75007 Uppsala, Sverige
Results of serum-HI tests for CPIV in 57 dogs

<table>
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<tr>
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<th>Number of dogs</th>
<th>Positive dogs</th>
<th>%</th>
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<tr>
<td>Dogs with symptoms</td>
<td>35</td>
<td>7</td>
<td>20%</td>
</tr>
<tr>
<td>Dogs in kennel</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Dogs at hospital</td>
<td>14</td>
<td>9</td>
<td>64%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>57</strong></td>
<td><strong>16</strong></td>
<td><strong>28%</strong></td>
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The CPIV antibody titres ranged from 1:8 to 1:256 and generally higher titre were found in dogs with clinical symptoms (figure 1).

![Bar Chart](chart.png)

Figure 1: Distribution of positive HI titre against CPIV in 16 of 57 dogs tested.

Titres less than 1:8 were considered negative

**Discussion**
The aim of the study was to confirm the presence of CPIV in the Icelandic dog population. To maximize detection, dogs living at a large kennel, dogs with symptoms of kennel cough and dogs spending considerable time at a veterinary hospital were tested for antibodies against the virus.

As the result show, antibodies against CPIV were found in 28% dogs tested, both in dogs with symptoms of respiratory disease and in dogs without symptoms as well. Many of the dogs with symptoms had been boarded shortly before at the large kennel, which homes some of the dogs also tested in the survey. None of the dogs living at the kennel, tested positive despite the fact that a large number of dogs boarded there every year develop clinical symptoms of respiratory diseases. The dogs at the kennel roam freely among the boarded animals and have every opportunity to contact and spread diseases. The possible explanation for the negative titres in the kennel dogs could bee that they have long ago made their acquaintance with the virus, youngest dog being 3 years old and the oldest 11 years old. Antibodies are found 10 days post infection and increase in amount up to 3 or 4 weeks after aerosol exposure. Levels decline thereafter and little or no antibody is present 3 or 4 months after exposure (Appel & Percy, 1970). There are however reports of cases where antibodies have persisted for more than 6 months and even for more than 2 years (Appel & Binn, 1987; Bittle & Emery, 1970). That could explain why there are dogs with positive titres, with no symptoms of disease. In a survey in USA no correlation was detected between the presence of antibody and the incidence of respiratory signs (Bittle & Emery, 1970). A Swedish survey has also showed relatively high titres in healthy dogs (Engelund et al., 2003).

Of the 35 sick dogs tested in current survey, seven dogs were positive and with higher titres than those not sick. Pared blood samples were not taken as the purpose of the present survey was not to determine the cause of the illness and therefore it is not possible to conclude if CPIV was involved or not.
Conclusion

In Iceland, the release of vaccines against infectious agents not native to the animal population in the country is prohibited (Reglugerð 665/2001). The present survey shows that antibodies against the virus, Canine parainfluenza, is present in the dog population in Iceland. Therefore an import and release of a new vaccine in Iceland containing *Bordetella bronchiseptica*, CAV-2 and CPIV should be possible from a legal point of view and should be considered.

Acknowledgements

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References


Reglugerð 665/2001 Um viðbrögð gegn smitsjúkdómum

