Dogs as potential carriers of infectious bursal disease virus

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In this study, the possibility that dogs could eventually be carriers of infectious bursal disease virus (IBDV) after having eaten (voluntarily or accidentally) IBDV-infected chicks has been evaluated. A single Beagle dog was fed chicks infected by a very virulent IBDV strain (vvIBDV). Afterwards, the presence and viability of IBDV in the faeces was assessed. Viable vvIBDV was detected in the dog’s faeces for 2 days after the initial ingestion, which indicates excretion of vvIBDV. Comparison by molecular techniques of the administered and excreted virus using reverse transcription-polymerase Chain reaction and enzymatic digestion confirmed that the initial virus maintained the same characteristics after being excreted. We believe that this study could be of great interest for a better understanding of the epidemiology of IBD disease on farms where dogs live close to avian facilities.

Introduction

Since its discovery by Cosgrove (1962) in the United States, infectious bursal disease (IBD) has had a relevant interest for the poultry industry worldwide. The reason is not only because of the great impact on the morbidity and mortality of affected birds, but also because of the immunosuppression induced by the virus, which increases the risk of secondary infections (Van den Berg, 2000). Control of the disease is often difficult because of the great number of variables involved (different poultry production and farming, characteristics of the virus, uneven antibody levels, types of vaccines, etc.), which usually lead to only a partial control of the virus activity on a farm. This, together with the occurrence of outbreaks caused by new infectious bursal disease virus (IBDV) strains with great pathogenicity, makes the control of the disease more difficult and the biosecurity measures to be applied more important (Chettle et al., 1989).

Several studies on the resistance of IBDV to ether and chloroform, to pH extremes, high temperature and to phenol or quaternary ammonium disinfectants have been conducted, and make understandable the reasons for the persistence of the virus on poultry farms (Lukert & Saif, 1997). The same authors stated that iodine, formalin and soaps with 0.05% sodium hydroxide inactivate the virus. It is important to point out, however, the potential diffusion of the virus through insects (Snedeker et al., 1967). IBDV was isolated from Alphitobius diaperinus 8 weeks after an outbreak of IBD and the virus remained infective, as was observed when susceptible chicks were fed with the infected insects. When the same experiment was performed with mosquitoes, the virus was also detected, but it had lost its infective potential (Howie & Thorsen, 1981). Another report also describes how IBDV was detected by agar gel precipitation from different tissues of rats captured on poultry farms (Okoye & Utche, 1986), but no data have been found on the role that dogs and other carnivores (which may be found inside or outside poultry farm facilities) could play in the epidemiology of IBD.

Materials and Methods

Very virulent IBDV

The virus used to perform the study was a very virulent (vv) IBDV strain, isolated in Mallorca (Spain) (Pagès-Manté et al., 1991) and referenced as 5939. It was molecularly characterized by Majó et al. (2002) as a vvIBDV (Genbank accession number AY083926). The
original isolate has been propagated in specific pathogen free (SPF)
birds. The bursae of the affected birds were recovered and homogenized
in phosphate-buffered solution (PBS) at pH 7.2. The suspension
obtained was then frozen and stored at -80°C.

The inoculum was prepared from a thawed vial, diluted at 1:10 (v/v)
with PBS. The titre of the administered virus was 10^5.3 median embryo
infectious doses (EID_{50}/ml). Each SPF chick was given 0.5 ml inoculum
by the oral route.

**Animals (chicks and dog)**

Nine 21-day-old chicks were used in the present study. They were
hatched from SPF eggs (Lohmann GmbH, Cuxhaven, Germany) and
kept in biological isolators (Domite Plastics, Belfast, UK). The birds
were fed with commercial foodstuff and water ad libitum; a 12-h light
schedule was used.

A 3-year-old female Beagle dog was used (Harlan France SARL,
Gannat, France). The dog was kept in a biological containment unit
during the experiment.

**Experimental design**

A group of four 3-week-old SPF chicks were individually identified and
inoculated with the strain 5939 of IBDV. Clinical signs were observed and
recorded daily for 5 days. Necropsy was performed on all dead
animals in order to study macroscopic lesions due to IBDV infection.
The spleen, liver, bursa of Fabricius and intestine were taken and stored at
-20°C. One sample of the bursa of Fabricius from each dead animal
was tested by reverse transcription-polymerase Chain reaction (RT-
PCR) for the presence of IBDV.

The tissues samples stored at -20°C were thawed at room temperature
and administered to the Beagle dog, which had been kept for 8 h with
restriction of feed. The whole carcasses were not administered to the
dog to prevent potential damage caused by eating the bones. Faeces
were recovered, before and after 24, 48 and 72 h after the administration of
the chick tissue samples. At the same time, clinical signs of the animal
were recorded daily for 7 days after the initial exposure to detect any
intestinal or general abnormality.

All faecal samples were tested for IBDV using the RT-PCR technique.
In the case of virus detection in faeces, these were homogenized in PBS
and centrifuged at 3000 x g for 5 min. The supernatant obtained was
then administered to five SPF chicks to confirm infectivity of the
detected virus. Inoculated chicks were maintained and observed for
5 days in a biological isolator.

**Clinical samples and extraction of viral RNA**

The spleen, liver, bursa of Fabricius and intestinal samples from infected
birds as well as the dog's faeces were homogenized mechanically in a
Stomacher (Stomacher Lab Blender Model 400 (BA 7021), London,
UK) and were resuspended with PBS (1:10 v/v). Once clarified by
centrifugation at 3000 x g for 5 min using a bench centrifuge
(Stomacher Sigma Model 4k-15C, Osterode am Harz, Germany) the
supernatant was used for the total RNA extraction. Negative and
positive controls using vaccinal and vvIBDV were included.

The viral content of the PBS suspensions of the dog's faeces was
titrated in chicken embryos by chorioallantoic membrane inoculation.

**Extraction of viral RNA, detection of IBDV by RT-PCR and digestion of
PCR products using restriction enzymes (restriction fragment length
polymorphism)**

The RNA extraction was performed using a commercial kit (RNasey
96) together with a plate centrifugation system (QIAGEN, Cedex,
France). The procedure was performed following the recommendations
of the supplier.

The synthesis and amplification of cDNA was performed in a single
step using the QIAGEN One-Step RT-PCR Kit® (QIAGEN) following
the recommendations of the supplier. Briefly, 5 μl RNA were transferred
to 45 μl RT-PCR mixture containing 50 mM reverse and forward
primers, which in turn amplified a RNA fragment of the hypervariable
region of the VP2 gene of IBDV. The conditions used for the RT-PCR
were adjusted following the instructions of the kit supplier. Conditions
for the PCR were as follows: one cycle of 50°C for 30 min, one cycle of
95°C for 15 min, 30 cycles of denaturization at 94°C for 30 sec,
annealing at 58°C for 30 sec and elongation at 68°C for 1 min. The
PCR was ended with a final cycle at 68°C for 7 min. The restriction
fragment length polymorphism procedure, which allows the differentia-
tion between vvIBDV and other IBDV strains, was performed by the
digestion of 5μl PCR product with the enzymes BspMI and SacI following the protocol previously published by Zierenberg *et al.*

**Results**

**IBDV infection of the SPF birds and sampling**

Clear clinical signs of IBDV infection were observed in the four inoculated birds. At 2 days post
inoculation (d.p.i.), three of the four inoculated birds showed depression, and one of them also
showed prostration, and no clinical signs were observed in the fourth bird. At 3 d.p.i., two of
the birds that showed clinical signs at 2 d.p.i. died, and the remaining two birds showed depression
and prostration. At 4 d.p.i. all the birds had died.

The bursae of all the birds that died were tested by RT-PCR and restriction fragment length poly-
morphism, and all were positive for vvIBDV.

**Inoculation of the dog, clinical signs and faeces testing**

The ingestion by the dog of tissues from birds that
died due to vvIBDV infection did not produce any
abnormal (neither digestive nor general) signs
during the 7 days after ingestion. The dog faeces,
which were recovered at 24 and 48 h after ingestion
of the affected tissues, were positive for IBDV when
tested by RT-PCR. Samples of faeces recovered
before the ingestion and 72 h after ingestion were
negative for IBDV by RT-PCR. The results of
testing on agarose gel the RT-PCR products
obtained from samples of faeces and tissues are
shown in Figures 1 and 2.

**Study of the viability of IBDV found in the dog
faeces, using chicks**

The virus titre of the 10% suspension of faeces was
10^{2.25} EID_{50}/ml at 24 h post inoculation, 10^{1.65}
EID_{50}/ml at 48 h post inoculation and no virus was
detected at 72 h post inoculation, confirming the
PCR results.

The virus recovered from the faeces was admin-
istered to five SPF chicks. No clinical signs were
observed at 1 and 2 d.p.i. At 3 d.p.i., one of the
birds had died, three showed depression and
prostration, and the fifth showed no clinical signs.
At 4 d.p.i., two more animals had died, and the
remaining two were depressed and prostrated up to
5 d.p.i. At necropsy, the two surviving birds showed
clear signs of IBDV infection.
Discussion

In this study we have demonstrated that a dog fed chicken tissues infected with IBDV excreted that virus in its faeces from 24 to 48 h post ingestion. Despite the low number of experimental animals used, the qualitative results of this trial are not affected. Our aim was to use as few animals as possible, but the use of more animals would not have changed the final conclusions. We have also demonstrated that the virus excreted by the dog remained infective and also maintained its original pathogenic characteristics. We have not evaluated the possible infection of the dog with IBDV (virus detection in the dog’s tissues, body temperature, microscopic lesions, etc.) because our objective was to check the possible role of dogs as carriers of IBDV, and not the effect of the virus in the dog. Nevertheless, this could be investigated in future experiments.

The mortality rate in SPF chicks inoculated with the original virus was higher than that of the faeces-isolated virus. There was a difference in mortality, but the surviving birds given the dog-passaged virus had clear IBD lesions, indicating that the virus affected all the inoculated birds. We believe that these differences in mortality were due to differences in titre between the virus inoculated to the first group of birds and the virus obtained from the dog faeces, which was inoculated to the second group of birds (a difference of more than 2 log₁₀), and not to a difference in pathogenicity.

We believe that this study is of great interest since it is common that dogs are kept inside or near poultry facilities. If dogs are fed with dead birds infected with vvIBDV, they could play an important role as a reservoir of the virus inside the facility, or spread the virus from one poultry facility to another. This fact is supported by data from Lukert & Saif (1997), in which they describe the chemical and physical characteristics of the virus, indicating its great resistance to different ranges of acidic pH despite being more sensitive to basic pH. The same authors also mentioned the great resistance of the virus to high temperatures. Godeau (1993) showed the pH in a dog’s digestive tract during digestion to be 7 in the oral cavity, pH between 1 and 7 in the stomach, and pH between 4.5 and 7 in the intestine. No bibliographic data...
have been found concerning the pH of the faeces, but we measured a pH between 6.5 and 7 in the faeces of several Beagle dogs kept at our facilities. None of these pH values is lethal for IBDV.

Other interesting data provided by Buttin & Sergheraert (1993) on the length of time of intestinal transit of food showed a correlation between the time of transit and the water content of the food. The transit time oscillated between 3 and 8 h in the stomach, 1 h in the small intestine and between 12 and 16 h in the large intestine, which would give a total persistence time of about 26 h. These data are in accordance with the results obtained in this study, where virus was detected at 24 and 48 h post-ingestion. With respect to the body temperature of a dog (Tennant, 1994), the values oscillate between 37.5°C and 39°C, which means that the virus would not be affected. Lukert & Saif (1997) established that IBDV remained viable after 5 h at 56°C, indicating its thermal stability. We do not know the effect of the dog’s gastric enzymes on the viability of the virus, but from the results obtained we believe that the saliva amylase, the glucoylic enzymes (maltase, sucrose and lactose), peptidases, and lipases would not inactivate the virus.

Considering the similarity of intestinal physiology of other wild or domestic carnivores, we do not know whether the same statement could be applied to all carnivores, including cats and foxes, which are frequently found around poultry houses. Okoye & Uche (1986) described an IBDV antigen that was found in rats captured on a farm with continual problems due to IBD. We are not sure whether our findings could be extended also to rats, because in the aforementioned paper the antigen viability was not demonstrated.

On the other hand, we would like to emphasize that the problem could be solved with better management of dead carcasses inside the poultry houses. Even in the Spanish legislation (Real Decreto 328/2003, recently published as BOE No 81) the management of dead carcasses is not given the consideration that we believe it requires. Methods of discarding of carcasses, including incineration, burying and composting, are covered, but it is not clearly stated how to transport dead bodies for disposal and this could represent a high risk for spreading the virus.

It would be interesting to assess IBDV content directly from cloacal swabs by RT-PCR, instead of using tissues or bursa of Fabricius, which requires opening of the bird to reach the affected organs. We believe conclusions similar to those stated by Jackwood & Sommer (1999) would be reached.

In conclusion, we think that this study has provided important new information to further our understanding of the epidemiology of IBD. Taking into account the difficulties in neutralizing the virus in poultry facilities, this could help in the prevention and elimination of the virus from a possible carrier of the disease and help to establish better biosecurity measures.

References


RéSUMÉ

Le chien: porteur potentiel du virus de la bursite infectieuse aviaire

Cette étude, évalue la possibilité que le chien soit éventuellement un porteur du virus de la bursite infectieuse aviaire (IBDV) après avoir mangé (volontairement ou accidentellement) des poulets infectés par des IBDV. Seul un chien, de race Beagle, a été nourri avec des poulets infectés par une souche hypervirulente de l’IBDV (vvIBDV). Ensuite, la présence et la viabilité de l’IBDV ont été évaluées dans les fèces. Le vvIBDV viable a été détecté dans les fèces du chien durant les deux jours qui suivirent l’ingestion, ce qui indique qu’il y a eu exécration du virus. La comparaison des virus administré et excréter, par des méthodes de biologie moléculaire utilisant la RT-PCR et une digestion enzymatique confirme que le virus excréter a les mêmes caractéristiques que le virus
initial ingéré. Nous croyons que cette étude peut avoir un intérêt important pour une meilleure compréhension de l'épidémiologie de l'IBD dans fermes où les chiens vivent proches des bâtiments d'élevage.

ZUSAMMENFASSUNG

Hunde als potenzielle Überträger des Virus der infektiösen Bursitis


RESUMEN

Los perros como portadores potenciales del virus de la bursitis infecciosa

En este estudio se evaluó la posibilidad de que los perros pudieran eventualmente ser portadores del virus de la bursitis infecciosa (IBDV) tras haber comido (voluntaria o involuntariamente) pollos infectados con IBDV. Un único perro de raza Beagle fue alimentado con pollos infectados con una cepa muy virulenta de IBDV (vvIBDV). Después, se evaluó la presencia y viabilidad de IBDV en las heces. Se detectó vvIBDV viable en las heces del perro durante dos días tras la ingestión, lo cual indica que existe excreción de vvIBDV. La comparación, mediante técnicas moleculares como la RT/PCR y la digestión enzimática, del virus administrado y el excretado, demostró que el virus mantenía las mismas características tras ser excretado. Pensamos que este estudio puede ser de gran interés para comprender la epidemiología de la bursitis infecciosa en granjas en las cuales los perros viven cerca de las instalaciones avícolas.