Very virulent infectious bursal disease virus (vvIBDV) in vaccinated broiler flock: Course of the disease, identification and characterisation of isolated strain*

H. M. Hafez¹, Christine Prusas¹ and R. Raue²

* Dedicated to Prof. Dr. Gerhard Monreal, on behalf of his seventy-fifth birthday

Introduction

Infectious bursal disease (IBD) is an acute, highly contagious viral disease of young chickens. It is most often found in highly concentrated poultry producing areas throughout the world. IBD is caused by a virus that is classified as a member of the genus Avibirnavirus of the family Birnaviridae (Leong et al., 2000), which is characterised by a bisegmented dsRNA genome (Müller et al., 1979, Kibenge et al., 1988). There are many different strains of the IBD virus (IBDV), which vary considerably in their pathogenicity and global distribution. Since 1988, outbreaks of IBD caused by very virulent (vv) IBDV strains occurred in broilers in several European countries, Asia, Africa and recently in Central and South America (Chettle et al., 1989; van den Berg et al., 1991; Eterradossi et al., 1992; Nunoya et al., 1992; Di Fabio et al., 1999; Zierenberg et al., 2000). These strains are antigenically similar to the classic virulent strains but can be distinguished by RT-PCR combined with restriction enzyme analysis (REA) or by genome sequencing (Eterradossi et al., 1997; 1999, Zierenberg et al., 2000, 2001). In contrast, “variant strains” with a different antigenic profile were described in the USA. Vaccination failure was incriminated to be responsible for the emergence of antigenic variation (Rosenberger and Cloud, 1986).

In general, IBDV is resistant to many disinfectants and environmental factors, and remains infectious for at least four months in the poultry house environment. Because of the resistant nature of IBDV, once a poultry house becomes contaminated, the disease tends to recur in subsequent flocks (Lukert and Saif, 1997). Hygienic measures alone are ineffective and vaccination is essential. Several vaccines are available. When they are given correctly, good immunity and protection can be achieved (van den Berg and Meulemans, 1991). Beside the proper application the major problem with the live vaccination of young chickens with maternally derived antibodies (MDA) is determining the proper time of vaccination, through monitoring of the antibody level in a breeder flock or its progeny.

The present paper describes an outbreak of IBD in vaccinated broiler flocks.

Material and Methods

Detection of viral antigens and antibodies

Detection of IBD antigen in the bursa of Fabricius (BF) was performed in an agar gel precipitation test. A portion of the bursa was homogenised, used as antigen and tested against a positive polyclonal antiserum. For serological investigations agar gel precipitation tests were used as well.

Virus isolation

The virus isolation trials were carried out in embryonated SPF-eggs and in chicken embryo fibroblasts using bursa homogenates from affected birds (Rosenberger et al., 1998).

Reverse transcription-polymerase chain reaction (RT-PCR)

For further typing of the isolated virus strains, RT-PCR combined with REA was performed and the variable region of the VP2 gene was sequenced as described previously (Zierenberg et al., 2000, 2001). These techniques allow to distinguish between field and vaccine strains.

Results

Clinical observations

In a broiler farm with about 180.000 birds kept in 6 houses birds were vaccinated against IBD via drinking water at day 17 of age, according to the advice of the hatchery, using TAD¹ Gumboro Vac Forte (Lohmann Animal Health). Each dose contained at least $10^{1.0}$ EID₅₀ embyo-adapted (bursa derived) IBDV. At day 36 only in two houses (1 and 2) with 56.500 birds a sudden onset of depression, vent pecking, whitish diarrhoea accompanied with increase in mortality were observed. On necropsy, haemorrhages were found in the thigh and pectoral muscles. The BF appeared oedematous, hyperaemic, and had a gelatinous yellowish transudate covering the serosal surface. The kidneys appeared swollen. Microscopically, lymphocytes, necrosis oedema, hyperaemia, and inflammatory cell infiltration were found in the BF.
Virological and serological examination

Trials of direct IBDV antigen detection using polyclonal antibodies in agar gel precipitation test revealed positive results.

In chicken embryo fibroblasts IBDV was isolated in the first passage. The isolation in embryonated SPF-eggs was successful after the second passage (Table 1). Testing of 10 serum samples from each house using Agar gel precipitation test at the time of slaughter revealed positive results of all samples.

Molecular biological identification and typing

As the used conventional isolation methods do not allow to distinguish between field and vaccinal strains, further investigations using RT-PCR in combination with REA or sequencing of the variable region in the VP2 gene were carried out.

The results after RT-PCR combined with REA revealed that the strain isolated in tissue culture showed a classic virulent IBDV genotype, whereas the analysis of the bursal material and the allantoic fluids of infected SPF-eggs indicated the presence of two IBDV strains, a classical virulent and a very virulent IBDV strain (Fig. 1). The obtained results are summarized in Table 2.

To get further information of the isolated strains, the variable regions of the VP2 gene were sequenced and the amino acids were compared (Table 3). In the case of GB887/01-B and GB887/01-E a faint additional sequence was visible indicating a second virus strain. However, the amino acid pattern of the main sequence of GB887/01-B and GB887/01-E revealed the vvIBDV genotype. As expected, the IBDV strain isolated from tissue culture (GB887/01-TC) showed the same amino acid pattern as the vaccine strain LC-75 (TAD® Gum-boro Vac Forte).

Economic parameters

The total mortality at the time of slaughter (day 39) was 6.1% and 5.1% in house 1 and 2, respectively. The total mortality rate in the non-infected flock (house 3) was 1.9%. The IBD related mortality rates were 4.5% (house 1) and 2.9% (house 2). In house 1, where the highest mortality rate was observed, a slight reduction in daily weight gain as well as in production number were observed (Table 4).

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Table 1. Results of virological examination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Designation of the isolate</th>
<th>No. of passages required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonated eggs</td>
<td>GB 887/01-E</td>
<td>2</td>
</tr>
<tr>
<td>Chicken embryo fibroblasts</td>
<td>GB 887/01-TC</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Summary of the results of RT-PCR combined with REA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Designation of the isolate</th>
<th>Judgment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursa homogenate</td>
<td>GB 887/01-B</td>
<td>classical virulent</td>
</tr>
<tr>
<td>Embryonated eggs</td>
<td>GB 887/01-E</td>
<td>classical virulent + very virulent</td>
</tr>
<tr>
<td>Chicken embryo fibroblasts</td>
<td>GB 887/01-TC</td>
<td>classical virulent</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the amino acids of selected positions of classical virulent (cv) IBDV, very virulent (vv) IBDV, the vaccine strain LC-75 and the investigated isolates

<table>
<thead>
<tr>
<th>Amino acid position</th>
<th>cvIBDV</th>
<th>vvIBDV</th>
<th>LC-75</th>
<th>GB887/01-B</th>
<th>GB887/01-E</th>
<th>GB887/01-TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>222</td>
<td>P</td>
<td>A</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>P</td>
</tr>
<tr>
<td>242</td>
<td>V</td>
<td>I</td>
<td>V</td>
<td>I</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>253</td>
<td>H</td>
<td>Q</td>
<td>H</td>
<td>Q</td>
<td>Q</td>
<td>H</td>
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<tr>
<td>256</td>
<td>V</td>
<td>I</td>
<td>V</td>
<td>I</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>279</td>
<td>N</td>
<td>D</td>
<td>N</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>284</td>
<td>T</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td>294</td>
<td>L</td>
<td>I</td>
<td>L</td>
<td>I</td>
<td>I</td>
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</tr>
<tr>
<td>297</td>
<td>S</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>299</td>
<td>N</td>
<td>S</td>
<td>N</td>
<td>S</td>
<td>S</td>
<td>N</td>
</tr>
<tr>
<td>330</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>

* between amino acid position 206 and 350
+ main sequence
GB887/01-B: Bursal material
GB887/01-E: strain isolated in embryonated SPF-eggs.
GB887/01-TC: strain isolated in chicken embryo fibroblasts.
Typical amino acids for the vvIBDV genotype are in bold.
Amino acids responsible for the adaptation to tissue culture are in italic.
houses, birds were vaccinated against IBD via drinking water at day 17 of age, according to the advice of the hatchery, using TAD® Gumboro Vac Forte (Lohmann Animal Health). At day 36 only in two houses (1 and 2) with 56,900 birds a sudden onset of depression, vent pecking, whitish diarrhoea accompanied with increase in mortality were observed. On necropsy, haemorrhages were found in the thigh and pectoral muscles. The BF appeared oedematous, hyperaemic, and had a gelatinous yellowish transudate covering the serosal surface. Trials of direct IBDV antigen detection using polyclonal antibodies in agar gel precipitation test revealed positive results. In chicken embryo fibroblasts IBDV was isolated in the first passage. The isolation in embryonated SPF-eggs was successful after the second passage. Further investigations using RT-PCR in combination with restriction enzyme analysis and sequences of variable region VP2 was carried out. The result revealed that isolated virus in tissue culture was classic virulent IBDV (Vaccinal strain). Examination of the bursa homogenate as well as the isolated virus in chicken embryo resulted in the detection of vIvBDV as well as the classic strain (Vaccinal strain).

The total mortality rate at the time of slaughter (day 39) was 6.1% and 5.1% in house 1 and 2, respectively. The total mortality in the non-infected flock (house 3) was 1.9%. The IBD related mortality rates were 4.5% (house 1) and 2.9% (house 2). In house 1, where the highest mortality rate was observed, a slight reduction in daily weight gain as well as in production number was detected. The results described here indicate either a failure in the vaccination procedure in these houses or that the birds of these houses might originated from other breeder flocks with different maternal derived antibody titres.

**Key words**
Infectious bursal Disease, Diagnosis, Vaccination, Vaccina
tion failure

**Zusammenfassung**

_Hochpathogenes Infektöses Bursitis Virus (vIvBDV) in geimpften Broilerherden: Verlauf der Erkrankung, Identifizie
gerung und Charakterisierung des isolierten Stammes_


Die gesamte Mortalitätsumrate zum Zeitpunkt der Schlachtung (39 Tag) lag bei 6.1% in Stall 1 bzw. 5.1% in Stall 2. Die ge
samte Mortalität der nicht infizierten Herde (Stall 3) betrug 1.9%. Die Mortalitätsraten, die durch IBD verursacht wurden, lagen bei

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**Table 4. Economic parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>House 1</th>
<th>House 2</th>
<th>House 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of birds</td>
<td>28,594</td>
<td>28,305</td>
<td>28,305</td>
</tr>
<tr>
<td>Age at vaccination (day)</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Age at onset of clinical signs</td>
<td>36</td>
<td>36</td>
<td>No signs</td>
</tr>
<tr>
<td>Total mortality rate (%)</td>
<td>6.1</td>
<td>5.1</td>
<td>1.9</td>
</tr>
<tr>
<td>IBD related mortality rate</td>
<td>4.5</td>
<td>2.9</td>
<td>(0.1)*</td>
</tr>
<tr>
<td>Daily weight gain (g)</td>
<td>43.23</td>
<td>44.79</td>
<td>43.35</td>
</tr>
<tr>
<td>Feed conversion rate</td>
<td>1:1.83</td>
<td>1:1.81</td>
<td>1:1.85</td>
</tr>
<tr>
<td>Production number</td>
<td>221.92</td>
<td>234.94</td>
<td>229.94</td>
</tr>
</tbody>
</table>

* non – IBD related mortality

**Discussion**

An effective IBDV control has to involve an effective vacci
cination program for breeder, broiler and pullet flocks. When the vaccine is applied correctly, good immunity and protection can be achieved (van den Berg and Meulemans, 1991). The vaccination program for each re
gion or company should be established on the basis of the evaluation of the circulated field virus strain, its anti
genicity and pathogenicity. In addition, several other as
perts such as the poultry density in an area and manage
tment or environmental associated stress should be taken into account.

Beside the proper application the major problem with the live vaccination of young chickens with MDA is deter
mining the proper time of vaccination, through monitoring of the antibody level in a breeder flock or its progeny (Luk
tert and Saif, 1997). However, if the MDA titres are not uniform in a flock, multiple costly vaccinations may be required (Kouwenhoven and van den Bos, 1994). Some causes of poorly uniform MDA titres are, poor vaccine adm
istration in broiler breeders and mixing of broilers from different breeder flocks. The best way to reduce these problems is to avoid as much as possible mixed hatches from different breeder flocks. When it is not possible, it is advisable to mix offspring from flocks with identical or very similar MDA titres (Kouwenhoven and van den Bos, 1994). Furthermore, specially in the USA and some parts of South America, the reason of the out
breaks in vaccinated flocks may be due to use of vaccine that does not include all types of “variant” strains.

The more frequent reason for outbreaks in vaccinated flocks is incorrect application of the vaccine. In the pres
tent field investigation birds in all houses are vaccinated at day 17 according to the advise of the hatchery based on antibody titre of the breeder flock. It is clearly that the chickens in the two houses (1 and 2) were infected with a vvIBDV strain accompanied with clinical signs, lesions and increased mortality rates in spite of the vaccination at day 17. The results described here indicate either a failure in the vaccination procedure in these houses or that the birds of these houses might originated from other breeder flocks with different MDA titres.

**Summary**

In a broiler farm with about 180,000 birds kept in 6 houses, birds were vaccinated against IBD via drinking water at day 17 of age, according to the advice of the hatchery, using TAD® Gumboro Vac Forte (Lohmann Animal Health). At day 36 only in two houses (1 and 2) with 56,900 birds a sudden onset of depression, vent pecking, mortality were observed. On necropsy, haemorrhages were found in the thigh and pectoral muscles. The BF appeared oedematous, hyperaemic, and had a gelatinous yellowish transudate covering the serosal surface. Trials of direct IBDV antigen detection using polyclonal antibodies in agar gel precipitation test revealed positive results. In chicken embryo fibroblasts IBDV was isolated in the first passage. The isolation in embryonated SPF-eggs was successful after the second passage. Further investigations using RT-PCR in combination with restriction enzyme analysis and sequences of variable region VP2 was carried out. The result revealed that isolated virus in tissue culture was classic virulent IBDV (Vaccinal strain). Examination of the bursa homogenate as well as the isolated virus in chicken embryo resulted in the detection of vIvBDV as well as the classic strain (Vaccinal strain).

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nation procedure in these houses or that the birds of these houses might originated from other breeder flocks with different maternal derived antibody titres.
4.5% (Stall 1) und 2.9% (Stall 2). In Stall 1 mit der höchsten Mortalitätsrate wurden eine geringe Reduktion der täglichen Gewichtszunahme sowie eine Reduktion der Produktionszahlen festgestellt. Die hier beschriebenen Ergebnisse sprechen dafür, dass entweder Fehler bei der Durchführung der Impfung unterlaufen sind oder die Tiere dieser Ställe aus anderen Elterntierherden stammten und abweichende maternale Antikörpertiter besaßen.

**Stichworte**

Infektiöse Bursitis, Diagnose, Vakzination, Impffehler

**References**


Correspondence: Prof. Dr. Hafez Mohamed Hafez, Institute of Poultry Diseases, Free University Berlin, Koserstr. 21, 14195 Berlin, Germany, e-mail: hafez@zedat.fu-berlin.de