ANTIGENIC CHARACTERIZATION
OF POLISH INFECTIOUS BURSAL DISEASE VIRUS STRAINS

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Antigen Capture-ELISA based on eight different neutralizing mouse monoclonal antibodies (Mabs) was used to study Polish infectious bursal disease viruses (IBDVs) isolated from two epidemics on the turn of 70/80s (early IBDV) and in the 90s (recent IBDV). They were compared to the Faragher 52/70 (F52/70) reference strain of European classical serotype 1 IBDV and to the 89/163 (typical) and 91/168 (atypical) French very virulent (vv) IBDV isolates. All Polish viruses, irrespective of the isolation date, exhibited antigenic profile which was different from the F52/70 reference strain (no binding of Mabs 3 and 4). Two of the early IBDVs did not react with Mab 5 and two of six recent IBDV did not react with Mab 8. The other recent Polish viruses had an antigenic reactivity similar to that of typical vvIBDV.

Key words: infectious bursal disease virus, IBDV, Antigen Capture–ELISA, monoclonal antibody.

Infectious bursal disease (IBD) is an important virus-induced, highly contagious disease of chickens (31). The disease causes heavy economical losses due to immunosuppression in subclinical cases or to acute cases associated with mortality, haemorrhages and bursal damage. Since the first report in the USA (3) the disease became widespread all over the world. In Europe it appeared for the first time in the middle of 1960s. Until 1987 the strains of the virus were of low virulence, they caused low mortality, and were satisfactorily controlled by vaccination. However, in 1987 some vaccination failures were described in different parts of the world (2, 10, 24, 28). In the USA, it was demonstrated that the new isolates had been affected by an antigenic drift against which classical IBD virus (IBDV) vaccines were not satisfactorily protective (11, 27). In Europe, the first cases of acute IBDV were described at the same time (2, 28). The acute forms of the disease, caused by very virulent strains, were then described in Japan in the early 1990s (12, 23) and they have rapidly spread all over the world (30). During the 63rd General Session of the Office International des Epizooties (OIE, Paris, 15-19 May 1995), it was estimated that IBD has a considerable socio-
economic importance at the international level, as the disease is present in more than 95% of Member Countries (6).

IBDV is a small, non-enveloped virus belonging to the family Birnaviridae. The genomes of the virus consist of two segments of double-stranded RNA contained within a single-shelled icosahedral capsid composed of four structural proteins (4). Two of these proteins, VP2 and VP3, are major immunogens. VP2 (MW 40-45 kDa) encoded by the larger genome segment, has major neutralizing, conformational epitopes within a central variable region. This part is named “VP2 variable domain” because most of the amino acids (aa) changes between antigenically different IBDVs are clustered in this area. Major differences in the reactivity of IBDV strains with neutralizing Mabs may be referred to aa changes in two hydrophilic aa sets termed peak A and B which flank VP2 variable domain (25, 29).

Antigenic diversity among IBDV isolates has been recognized since 1981 when serotypes 1 and 2 were defined on the basis of their lack of in vitro cross-neutralization (15). So far, virus strains that are pathogenic for chicken have all been shown to belong to serotype 1. Further antigenic differences have been demonstrated within this serotype since 1984, and the study of North-American IBDV isolates causing little mortality but significant immunosuppression has led to dividing the serotype into six subtypes which were originally differentiated by cross-neutralization assays using polyclonal sera (11). Studies based on Mabs subsequently demonstrated a growing number of modified neutralizing epitopes in the recent serotype 1 isolates from the United States, which were designated as “variant viruses”. It was hence suggested that North-American IBDV isolates might have been affected by an antigenic drift and the epidemiology of IBDV in the US has been defined since then by the natural occurrence of neutralizing monoclonal antibodies escape variants (26, 27).

In Poland first cases of IBD were diagnosed at the end of 60s but the real epidemic of IBD occurred at the end of 70s (1, 14, 16). Despite of a wide spreading of IBDV, the clinical form of the disease was rarely observed. The field strains isolated in 1978 and 1980 revealed low pathogenicity for SPF chickens (17, 18). However, they caused economic losses due to impaired growth and acquired immunodeficiency (19).

At the end of 1991 the first cases of acute form of IBD were diagnosed in western and central Poland. During the next year the disease spread rapidly throughout the whole country and affected broilers and laying pullets flocks with mortality up to 50% and 70%, respectively (20, 21). Pathotypic characterization of the IBDVs isolated in 1991-93 confirmed their high virulence (22). Mortality rates in challenged 4-week-old SPF chickens ranged from 40 to 100%. The aim of the present study was to compare antigenically early and recent Polish IBDV isolates in a Mab-based Antigen Capture-ELISA (AC-ELISA).

Materials and Methods

Viruses. Three early Polish isolates 78/GSi, 78/GSz and 80/GA were collected during the first epidemic of IBDV in Poland between 1978-80. Six recent IBDV strains were isolated in 1990-2000 from chickens with clinical symptoms of acute form of IBDV. The reference IBDV strains included in this study were the Faragher 52/70 (F52/70) strain as reference for the classical European serotype 1 strains, the French 89/163 strain as reference for the very virulent (vv) IBDV and the 91168 strain as an atypical vvIBDV (7). The viruses (isolates and strains) were propagated in 4-to-6-
week-old SPF chickens, with sampling of the infected bursae 4 days p.i. Bursal homogenates were prepared by blending the bursae w/v in phosphate buffered saline (PBS), then clarified v/v in Freon. All viruses were stored at -70°C.

**AC-ELISA.** The antigenic characterization was performed using a Mab based AC-ELISA according to Eterradossi (7). The study was performed in two steps, the first aimed at standardizing the amount of captured antigens (with polyclonal ascitic fluid), the second one at measuring Mab binding in a Mab-based AC-ELISA (7). Briefly, 96-well polystyrene plates were coated with a chicken anti-F52/70 polyclonal antiserum, diluted in PBS. Blocking was then achieved by incubating the coated plates overnight at 37°C with a blotto-tween solution containing 10% foetal calf serum and 0.02% sodium azide. Bursal homogenates were then incubated with the coated plates for 1 h at 37°C. After incubation the captured IBDV antigens were detected with mouse polyclonal anti-IBDV antibodies or with a panel of eight mouse anti-IBDV neutralizing Mabs 1, 3, 4, 5, 6, 7, 8 and 9 (ascitic fluids) followed by a goat anti-mouse alkaline-phosphatase conjugate (KPL, USA), both incubated for 1 h at 37°C. Optical densities (OD) were read at 405 nm after incubating an alkaline-phosphatase substrate (pNPP, Sigma, USA) for 1 h at 37°C. All immunological reagents used in each step of the assay were first equilibrated so that the capture of an undiluted infected bursal homogenate would yield an OD averaging the maximum reading value of the reader (3.000). In the next step the equilibration of the captured antigen was assayed; dilutions yielding 50% of the maximum OD were used in the antigenic typing. These dilutions ensure that differences in the intensity of binding of Mabs originate from true antigenic differences of the tested viruses, and not from the testing of different quantities of the same virus that may be captured at various level resulting from variation of the antigenic content in bursae infected by the same virus. Additionally, a few controls were included in each assay: the evaluation of the unspecific binding of mouse antibody (an equilibrated dose of the infected homogenates with a negative mouse ascitic fluid containing no anti-IBDV Mab), the control of the 50% effect that was to be yielded by the equilibrated antigens (an equilibrated dose of both the infected and the SPF homogenates with a mouse polyclonal ascitic fluid) and the control of the maximum effect (the undiluted infected and SPF homogenates with polyclonal ascitic fluid). The reactivity of the different Mabs was calculated as a percentage, according to the formula below (4, 6, 9).

\[
\text{Percentage of reactivity} = \frac{\text{OD}_{\text{Mab}} - \text{OD}_{\text{Negative ascitic fluid}}}{\text{OD}_{\text{Reference polyclonal}} - \text{OD}_{\text{Negative ascitic fluid}}} \times 100
\]

**Results**

In AC-ELISA, all bursal homogenates containing the recent Polish IBDVs proved to contain high amounts of IBDV antigens, and compared well with the reference F52/70-, 89/163- and 91/168-containing homogenates (optical densities ranging 2.2 to 2.9). On the contrary, all homogenates prepared from bursae infected with the early Polish isolates contained low amounts of antigens (optical densities ranging from 0.5 to 1.0).
Table 1 presents the reactivity of the eight anti-IBDV Mabs in paired AC-ELISAs performed with 12 viruses (the following marks are used: differences in the Mab reactivity of the tested viruses from F52/70 are evidenced as gray areas of the table, reduced binding (<25%) of the Mab with the tested viruses are evidenced as pale shading, lack of binding (<15%) as darker shading of the table). None of the tested Polish IBDV strains allowed significant binding of Mab 3 and 4 (mean reactivities of these Mabs ranging from 0-2% and 0-14%, respectively). This result suggests that the studied viruses, irrespective of their date of isolation, might have aa changes in the first major hydrophilic peak (peak A) of VP2 variable domain. This was a very interesting finding, for the early IBDV strains isolated before the emergence of the acute form of IBD all had shown a classical antigenicity (high reactivity versus all Mabs as a reference F52/70). Moreover, two early Polish viruses (78/GSi and 80/Ga) did not react with Mab 5 and also exhibited a two- to three-fold reduction in the binding of Mab 8 (mean reactivities were 31% and 21% for 78/GSi and 80/Ga, respectively, versus 2-3% in atypical vv IBDV or 82-88% in typical isolates). From previous studies, the lack of or a low reactivity with Mab 5 was considered as an indication of aa changes in the first minor hydrophilic peak of VP2 variable domain. Two of the recent Polish viruses (92/111 and 93/35) exhibited an antigenic profile similar to the atypical French 91/168 isolate as they did not react with Mab 8. This result suggested that aa changes may exist in their second major hydrophilic peak B. The 91/272, 94/48, 99/150 and 00/40 viruses had an antigenic reactivity similar to that of typical vvIBDV.

Discussion

Regarding recent Polish viruses, four of them had exactly the same antigenic profile as typical vvIBDV (no binding of Mabs 3 and 4). The present study could confirm previous pathotypic studies and demonstrate that vvIBDV is now present in Poland. Interestingly, two of the recent Polish viruses (isolates 92/11 and 93/35) appeared to be antigenically different from other vvIBDV, as did the 91/168 French atypical vvIBDV strain, because they all had a reduced ability to bind Mab 8. As shown for the French strain 91/168 responsible for this are aa differences located at the C-terminal end of peak B (Q324L in 91/168 in comparison to other typical vvIBDV) (8). As these atypical French and Polish vvIBDV-related isolates have been isolated during a relatively short period (1991-1993) and so far have not been isolated elsewhere, it is tempting to speculate that the same IBDV strains were exchanged between the two countries. However, the epidemiological links between the outbreaks in the two countries are not known and more isolates collected in the same areas / periods would be necessary to precise where the virus was originally prevalent. A 1989-1997 survey of French vvIBDV isolates suggested that the 91/168 strain caused only a sporadic outbreak in France and did not replace in this country the more typical 89163-like vvIBDV.
Table 1
Antigenic characterization of Polish IBDV strains in a Mab-based AC-ELISA

<table>
<thead>
<tr>
<th>IBDV strain</th>
<th>Reactivity in AC-ELISA with</th>
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<tbody>
<tr>
<td></td>
<td>Mab1</td>
</tr>
<tr>
<td>F52/70</td>
<td>72\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>71-72\textsuperscript{b}</td>
</tr>
<tr>
<td>89/163</td>
<td>58</td>
</tr>
<tr>
<td>typical vvIBD V</td>
<td>50-66</td>
</tr>
<tr>
<td>91/168</td>
<td>62</td>
</tr>
<tr>
<td>atypical vvIBD V</td>
<td>61-62</td>
</tr>
<tr>
<td>78/GSi</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>26-35</td>
</tr>
<tr>
<td>78/GSz</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>52-70</td>
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<tr>
<td>80/GA</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>16-18</td>
</tr>
<tr>
<td>91/272</td>
<td>68</td>
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<td></td>
<td>45-91</td>
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<tr>
<td>92/111</td>
<td>82</td>
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<td>66-98</td>
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<td>93/35</td>
<td>72</td>
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<td>66-78</td>
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<td>94/48</td>
<td>65</td>
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<td>62-67</td>
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<td>99/150</td>
<td>89</td>
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<td>97-80</td>
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<td>00/40</td>
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<td>71-72</td>
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\textsuperscript{a}: Mean result from two repeated tests.
\textsuperscript{b}: Range in two repeated tests.
Unexpected results were also obtained with the 78/GSz, 78/GSi and 80/GA early Polish viruses. Indeed, these isolates did not react with Mabs 3 and 4 and the two latter viruses also reacted poorly, if at all, with Mabs 5 and 8. Hence, the neutralizing epitopes present on the early Polish IBDVs were clearly different in comparison with both the European F52/70 reference strain and the recent vvIBDV isolates from Poland.

With respect to strain identification in the laboratory, the results concerning early Polish viruses are interesting as several previous studies had suggested that the lack of reactivity to Mabs 3 and 4 in AC-ELISA might be a character of very virulent and variant IBDVs and help in differentiation between classical and recent isolates in addition to pathogenicity testing (9). The present results indicate that early Polish IBDVs isolated between 1978-80, hence before the identification of variant and very virulent strains, may also not react with Mab 3 and 4, in spite of being early strains with a low pathogenicity. It should be remembered that bursal homogenates contaminated with the early Polish isolates contained low amounts of IBDV antigens, as compared with homogenates containing pathogenic strains such as F52/70 or the vvIBDVs. Hence, as precised previously (6, 9), both the high antigen content in bursal homogenates and the lack of binding of Mabs 3 and 4 should be considered as relevant in the presumptive identification of vvIBDVs. The identification should always be definitively assessed by pathogenicity testing (8).

With respect to IBDV epidemiology, the present results of testing early Polish isolates are also interesting. Indeed, the current idea is that early IBDVs, prevalent worldwide until the emergence of the US variants and vvIBDVs, were “classical” ones with more or less the same antigenic profile as strain F52/70. This theory stems from the antigenic and genetic study, firstly of a limited number of early pathogenic viruses which have been maintained as reference strains (e.g. the 002/73, STC, CuI and F52/70 strains), and secondly of some IBD attenuated vaccine strains which have been developed from several early IBDV isolates (Lukert strain, D78, etc). However, the extent to which early IBDVs were actually antigenically and genetically homogeneous is not known precisely, as the number of available early IBDV strains is, unfortunately, limited. Similarly, it is not known whether other IBDV strains, possibly involved in subclinical infections, were present in Europe before the F52/70-like viruses were isolated. These points might be important to investigate, as the epidemiological origin of the IBDV strains that have recently emerged in Europe is still unknown. The present report showing that early Polish isolates appear antigenically original suggests that more diversity than currently admitted might have existed in Europe prior to the emergence of the vvIBDV. Such an assertion is in accordance with a recent report by Mato et al. (12) showing that several early Hungarian IBDV isolates collected in the late 70s and early 80s belong to a genetic cluster significantly different from the “classical” viruses. Analysis of more early IBDV isolates is necessary to get a more precise insight into the actual epidemiology of IBD.

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References