

PATHOGENICITY OF CZECH ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS

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Abstract

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An epidemics of infectious bursal disease (IBD) affected chicken farms in the Czech Republic in the mid nineties. Characteristics of six strains of infectious bursal disease virus (IBDV), isolated from broiler chickens, laying hybrid pullets, and chickens kept on small farms, were compared using standard methods of assessment of lymphatic tissue (cloacal bursa, thymus, spleen) index and morphological lesions indicative of immunosuppression, and haematological tests. The results of the investigations confirmed the presence of pathogenic IBDV strains in the local chicken population; four of the isolates induced in susceptible chickens clinical IBD manifested by inflammatory and degenerative lesions in cloacal bursa, thymus and spleen. Irrespective of the severity of the clinical disease, all the isolates induced significant ($p < 0.05$) bursal atrophy on post-infection days 3 to 6 and a serious damage to the two central lymphatic organs. The effect of infection on haematopoietic tissues was manifested by shifts in the leukogram and in percentages of immature and mature lymphocytes. Infection by some of the isolates resulted in early predominance of heterophils and an increase in the number of mature lymphocytes. Increased number of lymphocytes and a higher percentage of immature lymphocytes were found in chickens recovered from the infection.

Although the isolates differed from each other in the indexes of pathogenicity, cloacal bursa, and thymus, they showed similar immunosuppressive activity in regard to the primary lymphatic tissues. The IBDV isolates can be classified as standard strains differing in virulence. None of the isolates showed characteristics of a variant IBDV strains.

Domestic chickens, infectious bursitis, infectious bursal disease virus, pathogenicity, immunosuppression, IBDV isolates

Epidemics of infectious bursal disease (IBD), affecting in the eighties and nineties most of the countries with advanced poultry industry, extended from Slovakia to Czech poultry farms in the mid nineties (Pospíšilová 1997). Outbreaks of IBD caused by virulent strains were reported from European countries (Box 19889; Chettle et al. 1989; Van den Berg et al. 1991), western Africa (Cissé et al. 1989; Etteradossi et al. 1999), the United States (Rosenberg and Claud 1986; Saif et al. 1987; Sharma et al. 1989, Ismail et al. 1990), and Japan (Nuoga et al. 1992; Tsukamoto et al. 1992). In addition to the standard IBDV strains, domestic chickens can be infected by local variant strains causing bursal atrophy without signs of inflammation and damage to thymus (Rosenberg 1987); the infections are accompanied by severe suppression of humoral and cell-mediated immunity (Sharma et al. 1989). Immunisation with vaccines prepared of a standard strain does not protect against challenge with variant strains (Craft et al. 1990). The first outbreaks of acute bursitis in the Czech Republic were diagnosed in broiler chicken operations, flocks of hybrid laying pullets, and sporadically on small farms. Since outbreaks were reported also from vaccinated flocks, the question whether variant strains of IBDV circulate in the Czech chicken population has been raised.

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Six IBDV strains isolated from field outbreaks were tested for pathogenicity and immunosuppressive activity in susceptible chickens. The objective of our study was to compare the characteristics of the isolates using standard methods of assessment of lymphatic organs index, lesion scoring, immunosuppressive effects, and haematological parameters.

Materials and Methods

Chickens purchased from a controlled flock (Striped Leghorns, Czech Academy of Sciences, Prague) free of antibodies to IBDV were used in the experiments.

Six strains of IBDV (A through F) were isolated from field cases of clinical infectious bursal disease in broiler chickens (strains B, D, and F), a laying pullet flock (strain A), and on small farms (strains C and E). Inocula were prepared from samples of bursal tissue by the standard method (Rosenberger 1989) and each isolate was passaged four times in susceptible chickens. The chickens were inoculated intranasally with 0.1 ml of supernatant of the 4th passage. Titres of the inocula ranged from 10^4 to 10^5 EID₅₀. Blood samples for the enumeration of red and white blood cells by the dilution method were collected from all the experimental and the control chickens after decapitation into tubes containing an oxalate evaporation residue as described by Wintrobe. Blood smears stained panoptically with Papanicolau were used for qualitative assessment of the leukogram.

Blood serum samples obtained from the experimental and the control chickens were tested for the presence of antibodies to IBDV by ELISA using the commercial IDEXX kit.

Samples of lymphatic tissues (bursa, thymus, spleen) were fixed in 10% neutral formaldehyde solution, processed by the conventional paraffin technique, and stained with haematoxylin and eosin. Pathogenicity of four selected IBDV isolates (A, B, C, D) was assessed in terms of bursal and thymus indexes, score of microscopic lesions in the central lymphatic organs, and pathogenicity index calculated from morbidity and mortality data. The first criterion for the assessment of morphological changes indicative of immunosuppression was relative weight (index) of bursa and spleen calculated using the formula organ weight (g) \times 1000 / body weight (g).

Morphological lesions in lymphatic tissues indicative of immunosuppression were assessed as recommended by Halouzka (1991). The degree of immunosuppressive (Si) changes in bursa, thymus, and spleen was scored numerically from 0 to 4. The index of immunosuppression (Is), calculated as the ratio between the sum of scores of immunosuppressive changes in the individual chickens and the number of examined chickens, was used for the general assessment of histological lesions in lymphatic tissues.

Procedure of Experiment 1

Twenty-four-day-old chickens were divided into 2 groups of 24 isolated from each other. One of the groups was infected with one of the IBDV isolates (A through F) and the other served as controls. Four chickens of the experimental and the control groups were collected daily, weighed, sacrificed by decapitation after collection of blood samples for haematological examination and necropsied. Spleens and bursae were weighed to calculate their relative weights and thymic, bursal and splenic samples were collected for microscopic examination. The last group of four chickens was tested only serologically on post-infection day 24.

Procedure of Experiment 2

Four IBDV isolates (A – D) were selected for pathogenicity tests considering their characteristics established in Experiment 1. Forty 4-week-old chickens free of maternal antibodies to IBDV were divided into five groups of eight of which four were inoculated intranasally with 0.1 ml of supernatants of the strains A, B, C, or D, respectively, and one served as the control group. State of health of the birds was monitored for 10 days. Thereafter, the survivors were sacrificed by decapitation and examined. Data on body weight, morbidity, mortality, and bursal, splenic, thymic, and pathogenicity indexes were recorded.

The results were processed using the software StatPlus Version 1 (Matoušková et al. 1994).

Results

Clinical signs typical of acute IBD were observed from day 2 to day 5 after the infection in chickens with the strains A, B, D, or F. Significant body weight loss was recorded in chickens infected with the strains B, D, or F. Clinically inapparent IBD developed in chickens infected with the strains C or E.

Postmortem findings in dying and sacrificed chickens corresponded to the respective stage of acute IBD. Marked bursitis was found particularly in the group infected with the strain A. All the infected groups developed bursal atrophy from postinfection day (hereinafter only day) 4. Moreover, atrophy of thymic lobes was observed at the same time in the chickens infected with the strains A, B, C, D, or E, and pale bone marrow in those infected with the strains B, C, D, or F.

Significant increases in relative weights of bursa and thymus were recorded on day 2 in the chickens infected with the strain A; significant ($p < 0.05$) bursal atrophy was recorded from day 3 in the chickens infected with the strains E or F, and from day 4 in those infected with the strains A, B, C, or D. Spleen swelling developed in the infected chickens from day 2 (Table 1).

Table 1
Mean bursal and spleen weight indices at days 2 through 6 after infection with IBDV isolates

IBDV	Mean bursal weight indices					Mean spleen weight indices				
	2	3	4	5	6	2	3	4	5	6
A	5.1 ^a	3.1	2.3 ^b	2.2 ^b	1.6 ^b	2.5 ^a	2.3 ^a	3.0 ^b	3.3 ^a	2.1
Control	3.4	3.1	3.9	3.6	3.4	1.4	1.2	1.2	1.3	1.6
B	3.4	2.9	2.7 ^a	2.0 ^a	1.5	2.1 ^a	1.9	1.7 ^a	3.9 ^b	2.2
Control	3.1	3.0	4.2	3.3	5.6	1.2	1.4	1.1	1.7	2.1
C	4.0	2.6	2.6 ^a	2.5 ^b	2.2	2.9 ^a	2.6 ^a	3.3 ^b	4.2 ^a	4.0
Control	3.6	3.5	4.6	3.6	4.6	1.5	1.2	2.1	1.9	3.1
D	2.8	2.9	2.5 ^a	3.0 ^b	2.1	2.8 ^a	2.1 ^b	2.3	3.0	3.9
Control	3.6	3.5	4.6	3.6	4.6	1.5	1.2	2.1	1.9	3.1
E	3.6	2.6 ^b	2.5 ^a	2.0 ^a	1.8 ^a	2.4 ^b	2.6	3.9 ^b	3.0	2.4 ^b
Control	3.6	4.6	3.7	3.8	4.1	1.8	2.0	1.8	2.1	1.5
F	2.2	2.5 ^b	2.5 ^a	1.9 ^a	1.9 ^a	2.0	2.4	2.6	2.8	2.3 ^b
Control	3.6	4.6	3.7	3.8	4.1	1.8	2.0	1.8	2.1	1.5

a,b: difference significant at $p < 0.01$ and $p < 0.05$, respectively

Bone marrow anaemia was associated with a change in erythrocyte counts in all the infected groups from day 2. At the same time, a shift in the leukogram occurred (Table 2);

Table 2
Effects of infection with IBDV isolates on leukogram at post-infection days 2 through 6

IBDV isolate	Lymphocytes (%)					Heterophils (%)				
	2	3	4	5	6	2	3	4	5	6
A	42	45	83 ^a	71 ^b	62	55	51	16 ^a	27 ^b	34
Control	57	58	58	53	63	38	39	40	45	34
B	35 ^a	45	67	73 ^a	71	64 ^a	53 ^b	32	24 ^a	27
Control	59	61	67	51	61	37	34	28	44	37
C	26 ^a	49	59	61	74 ^b	74 ^a	49	41	36	23 ^b
Control	57	63	55	57	65	40	33	44	40	34
D	33 ^a	55	nt	48	70	65 ^b	45	nt	49	26
Control	57	63	55	57	65	40	33	44	40	34
E	31 ^a	51	67	58	63	67 ^a	48	30	39	33
Control	59	56	62	51	64	37	41	35	47	31
F	25 ^a	48	68	69 ^a	71	73 ^a	50	30	28 ^a	25
Control	59	56	62	51	64	37	41	35	47	31

a,b: difference significant at $p < 0.01$ and $p < 0.05$, respectively

the initial heterophilia and lymphocytopenia observed in the chickens infected with the strains B, C, D, E, or F from day 2 was followed by lymphocytophilia and heteropenia. Another shift occurred in percentages of mature and immature lymphocytes. A higher percentage of mature small lymphocytes observed in chickens infected with the strains A, D, or F at day 2 was followed by an increase in the percentages of immature medium and large lymphocytes in chickens infected with the strains A, B, or E. No changes in the counts of mature and immature lymphocytes were observed in the group infected with the strain C.

Results of microscopic examination of lymphatic organs are presented in Table 3. The infection with IBDV induced serious degenerative and inflammatory lesions in bursal, thymic and splenic lymphatic tissues, disintegration of all bursal follicles, total lymphocyte depletion, and fibrous degeneration, and atrophy of bursa. The damage to thymus consisted in thinning of lobular cortex, lymphocyte depletion, and hyperplasia and hypertrophy of Hassall bodies. Hyperaemia and lymphocyte depletion were found in the spleen. No among-strain differences in effects of the isolates on lymphatic organs were observed.

Table 3
Mean lesion scores in lymphatic organs at post-infection days 2 through 6

	IBDV isolate	Mean lesion scores at days				
		2	3	4	5	6
F. bursa	A	4.0*	4.0	4.0	4.0	4.0
	B	3.8	4.0	4.0	4.0	4.0
	C	4.0	4.0	4.0	4.0	4.0
	D	4.0	4.0	4.0	4.0	4.0
	E	4.0	4.0	4.0	4.0	4.0
	F	3.3	4.0	4.0	4.0	4.0
Thymus	A	1.0	2.0	2.5	3.0	2.8
	B	1.0	2.0	3.5	3.8	2.3
	C	2.3	3.3	2.5	2.5	2.5
	D	1.0	2.5	2.3	2.0	2.0
	E	2.0	2.8	3.0	2.0	2.0
	F	1.3	2.3	2.8	2.8	2.8
Spleen	A	1.3	2.0	2.5	2.5	2.0
	B	1.3	2.5	3.3	2.5	2.5
	C	2.0	3.0	2.5	2.5	2.0
	D	1.3	2.5	2.5	2.0	2.3
	E	1.5	2.5	3.0	2.0	2.0
	F	1.5	2.0	2.5	2.0	2.8

Seroconversion was demonstrated by ELISA at day 6 and equal antibody levels were found at day 24 in all the experimental groups. Results of pathogenicity tests of the four selected IBDV isolates are shown in Table 4.

Table 4
Pathogenicity of IBDV isolates A, B, C and D

Isolate IBDV	Mean body weight (g) of chickens		Morbidity	Mortality	Mean bursal weight index	Mean thymic weight index	Pathogenicity index
	0	10					
A	223	208	8/8	4/8	2.1	2.2	0.71
B	230	127	8/8	8/8	2.0	0.9	0.90
C	219	274	3/8	0/8	1.1	3.2	0.25
D	211	128	8/8	4/8	1.8	1.2	0.49
Control	223	322	0/9	0/9	5.0	5.1	0

The field isolates A, B, C, and D induced serious damage to both central lymphatic organs and subsequently bursal and thymic atrophy. The infection of 4-week-old susceptible

chickens with the strains A, B, or D induced clinical IBD with a high morbidity and mortality. The isolates showed characteristics of standard IBDV strains differing from each other in virulence and pathogenicity indexes.

Discussion

Six strains of IBVD isolated on various farms from cases of acute infectious bursal disease were tested in susceptible chickens with the objective to describe characteristics of field isolates, to detect possible circulation of variant strains in the local poultry population, and to assess their pathogenicity, immunosuppressive activity, and haematopoietic effects.

Infections of susceptible chickens with the isolates A, B, or D induced clinical signs and lesions typical of IBD manifested by bursitis and subsequent bursal and thymic atrophy. Immunosuppressive effects of the IBDV isolates were assessed on the basis of relative weight changes and lesion score in lymphatic organs. It can be concluded that irrespective of the severity of clinical disease infection by any of the isolates induced significant bursal atrophy accompanied by severe damage to lymphatic tissue, atrophy of plicae and fibrous degeneration of the organ.

Serious microscopic lesions were observed in lymphatic tissue of thymus, particularly in chickens infected with the strains A or D. Damage to lymphatic tissue of thymus was observed in birds infected by standard strains of IBDV (Sharma et al. 1995), or in association with the potential of IBDV to penetrate into other-than-bursal tissues of the immune system incl. haematopoietic tissues (Tanimura et al. 1995) in virulence-dependent potential. Cloacal bursa is the target organ for standard, pathogenic, and variant strains of IBDV which induce similar forms of bursal atrophy. Absence of serious inflammatory bursal lesions was reported from domestic poultry populations infected by pathogenic or variant strains of IBDV (Rosenberger et al. 1987; Sharma 1998). Variant strains show a higher immunosuppressive potential than standard strains (Craft et al. 1990) and induce rapid bursal atrophy with only minimum inflammatory lesions (Rosenberger et al. 1987). Infection of susceptible chickens by virulent IBDV strains results in a high concentration of viral antigen in bursa, serious lymphocyte depletion in bursa and spleen, and subsequent development of a highly contagious disease (Snyder 1992).

Haematological investigations carried out within our experiments demonstrated a certain effect of the infection with the IBDV isolates on the haematopoietic system of the chickens. Considering the character of the infection and the affinity of the causative agent to immature precursors of B lymphocytes particularly in bursa and to a lesser extent also in the spleen, thymus and caecal lymph nodes, the changes in the proportions of mature and immature lymphocytes and in the leukogram were expected. The observed shifts in the leukogram were probably due to degenerative and inflammatory processes in lymphatic tissues and to virulence of the tested IBVD isolates manifested by their potential to infect and destroy bone marrow monocytes and macrophages, but not erythrocytes, thrombocytes, and granulocytes (Müller et al. 1979, Tanimura et al. 1995). In this regard the known variability of physiological values of the blood picture in birds and the potential of the avian bone marrow to compensate losses of blood cells rapidly should be considered.

The isolates A, B, C, and D were selected for pathogenicity tests on the basis of results of previous experiments in susceptible chickens. Although the four isolates differed from each other in pathogenicity, bursal, and thymic indexes, their immunosuppressive effects on primary lymphatic organs were similar. Experimental infection of susceptible chickens induced typical acute bursitis of various severity, and macroscopic and microscopic lesions in thymus and spleen. No unexpected effects on the morphology of bursa, antibody response, function of the immune system, or haematopoiesis were observed. The isolated strains of infectious bursitis virus can be classified as standard strains differing in virulence only.

It can be concluded that pathogenic strains of IBDV differing in virulence were isolated on local poultry farms. Characteristics indicative of a variant strain could not be demonstrated in any of the IBDV isolates. The strains showed different pathogenicity as manifested by clinical or subclinical infections, but induced similar immunosuppressive effects and damage to haematopoietic tissues.

Patogenita izolátů infekční burzitidy v chovech drůbeže v České republice

V polovině 90. let postihla chovy kura domácího v České republice (ČR) vlna onemocnění infekční burzitidou (IBD). Z terénních případů akutního onemocnění ve výkrmu brojlerových kuřat, odchovu kuřic nosných hybridů a drobných chovů kura domácího bylo postupně izolováno šest izolátů viru infekční burzitidy (IBDV). Naším záměrem bylo porovnat vlastnosti izolovaných kmenů IBDV pomocí standardních metod hodnocení indexu lymfatických orgánů (Fabriciova burza, thymus, slezina), morfologických imunosupresivních změn a hematologického vyšetření.

Studium vlastností šesti terénních izolátů IBDV potvrdilo přítomnost patogenních kmenů v populaci kura domácího; čtyři testované izoláty vyvolávaly u vnímavých kuřat klinické onemocnění doprovázené zánětlivými a degenerativními změnami v kloakální burze, thymu a slezině. Bez ohledu na průběh onemocnění byla po infekci všemi izoláty zaznamenána statisticky významná atrofie F. burzy ($p < 0,05$) 3. až 6. den po infekci s vážným poškozením obou centrálních lymfatických orgánů. Vliv infekce na hematopoetickou tkáň se projevil změnami v leukogramu a v zastoupení zralých a nezralých lymfocytů. Po infekci některými izoláty bylo na začátku onemocnění zjištěno v leukogramu vyšší procento heterofilů a zralých lymfocytů. Po uzdravení kuřat bylo naopak pozorováno vyšší procento lymfocytů, zejména jejich nezralých forem.

Přestože se izoláty lišily v indexu patogenity a indexu F. burzy a thymu, měly podobné imunosupresivní vlastnosti ve vztahu k primárním lymfatickým orgánům. Izolované kmeny IBDV lze na základě testování považovat za standardní kmeny s různým stupněm virulence. Žádný z izolátů neměl vlastnosti variantního kmene IBDV.

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