VIROLOGICAL CHARACTERISTICS OF BULL SEMEN

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Ninety semen samples collected from bulls in 7 breeding farms were examined for the presence of different viruses. The result of the tests was positive in 33 out of 90 (36.7 per cent) examined bulls. In most cases (60.6 per cent) ECBO virus was isolated from virus positive animals. IBR/IPV virus was isolated in 5 farms from 11 bulls (33.3 per cent), and PI-3 virus from 4 bulls (12.1 per cent). Reo-1 and RS viurses were encountered only in 2 bulls (3 per cent). Reo-2 and VDMD viruses were not encountered in these farms.

Diseases of the genital system, their early diagnosis and prevention are of great practical importance because of extensive development of artifical insemination. Investigations performed up to now showed that microorganisms which are of importance to bovine epizootic infertility are *Chlamydia* (*Psittacosis—Lymphogranuloma—Venereum—Trachoma*—Group—PIT) (10, 11, 13), *Mycoplasma* (4, 5, 15, 17) and viruses belonging to different systematic groups. Other viruses causing lesions in bovine genital organs are enteroviruses isolated often from the prepuce and semen of bulls showing decreased fertility (1, 7) and IBR/IPV virus, belonging to a Herpes group, which causes posthitis and vaginitis (5). These viruses are of importance in disturbances of fertility in animals (2, 3, 6, 12, 16) and may be transmitted to females by semen or preputial secretions and also to calves by a placental route. In a similar way, can be also transmitted PI-3 virus, which sometimes may be an aetiological agent of infections of bovine genital system. The purpose of this work was to identify some viruses isolated from the semen of bulls originating from different breeding farms.

Material and Methods

Virological examinations were carried out with 90 semen samples collected from bulls originating from different breeding centres. The semen was obtained from healthy bulls, those suspected for infection with *Chlamydia* microorganisms or from animals which recovered from the infection. The semen samples were frozen and stored at — 20°C until used.

Virus isolations were carried out in calf kidney and testicle cell cultures which were multiplicated in Parker's and Hanks' nutrient fluids (1:1) supplemented with 10 per cent calf serum. Just before infection of the cultures, semen samples were thawed and frozen three times and diluted 1:10 with phosphate-buffered saline solution (PBS). Ten tubes, containing each cell culture, were inoculated with this material in such a way that its final dilution was 1:10. The cell cultures were incubated at 37°C and examined daily until the first cytopathic effects (CPE) appeared. Then, from each semen sample examined, three successive passages were made into calf kidney and testicle cell cultures. Infection of the examined semen with viruses was evaluated taking into consideration the occurrence of CPE. Identification of isolated viruses was carried out using the material from the third passage. It was examined by the direct and indirect immunofluorescence tests using complement-anticomplement system, alpha seroneutralisation test with known diagnostic sera and haemadsorption (HAD) and haemagglutination (Ha) tests. Immune sera against ECBO, IBR/IPV, PI-3, Reo-1, Reo-2, and RS viruses were prepared in rabbits. They were injected intravenously with 8 increasing doses of viruses, ranging from 0.5 to 2.0 ml, at 4 to 5-day intervals.

Results and Discussion

Examination of semen samples collected from bulls originating from 7 farms showed that viruses were present in 33 bulls (36.7 per cent). The detailed results are presented in Table 1. The relative number of infected bulls in particular groups ranged from 7.7 to 80.0 per cent. The greatest number of bulls was infected with ECBO virus. In all the examined farms, the percentage of infection with this virus, calculated in relation to the total number of animals reacting positively, was 60.6 per cent. Out of 33 infected bulls, single infections with ECBO virus were found in 16 bulls (48.5 per cent) and mixed infections — in 4 bulls: 3 bulls were infected with IBR/IPV and ECBO viruses (9.1 per cent) and one bull with PI-3 and ECBO viruses (3 per cent). IBR/IPV virus was isolated in 5 farms from 11 bulls (33.3 per cent). Single infections were found in 8 cases (24.2 per cent) and mixed infections with IBR/IPV and ECBO viruses — in 3 cases (9.1 per cent). PI-3 virus was isolated in 3 farms from 4 bulls (12.1 per cent). Single infections were found in 3 bulls (9.1 per cent) and mixed infections with PI-3 and ECBO viruses — in 1 bull (3 per cent). Reo-1 and RS viruses were isolated in 2 farms in single cases only (3 per cent). Reo-2 and VDMD viruses were not found at all.

The results of these investigations showed that mixed infections were found in 4 bulls and in the remaining cases, i.e. in 29 bulls, only single infections with viruses belonging to different systematic groups were encountered.

Some properties of viruses isolated from bull semen were shown in Table 2. ECBO virus isolated most frequently from bull semen produced CPE in calf kidney and testicle cell cultures 48 to 72 hours after inoculation. IBR/IPV virus produced CPE 72 to 96 hours after inoculation of calf kidney and testicle cell cultures. PI-3 virus produced CPE and showed both haemagglutinating and haemadsorbing properties 48 to 72 hours and 72 to 96 hours after inoculation of the cell cultures. Reo-1 and RS viruses multiplicated only in kidney cell cultures and produced CPE 96 to 120 hours after inoculation.

Table 1
Results of virus isolation from bull semen

Number of examined animals in particular farms	Animals reacting positively		Isolation of viruses from bulls reacting positively													
			ECBO		IBR/IPV		IBR/IPV ECBO		PI-3		PI-3 ECBO		Reo-1		RS	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
26	2	7.7	1	50.0	—	—	—	—	1	50.0	—	—	—	—	—	—
5	4	80.0	2	50.0	—	—	2	50.0	—	—	—	—	—	—	—	—
23	10	43.5	6	60.0	4	40.0	—	—	—	—	—	—	—	—	—	—
7	3	42.9	2	66.6	—	—	—	—	—	—	—	—	1	33.3	—	—
11	6	54.5	3	50.0	1	16.6	—	—	1	16.6	1	16.6	—	—	—	—
8	3	37.5	1	33.3	2	66.6	—	—	—	—	—	—	—	—	—	—
10	5	50.0	1	20.0	1	20.0	1	20.0	1	20.0	—	—	—	—	1	20.0
Total 90	33	36.7	16	48.5	8	24.2	3	9.1	3	9.1	1	3.0	1	3.0	1	3.0

Table 2
Serological properties of viruses isolated from bull semen

Viruses	Number of positive results	CPE, hours after infection		HAD	Ha	SN test with homological sera
		kidney	testicles			
ECBO	16	48—72 +	48—72 +	—	—	+
IBR/IPV	8	72--96 +	72—96 +	—	—	+
PI-3	3	48—72 +	72—96 +	+	+	+
Reo-1	1	96—120+	96—120—	—	—	+
RS	1	96—120+	96—120—	—	—	+

+ positive result — negative result

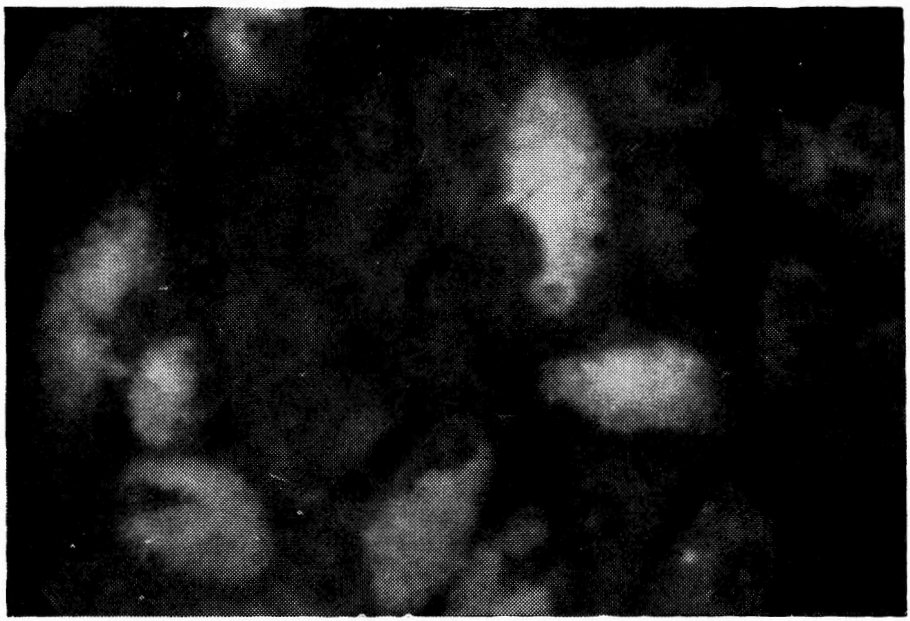

Fig. 1. Calf kidney cell culture stained with conjugate, 48 hours after infection with examined bull semen. Characteristic fluorescence in cell plasma is seen. ECBO virus.

Virological investigations of bull semen showed that the examined semen samples were infected with viruses belonging to different systemic groups. This finding suggest that a proper method of control should be accepted, especially in case of bulls from which PI-3 and IBP/IPV viruses were isolated, in order to prevent the transmission of these viruses by the semen (9, 14, 16).

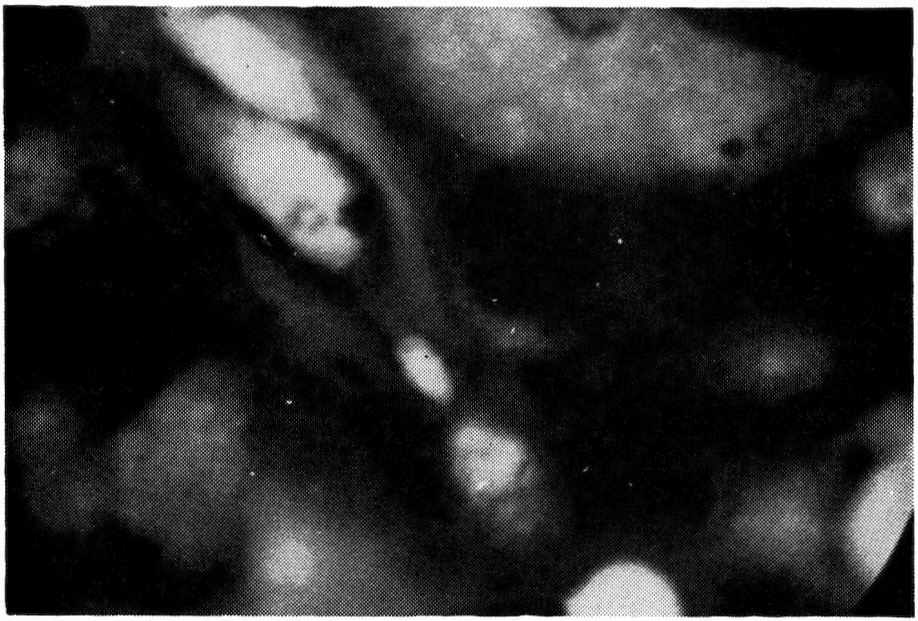

Fig. 2. Calf kidney cell culture stained with conjugate, 48 hours after infection with examined bull semen. Fluorescence in the nucleus and cell plasma in seen. IBR/IPV virus.

Fig. 3. Calf kidney cell culture stained with conjugate, 48 hours after infection with examined bull semen. Granular fluorescence in cell plasma is seen. PI-3 virus

Fig. 4. Calf kidney cell culture stained with conjugate, 43 hours after infection with examined bull semen. Perinuclear fluorescence is seen. Reo-1 virus.

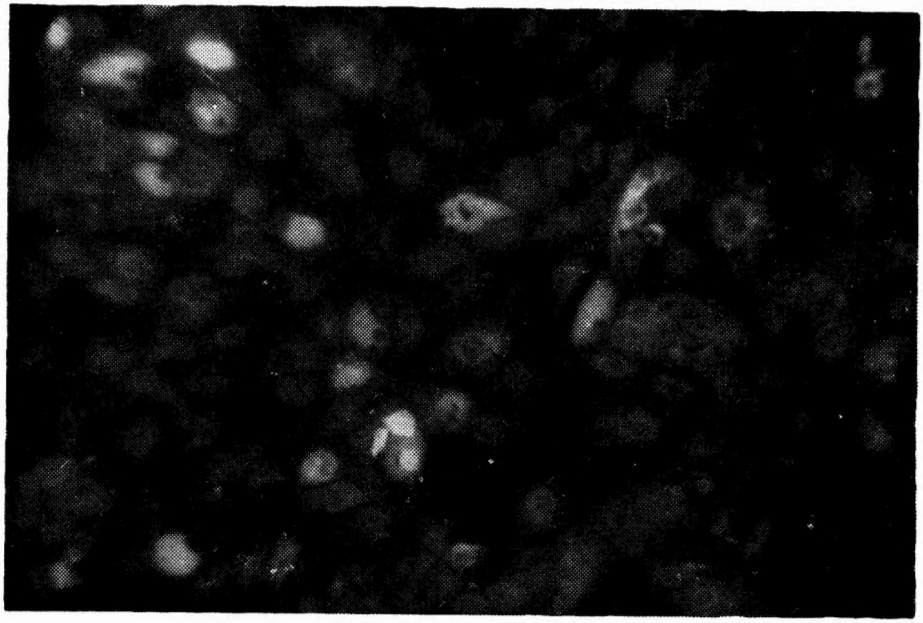

Fig. 5. Calf kidney cell culture stained with conjugate, 48 hours after infection with examined bull semen. Uniform fluorescence in the plasma of single cells in seen. RS virus.

The present investigations revealed no correlation between health conditions of the examined bulls and the frequency of virus isolation from their semen. It seems, however, that further investigations carried out with greater number of semen samples collected several times from the same bull in different period of time would enable to determine the exact course of virus infection.

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