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Full Length Research Paper

Development of the scheme to prepare specimens for sheep pox virus indication in objects of environment

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While isolating sheep pox virus from samples of air, water, hay and grain experimentally infected with this agent various filters and eluting media were tested. Possible usage of enzyme-linked immunosorbent assay (ELISA) for specific indication of the sheep pox virus in various objects of veterinary supervision was investigated.

Key words: Specific indication, objects of veterinary supervision, concentration, elution, virus, antigen, enzyme-linked immunosorbent assay.

INTRODUCTION

Most valuable are the methods for specific indication of viruses in diverse objects of environment that enable to detect a pathogen and to specify it during a short time interval. Specific indication includes sampling and specimen preparation, isolation and indication of the agent with the help of a rapid method of assay. For solving this problem it is important to improve the methods of specimen preparation. In this respect it is worthy to mention the methods of specimen concentration from large volumes of fluids or air with use of optimal filters and eluting media that can markedly enhance the effectiveness of indication of viral agents (Ballad and Zorikhina, 1983; Belfort et al., 1982; Dzantiyev and Yegorov, 1982; Dmitriyeva, 1977; Koromyslov and Avilov, 1985; Mishchenko, 1997; Nupen and Grabow, 1985; Samuilov, 1999).

The purpose of our research was to develop optimal methods of specimen preparation for specific indication of sheep pox virus in objects of environment and veterinary supervision as well as for detection of its antigen in ELISA.

MATERIALS AND METHODS

In the course of the study the sheep pox virus, strain "NISKhI", was used in the form of cultural suspension with infectious activity 5.5 - 6.0 lg TCD_{50/cm^3}. Air, water, hay and grain were sampled as objects of environment.

Sampling and specimen preparation

Water samples of 0.5 - 5 L in volume were contaminated with various doses of the sheep pox virus and concentrated with the help of a vacuum pump or DCD using different filters. The viruses were eluted from the filters to various eluting media at continuous stirring. The resulted effluent was centrifuged at 3000 g for 20 min and the supernatant was used in assays. Samples of hay or grain (100 - 500 g) experimentally contaminated with the sheep pox virus in various doses were flooded with 0.5 - 2.5 L of PBS, thoroughly stirred for 5 - 10 min, then the fluid was poured out, filtered through gauze and concentrated with the help of filters. The virus was eluted from filters with 0.01 M PBS supplemented with 0.5% Tween 80. The sheep pox virus aerosol was sampled to the filters FPP-15-1.5 or NEL-3 with the help of a sampler. The samples of the virus containing material were eluted from filters in the media with various additions.

The developed direct ELISA is carried out according to the following conditions:

- Sensibilization of polystyrene U-bottomed plates with the specific immunoglobuline diluted with carbonate-bicarbonate buffer, pH 9.5 for 18 h at 4ºC;
- Interaction of the tested and control antigens with immunoglobuline fixed on polystyrene for 18 h at 4ºC;
- Interaction of the virus-specific conjugate with the antigens for 50 - 60 min at 37ºC and then interaction of the substrate with conjugate for 30 - 60 min at room temperature.

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Abbreviations: PBS – phosphate buffer saline; FPP-15-1.5 – Petryanov's filter; VP-20 – Vladipore filter; ELISA – enzyme-linked immunosorbent assay; CBB – carbonate bicarbonate buffer; DCD – differential concentration device; BEB – beef extract broth; BSA – bovine serum albumin.

Table 1. Effectiveness of different eluting media in sheep pox virus concentration on millipore filters.

Eluting Media	Infecting dose, TCE_{50/cm^3}	ELISA of Water Samples	
		3 L	5 L
5% solution of normal bovine serum in 0.1 M PBS	100	0/3	1/3
	1000	1/4	2/4
1% solution of sodium dodecyl sulphate	100	0/3	0/3
	1000	0/3	0/3
5% solution of BEB in 0.1 M PBS	100	0/3	0/3
	1000	1/3	1/3
1% solution of NaCl in 0.05 M PBS with 0.1% Tween 80	100	3/3	3/3
	1000	3/3	3/3
1% solution of NaCl in 0.05 M PBS with 0.5% Tween 80	100	3/3	3/3
	1000	3/3	3/3
1% solution of NaCl in 0.05 M PBS with 1% Tween 20	100	3/3	3/3
	1000	3/3	3/3
1% solution of NaCl in 0.05 M PBS with 0.5% Triton X-100	100	0/3	0/3
	1000	0/2	0/3
1% solution of NaCl in 0.05M PBS with 1% Triton X-100	1000	0/3	0/3
1% solution of NaCl in 0.05M PBS with 1% BSA	100	2/3	3/3
5% solution of BEB in 0.05M PBS with 0.5% Tween 80	100	3/3	3/3
	1000	3/3	3/3

Note: Numerator – number of positive samples; Denominator – number of tested samples. ovine serum albumin.

RESULTS

At the initial stage of our research aimed at development of effective scheme for specimen preparation in the procedure of sheep pox virus indication the experiments were carried out to choose optimal filters and eluting media for concentration of environmental samples (air, water). For concentration of water samples Millipore, Vladipore and Capron filters with pore size 0.22 μm were tested, and for aerosol sampling Petryanov's tissue filters FPP-15-1.5, NEL-3 and VP-20 were tested. Media containing normal calf blood serum, beef-extract broth (BEB), bovine serum albumin (BSA), Tween 80, Tween 20, Triton, sodium dodecyl sulphate, sodium chloride, and potassium thiocyanate in various concentrations were tested as eluting fluids. Effectiveness of using different filters and eluting media for concentration of samples taken from objects of veterinary supervision was assessed by assaying these specimens in ELISA. In the first series of experiments the effectiveness of different media in elution of sheep pox virus concentrated from infected water samples to Millipore filters was determined. Water in volume of 5 liters was contaminated by sheep pox virus in doses of 100 and 1000 TCE_{50/cm^3}, stayed at room temperature for 10 min and concentrated to a filter with the help of a differential concentrator device. Then it was eluted from the filter with the help of different media. The results of this study are presented in Table 1.

Table 1 data show that effectiveness of sheep pox virus indication depends on infecting virus dose, volume of a tested specimen and on composition of an eluting medium. The best results were obtained in examination of water samples infected with 100 and 1000 TCD_{50/cm^3} when the sheep pox virus was eluted from Millipore filters with 1% NaCl solution in 0.05 M PBS at various concentrations of Tween 80 and Tween 20 and with 5% solution of BEB in 0.05 M PBS with 0.5% Tween 80. However, automatic ELISA reading-out showed higher optical density of the tested specimens when the antigen was eluted from filters with 5% solution of BEB and 1% NaCl solution in 0.05% PBS with 0.5% Tween 80. Because of this in the further experiments the above stated media were used to concentrate liquid samples and to assess effectiveness of other filters. In the next series of experiments aimed at assessing effectiveness of Vladipore 2 filters and Capron micropore membranes in concentration of liquid specimens eluting media with BEB and NaCl were used. Table 2 shows the results of the research.

Data in Table 2 shows that the effectiveness of the tested filters in concentration of liquid specimens is the same both in experiments with BEB medium and with NaCl solutions. However automatic reading-out of ELISA results has shown that optical density of tested samples is higher when Capron filters and BEB containing eluting medium are used for concentration. So, in further experiments on liquid sample concentration Capron filters and BEB containing eluting medium were used.

To select effective media for elution of air samples contaminated with sheep pox virus media that contain normal calf serum, BEB and lactalbumin hydrolyzate (LAH) as well as different methods of virus elution from filters FPP-

Table 2. Comparative effectiveness of Vladipore filters and Capron membranes in concentration of sheep pox virus from samples of water experimentally infected with the agent.

Filters	Eluting Media	Infecting Dose for Water (TCD_{50/cm^3})	ELISA Results
Vladipore	1% NaCl solution in 0.05 M PBS with 0.5% Tween 80	100	3/3
Capron membrane		100	3/3
Vladipore	5% solution of BEB in 0.05 M PBS with 0.5% Tween 80	100	4/4
Capron membrane		100	5/5

Note: Numerator – number of positive samples; Denominator – number of tested samples.

Table 3. Comparative effectiveness of the tested eluting media and methods for sheep pox virus desorption from filters FPP-15-1.5.

Desorption Methods	Eluting Media	Virus Titer in Initial Suspension, lg TCE_{50/cm^3}	Virus Titer in Effluent, lg TCE_{50/cm^3}	Virus Inactivation and Retaining on Filters, lg TCE_{50/cm^3}
Pipetting	5% solution of normal bovine serum in 0.1 M PBS	5.73±0.21	5.09±0.11	0.64
	5% BEB solution in 0.01 M PBS with 0.5% Tween 80	5.73±0.21	5.1±0.15	0.63
	10% BEB solution in 0.01 M PBS with 0.5% Tween 80	5.73±0.21	5.08±0.23	0.65
	0.5% LAH solution in Hanks solution	5.73±0.21	4.76±0.31	0.97
Shuttling	5% solution of normal bovine serum in 0.1 M PBS	5.73±0.21	5.0±0.25	0.63
	5% solution of BEB in 0.01 M PBS with 0.5% Tween 80	5.73±0.21	5.11±0.25	0.62
	10% BEB solution in 0.01 M PBS with 0.5% Tween 80	5.73±0.21	5.08±0.14	0.65
	0,5% LAH solution in Hanks' solution	5.73±0.21	4.81±0.27	0.92

15-1.5 have been tested.

For this purpose 0.5 ml of virus suspension was layered on filters, filters were stayed at room temperature for 1.5 h, and then immersed in tested eluting solutions, pipetted for 1 - 2 min and kept for 1 h at (4 ± 1)°C. The other part of samples was shuttled for 1 h at (4 ± 1) °C. After interaction samples were thoroughly stirred with a pipette for at least 2 minutes, filters were squeezed and the supernatant was poured off and titrated in lamb kidney cell culture. Degree of virus inactivation and its retaining on filters was assessed by titer differences resulted from titration of initial materials and tested samples. Results of these experiments are presented in Table 3.

The data in Table 3 evidence that effectiveness of tested methods for sheep pox virus elution from filters FPP-15 -1.5 is nearly the same. Among all tested eluting media those which contain 5% of calf serum and 5% of BEB appeared to be the most effective. However in ELISA effluents containing 5% of serum caused nonspecific background coloring in negative controls that is why 5% solution of BEB was used as eluting medium in the further experiments. In the subsequent study effectiveness of some filter models in sampling air experimentally infected with the sheep pox virus was assessed. For this purpose the virus was dispersed in a vertical dynamic chamber (VDC) with the help of an aerosol inhaler. Virus concentration in the chamber in the course of dispersion and in aerosol samples was assessed by uranin marker. Aerosol was sampled onto FPP-15-1.5, VP-20 and NEL-3 filters for 1.5 hour with the help of a vacuum pump with filter nozzle. The virus from filters was eluted into 5% solution of BEB at (4 ± 1)°C for 1.5 h. Effluents were assayed in ELISA. The results of these experiments are presented in Table 4.

The data of Table 4 show that effectiveness of sheep pox virus aerosol sampling does not depend on the kind of the filter. The major parameter demonstrating effectiveness of virus indication is the virus concentration in aerosol. The above described results of experiments aimed at optimization of the specimen preparation scheme for specific indication of sheep pox virus antigen in environment samples and samples of objects under veterinary supervision allow making a conclusion that Capron filters are most suitable for concentration of liquid samples from various test-objects, and FPP and NEL-3 filters are the best ones for aerosol sampling. The medium containing 5% BEB is the most effective medium for virus elution from a filter. Upon optimization of the scheme for specimen preparation experimental indication of sheep

Table 4. Comparative efficiency of some filter models for sheep pox virus aerosol sampling.

Filters	Virus Concentration in Aerosol (TCE_{50/cm^3})	ELISA Results
FPP-15-1.5	5.9±0.63	1/3
	9.7±1.48	2/3
	12.1±1.23	6/6
	25.8±1.93	3/3
	42.8±1.38	6/6
	116.6±1.43	3/3
VP-20	5.9±0.63	1/6
	8.4±1.14	2/3
	12.1±1.23	6/6
	42.8±1.38	6/6
	116.6±1.43	3/3
NEL-3	5.2±1.14	2/3
	8.3±1.31	4/6
	21.0±1.52	6/6
	40.0±1.83	6/6

Note: Numerator – number of positive samples; Denominator – number of tested samples.

Table 5. Sheep pox virus indication in objects of environment and objects under veterinary supervision.

Samples	Infecting Dose, $TCE_{50/cm^3/g}$ or $TCE_{50/L}$	Number of Tested Specimens	Number of Positives	Antigen Titer in ELISA
Water	100	16	11	1:2-1:8
Water	500	3	3	1:4-1:16
Grain	100	3	3	1:2-1:8
Grain	500	3	3	1:4-1:16
Hay	100	3	3	1:2-1:8
Hay	500	3	3	1:16-1:64
Air	5	21	12	1:5-1:10
Air	10	12	12	1:5-1:10
Air	50	18	18	1:20-1:40

pox virus in various environmental objects and objects under veterinary supervision was carried out. After concentration and elution the samples of water, hay and grain contaminated with the sheep pox virus in doses of 100 and 500 $TCD_{50/cm^3/g}$ and samples of air contaminated with the virus in doses of 5, 10 and 50 $TCD_{50/L}$ were assayed in ELISA. The results of the experiments are presented in Table 5.

Table 5 data evidence effectiveness of the optimized scheme of specimen preparation for sheep pox virus indication in various objects of environment. ELISA allows detecting sheep pox virus in aerosol samples in dose of 10 $TCE_{50/L}$ and over, in samples of water, hay and grain in dose of 100 $TCE_{50/cm^3/g}$ and over.

DISCUSSION

Indication of infectious agents in objects of environment allows veterinary specialists to duly conduct control measures among animals and birds prior to emergence of a pesthole. Our research aimed at development of an effective scheme for preparation of specimens for the sheep pox virus indication started with experiments in selecting optimal filter models and eluting media for concentration of samples of environmental objects (water, air). Solving these problems is basic in development of an effective scheme for sample preparation and can significantly increase efficiency of viral agent indication (Anufriyev et al., 1973; Ballad and Zorikhina, 1983; Belfort et al., 1982; Dzantiyev and Yegorov, 1982; Dmitriyeva, 1977; Koromyslov and Avilov, 1985; Mishchenko, 1997; Nupen and Grabow, 1985; Samuilov, 1999; Khramov, 2005; Lewis et al., 1985). The conducted research has shown that the effectiveness of sheep pox virus indication depends on infecting dose of the virus, volume of a tested sample prior to concentration and composition of the eluting medium. The best results were demonstrated when the sheep pox virus was eluted from a Millipore filter with 1% NaCl solution in 0.05 M PBS with 0.5% Tween 80. Testing effectiveness of Vladipore-2 filt-

ers and Capron micropore membranes in concentration of liquid samples with use of eluting media containing BEB and NaCl has shown that optical density of the specimens in ELISA is higher when they have been concentrated with Capron filters and eluting media containing BEB. Experiments in selecting optimal media for elution of air samples infected with the sheep pox virus have shown the effectiveness of the tested methods of elution from FPP -15-1.5 filters to be almost the same. Among tested eluting media the media containing 5% BEB demonstrated higher effectiveness. Study in usefulness of some filter models for air sampling has shown that efficiency of sampling air infected with the sheep pox virus does not depend on the model of filters. Key index of virus detection efficiency is concentration of the virus in aerosol. On the basis of the above-stated results of studies on development of an optimal scheme for preparation of specimens for specific indication of the sheep pox virus antigen in samples of environmental objects and objects of veterinary supervision one can conclude that for concentrating liquid samples of various test objects Capron filters are the best, for aerosol sampling FPP or NEL-3 filters are most effective. The medium containing 5% BEB is shown to be the most efficient one for virus elution from filters.

Upon developing optimal scheme for preparation of specimens it appeared possible to detect the sheep pox virus antigen in specimens from objects of environment in the following concentrations: 10 $ТСД_{50/dm^3}$ and higher in aerosol samples, 100 $ТЦД_{50/cm^3/g}$ and higher in samples of water, hay and grain. Similar studies on indication of rinderpest and foot-and-mouth disease virus antigens in samples from objectsof environment were carried out by Anufriyev (Anufriyev et al., 1973) and Mishchenko (Mishchenko, 1997). They have shown that effectiveness of the antigen indication is rather high at the identical density of infection of hay, grain and water with virus containing preparations.

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