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COMPARISON OF THE AVIDIN-BIOTIN AND POLYMER DETECTION SYSTEMS FOR RAPID RECOGNITION OF PESTE DES PETITS RUMINANTS (PPR) VIRUS *IN SITU*

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Summary

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The present study compares two of immunochemical detection techniques, the avidin-biotin complex (ABC) and the polymer-conjugated system (PCS), in freshly frozen tissue sections (intestine, lungs, tongue, lymph node, spleen), and epithelial cells from ocular swabs. The PCS is a set of PicTure^R kits elaborated by Zymed Co., USA for "one-step" immunohistochemical (IHC) antigen detection method. Peste des petits ruminants (PPR) virus antigen was apparently demonstrated in frozen tissue sections and in epithelial cells by both ABC and by PCS without any background staining.

Key words: immunochistochemistry, peste des petits ruminants

INTRODUCTION

The present study compares two versions of immunochemical (IHC) detection of peste des petits ruminants (PPR) antigen in frozen tissue sections (intestine, lungs, tongue, lymph node, spleen) including epithelial cells (ocular swabs) – by the avidin-biotin complex (ABC) and the polymer-conjugated systems (PCS).

According to the our earlier reports (Eligulashvili et al., 1999; 2000) an IHC method was successfully employed for the identification of PPR virus antigen in formalin-fixed, paraffin-embedded tissues as well as in cryostat sections prepared from materials taken from cases of natural infections. Similar results have been published elsewhere (Alcigir et al., 1996).

The PCS is a set of PicTure^R kits elaborated by Zymed Co., USA containing multiple horseradish peroxidase *(*HRP) and Fab fragments that provide high sensitivity. The PCS is a set for use "one-step" antigen detection IHC method in frozen tissue preparations. This method does not need any preliminary blocking, except endogenous peroxidase. The complete procedure starting from the material processing of the till its microscopic inspection takes about 2.5-3.5 hours for tissue sections and up to 5 hours – for epithelial cells in smears.

MATERIALS AND METHODS

Freshly frozen (2-methylbutane in liquid nitrogen) ovine tissues (tongue, spleen, intestine, lungs, lymph node) were cryostat sectioned (thickness 6 μm), placed on poly-L-lysine coated glass microscope slides (Zymed Co., USA) and dried for 10-15 min at room temperature (RT).

Swab material was centrifuged for 10 min at low speed (70-90 x g); the sedi-

ment (epithelial cells) was resuspended in a small volume of phosphate buffer saline (PBS), and a drop of the material was placed on poly-L-lysine coated microscope glass slides and then was dried at RT (or in a dry incubator at 37° C). Slides bearing the sections and swab's cell were fixed in cold acetone (10 min. at –20° C).

Endogenous peroxidase was inactivated in peroxo-block solution elaborated by Zymed Co., USA (ready to use), for 45 s according to manufacture's instructions. The sections were then rinsed three times in PBS + 0,05% Tween 20 (PBST) for 2-3 min, incubated with blocking solution – 10% nonimmune goat serum at RT in humid chamber, for 10 min (using the ABC procedure) and then incubated for 30 min at RT with a 1/50 dilution of the primary monoclonal antibody to the N protein of the PPR virus manufactured by CIRAD/EMVT Montpelier (Libeau et al., 1994). After the washing (3 times for 2-3 min in PBST) we used ABC and in parallel, PicTure^R kits (PCS) for IHC staining, using 3-amino-9-ethylcarbazole (AEC) as substrate.

In the following steps of the ABC procedure, reagents were supplied by Zymed Co., USA: after Moab's addition of Biotin – Goat – Anti - Mouse – IgG (ready to use) for 10 min at RT, followed by 3 washes in PBST, Streptavidin Peroxidase (ready to use) was added for 10 min, followed by 3 washes in PBS and AEC substrate - for 6-8 min. After washing the preparations were counterstained with Hematoxylin solution - 2 min followed by washing in tap water for 5-10 min. GVA-Mount was used to cover the slides with cover glasses.

In contrast to the ABC in the PicTure^R kit (PCS) we used only Polymer solution for 10 min according the manufacture's instructions.

In 2001, 86 tissue samples and 21 smears from 53 animals (sheep and goat), collected from different regions of Israel, were checked by IHC-ABC . All of them were checking by Polymerase Chain Reaction (PCR) also and some of them – with PCS (12 swabs and 44 tissue samples). Many samples were in decayed condition.

RESULTS

The PPR antigen was visualized in tissue sections and in epithelial cells with the ABC system, and with the PCS without any background staining. We obtained the same cytopathogenic results in cryostat sections of intestine (Fig. 1), lungs, lymph nodes, spleen with both techniques. In many cases the signal in the ABC system was of lower intensity and contrast than seen in the PCS under the same conditions (Fig. 1 and 2).

In the frozen tissue sections from lungs and intestine, more aggregates of PPR antigen than in the other organs were obtained.

In the swabs taken during the "early phase" of disease (the first symptoms) specific staining of epithelial cells was found (Fig. 2). The staining in the ocular swabs was the same as in cryostat sections. In the negative control no signal was found (staining of cells or background) (Fig. 1 and 2).

From the 86 checked samples we received the same results by two methods (IHC and PCR). Only one sample from ocular swabs was negative by IHC and slightly positive by PCR.

DISCUSSION

As shown by this study, the two techniques of immunochemical analysis are equally suitable for laboratory confirma-

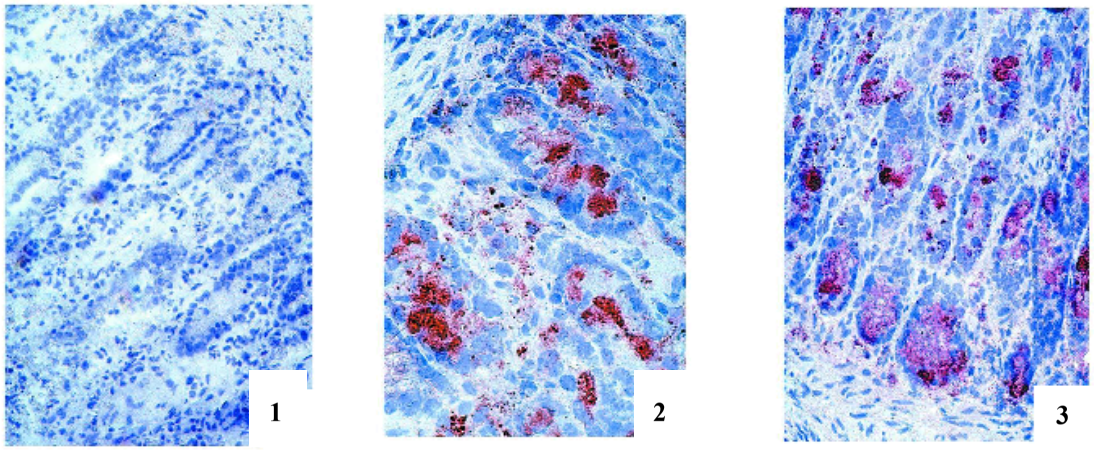

Fig. 1. Immunohistochemistry detection of PPR virus antigen on cryostat section by ABC and Polymer detection system: 1) intestine – negative control; 2) intestine – ABC system; 3) intestine – Polymer system.

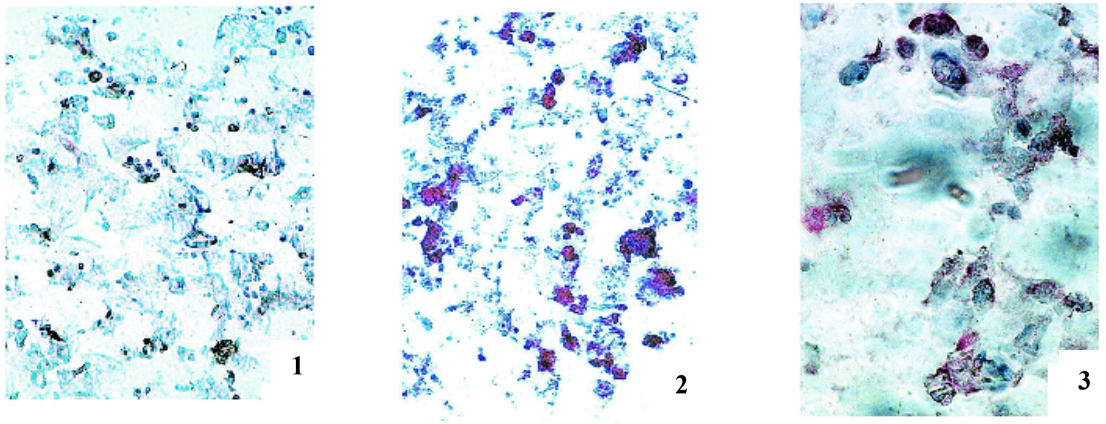

Fig. 2. Immunohistochemistry detection of PPR virus antigen in epithelial cells from ocular smears by ABC and Polymer systems: 1) ocular swabs – negative control; 2) ocular swabs – ABC system; 3) ocular swabs – Polymer system.

tion of PPR infection. We made no particular experiments for a comparative determination of sensitivity. However, in a simultaneous study of one of the specimens, an unusually stained cells (up to 10-15 cells per preparation) were found, however localization of the paint was not specific for the PPR virus. We had the impression that the sensitivity of the test with ABC slightly exceeded that provided by the polymer detection system, but it does not affect to final results. Both techniques may be suitable for rapid laboratory diagnosis of PPR virus. PCS is simpler then ABC.

The results confirmed our view that the selected marker antigen of one of the N protein epitopes does meet the requirements. It always occurs in PPR infection, resists abundant fixation (acetone 5 to 10 min), and is stable on autolysis.

Suitable organs for laboratory diagnosis of PPR infection by both (ABC and PCS) IHC techniques are lungs and intestine.

Of particular significance is the fact that the virus-specific antigen can be detected in the epithelial cells from ocular swabs in both variants of the method.

This noninvasive procedure markedly simplifies the collection and transport of material and provides an early (ocular swabs) and rapid (tissue sections) diagnosis.

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