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DETECTION OF POULTRY INFECTION WITH THE VIRUS OF MAREK'S DISEASE USING DIFFERENT LABORATORY METHODS

MAJA GAGIĆ*, S.ĐURIŠIĆ*, RUŽICA AŠANIN**, and T. PALIĆ**

*Scientific Institute for Veterinary Medicine, Novi Sad **Faculty of Veterinary Medicine, Belgrade

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In this paper several laboratory methods for detecting the virus of Marek's disease (MD) in poultry are described.

Pathogenic MD virus was isolated in cultures of duck fibroblasts, while vaccinal virus was grown in culturs of chicken fibroblasts. The cytopathogenic effect of vacinal virus was more intensive and could be vizualized faster than that of the pathogenic strain used for infection of the chickens.

In the serum of infected chickens, antibodies for "A" antigen of the MD virus were confirmed by agar gel precipitation, whereas in the vaccinated group no antibodies for this virus were found. In the feather follicles of infected chickens the MD virus could be confirmed by gel immunodiffusion, whereas the vaccinal virus was not detected by this technique.

When determining the extent of chicken infection with MD virus culates in farms, besides virus isolation in cell cultures, it would be useful to employ other laboratory methods also. Our report deals with the advantages and disadvantages of some of these methods.

Key words: Marek's disease, virus, feather follicle, cell culture, immundiffusion, "a" antigen, antibody.

INTRODUCTION

Marek's disease (MD) is an infectious lymphoproliferative disease of birds caused by a Herpesvirus (Churchill and Biggs, 1967). The great economic losses in the poultry industry due to MD were reduced worldwide by the introduction of vaccination (Witter et al., 1970) with turkey herpesvirus (HVT). The appearance of very virulent viruses necessitated the application of bivalent vaccines (Schat et al., 1978) of the SB-1 strain in combination with HVT. A vaccine made from an attenuated strain of MD virus belonging to serological group 1 is used in Europe (Rispens et al., 1972, De Boer et al. 1987).

Because of occasional outbreaks of the disease in our country, even in vaccinated flocks, there arose a need for new diagnostic methods in addition to

the already existing ones (clinical picture, pathoanatomic examination and pathohistology).

Therefore, we decided to check the efficiency of several different laboratory methods for the detection of the virus under experimental conditions of infection and vaccination.

MATERIALS AND METHODS

Chickens

Fifty day old chickens of the Hybro line were housed in one of the boxes of the experimental block of the Veterinary Institute. The chickens were infected on the first day of life with NS/91 virus of MD isolated from chicken embryo fibroblast (CEF) cultures. The second group containing 50 chickens was vaccinated on the first day of life with turkey herpesvirus (HVT), Pliva Marikal serial no. 2211041. The third, control group, contained 30 non-vaccinated and - infected chickens of the same line. All chickens in the experiment (except for the controls) were individually labelled.

Detection of viremy

The state of viremy was checked on days 14 and 42 post infection and vaccination, respectively, in the first and second group of chickens, while in the controls it was done only on the 42nd day of life.

Viremy was demonstrated by virus isolation from lymphocytes of the peripheral blood of the chickens, using Lymphoprep (Nyegaard Co. A/S, Oslo, Norway). Pathogenic virus was demonstrated in cultures of duck embryo fibroblasts (DEF), while vaccinal virus was demonstrated in chicken embryo fibroblasts (CEF). When a cytopathogenic effect, was observed no matter its intensity, viremy was marked as positive.

Demonstration of the virus in cell cultures using monoclonal antibodies

Monoclonal antibodies for all three virus serotypes were produced in the Avion Disease and Oncology Laboratory, 3606 E. Mount Hope Rd., Lansing, Michigan USA (Lee et al., 1983).

Indirect immunofluorescence was performed as a standard procedure. A mixture of acetone and alcohol (6 parts of acetone and 4 parts of 95% alhocol) was used for cell fixation. Monocional antibodies were diluted according to the recommendation of the producer in the ratio 1:100. Anti murine antibodies diluted in the ratio 1:50 were used as the conjugate. Before reading the cells 3-4 drops of a mixture of glycerine in PBS in the ratio 1:2 were added.

Demonstration of the virus in feather follicle epithelial cells

Five feathers from the breasts of each infected and vaccinated chicken were taken as samples at two week intervals, i. e. 14, 28, 42 and 56 days of age. Follicles were cut at the length of 5 mm and put into agar gel around the central hole which contained hyperimmune serum. The distance between serum and the feathers was 4 mm.

Demonstration of "A" antigen in sera of chickens

In order to demonstrate "A" antigen for MD virus at the same time intervals, samples of blood from all three groups of chickens were collected by wing vein puncture. For that purpose we used an agar gel precipitation fest (AGPT) with a previously prepared antigen labelled MNS/91. For comparison we also used on antigen from Weybridge (No. 0156/03). Monovalent serum labeled AsMD was used as the positive control serum, which was obtained after infection of chickens with a pathogenic MD in a previous trial, as well as antiserum (0157/03) from Weybridge.

RESULTS

We noticed differences in the cytopathogenic effect (CPE) during demonstration of viremy of vaccinal and pathogen viruses by isolation on cell culture. Vaccinal virus formed foci on the third day post inoculation (Fig. 1). On the fifth day foci on the CEF cultures were much bigger (Fig. 2). These foci

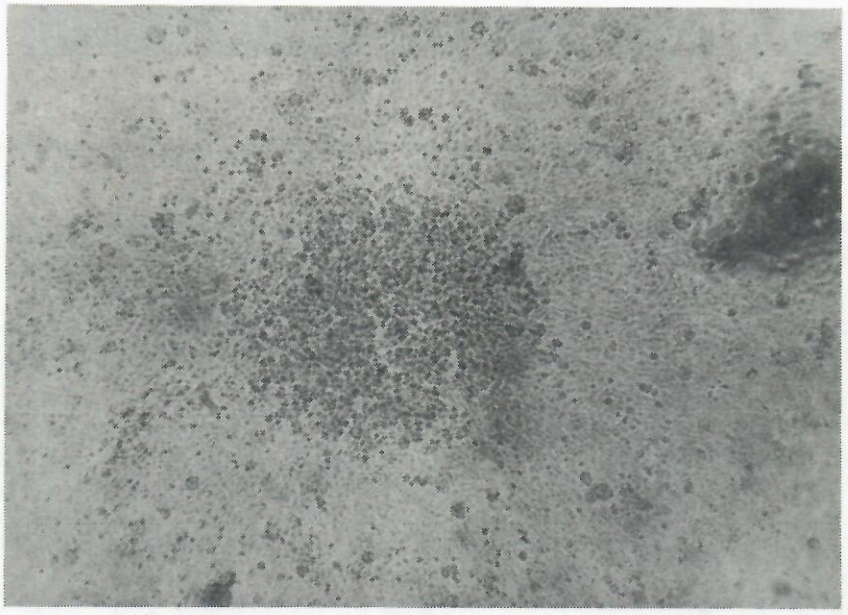

Figure 1. Foci on CEF culture 3 days after infection with lymphocytes separated from the blood of a chicken vaccinated with HVT vaccine

consisted of shiny round cells among which giant balloon-like cells could be noticed. Isolation of vaccinal virus was possible in 79,1% of the chickens examined. During the 6th week vaccinal virus could be isolated in 88,8% of the chickens (Chart 1).

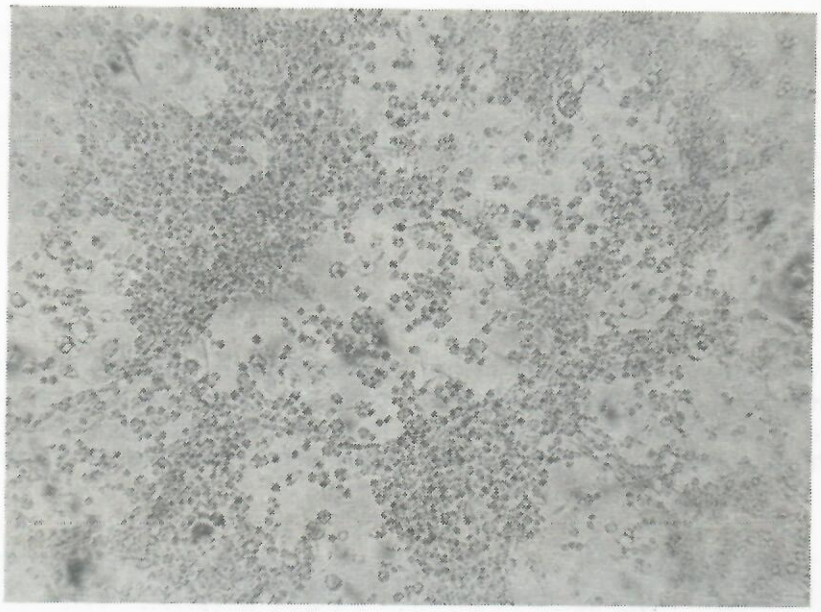

Figure 2. Foci on CEF culture 5 days after infection with lymphocytes from the peripheral blood of a chicken vaccinated with HVT vaccine

Figure 3. Foci on DEF culture 5 days after infection with lymphocytes separated from the peripheral blood of a chicken infected with MDV

Pathogenic virus formed many smaller foci in DEF cultures which consisted of groups of round shiny cells unequally arranged (Fig. 3). Giant cells in this culture were present in a smaller number and usually with out vacuoles, as was the case with vaccinal virus. Isolation of pathogenic virus was possible during the second week of infection in 70% of the chickens, while during the 6th week the percentage was 84% (Chart 1).

Using monocional antibodies we confirmed that the isolated pathogenic virus belonged to serotype 1. Pathogenic virus did not give positive findings by the method of indirect immunoflorescence with monoclonal antibodies for serotypes 2 and 3.

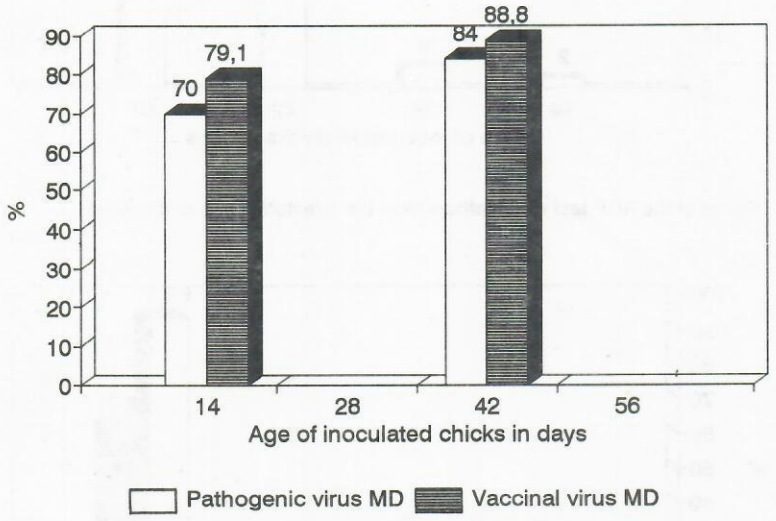

Chart 1. Isolation of a pathogenic and vacinal MD virus

The immunodiffusion test with feathers was positive only in the group of infected chickens. During the second week of infection one chicken had the virus in its feathers (2%), while in the fourth week two chickens were positive (4%) (Chart 2). During the 6th and 8th week most of the chickens were positive (68,5% and 77,7%, respectively). In the vaccinated and control group of chickens MDV was not found in the feather follicles.

It was possible to detect antibodies for "A" viral antigen with antigen MNS/91 earlier than with the antigen from Weybridge (0156/03; Chart3). During the fourth week only one chicken was positive (2%), and in the 6th week 4 chickens out of 48 were positive (8,3%). However, eight weeks post infection 93,3% of the chickens had antibodies for MD in blood sera as detected with antigen MNS/91. The antigen from Weybridge gave 62,2% positive findings at the same age of the chickens.

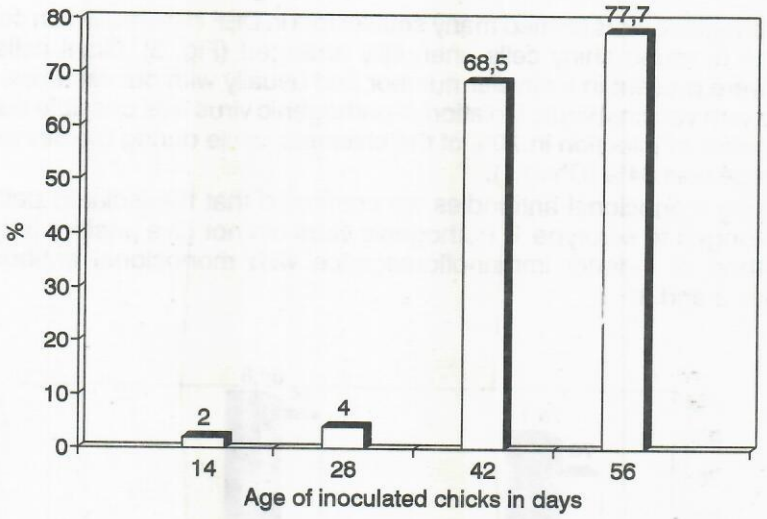

Chart 2. Result of the AGP test with feathers from the infected group of chickens

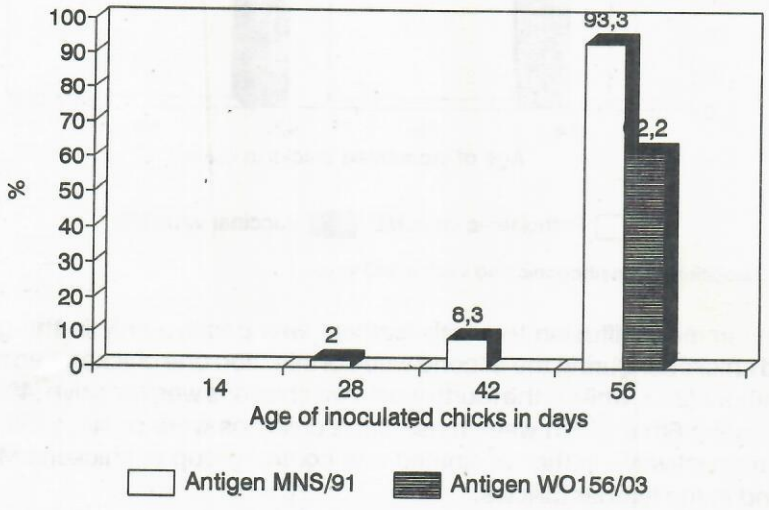

Chart 3. Results of the AGP test with antigens MNS/91 and WO 156/03

DISCUSSION

The cytopathogenic effect due to vaccinal and pathogenic virus could be differentiated according to its intensity and the appearance of foci in cell cultures. However, when examining field blood samples microfoci of the pathogenic virus

were sometimes masked by changes in cell cultures caused by vaccinal strains. Vaccinal HVT virus could be demonstrated during 70 weeks (Witter et al., 1978) only in a small percentage in comparison to the same opportunity a few weeks post vaccination. Since, under natural circumstances, it is not known when chickens acquire infection with pathogenic virus, even if we have established that it mostly happens at an early age, there are difficulties in the isolation of pathogenic virus directly from infected chickens. The weak spread of the virus in the cell culture, which may be explained by the facts that MDV is bound to the cell and that the virus is poorly released into the medium (Payne, 1985), makes it even more difficult to identify a strain judging only by the appearance of CPE.

Therefore, the diagnosis of MD was greatly helped by monoclonal antibodies (Lee et al., 1983) which enable the determination of the serotype of a certain virus. This paper confirms that the pathogenic virus isolated during determination of the state of viremy of infected chickens belongs to serotype 1. However, difficulties in routine diagnosis are still present because the vaccinal attenuated strain CVI-988 belongs to the same serotype. Attempts have been made to solve this problem elsewhere by introducing ELISA tests based upon monoclonal antibodies (Lee et al., 1992).

Concerning results obtained here, determination of virus in feather follicles of infected chickens, as well as antibodies to "A" antigen in sera of the same chickens, may serve for making a diagnosis. Using a radial immunodiffusion test (RID) Rusov et al., (1992) confirmed the presence of the virus in feather follicles of infected chickens, but it was concluded that vaccinal viruses could also be detected in feather follicles. In those experiments vaccinal virus (HVT) could not be found in any of the samples of feathers of infected chickens during 8 weeks. Rangga et al., (1982) managed to find non-oncogenic virus only in genetically susceptible lines of chickens in 3% of feather samples. Cho (1975) found vaccinal virus only in the second week after vaccination with HVT vaccine which was applied in high doses which are not common for a commercial vaccine. Finally, it is known that vaccinal virus is weakly replicated in feather follicles, especially HVT. Its weak horizontal spread in a flock is important and this is supported by the results obtained in this work (Purchase et al., 1972).

Antibodies for "A" viral antigen were found in a high percentage only in the infected group of chickens. Chickens which died during the experiment prior to 8 weeks old did not have antibodies for "A" antigen in their sera. During the 8th week most of the infected chickens were positive, which agrees well with the results of Sharma et al. (1973) who managed to detect antibodies only in the 6th and 8th week.

All the methods mentioned in this paper may undoubtedly assist in making a diagnosis of MD in the field. This is especially easy if there are characteristic clinical and pathoanatomic symptoms in the flock. In a previous work (Gagić et al., 1992) we showed that an outbreak of disease could be predicted from the results of examination of sera from the farms if precipitins to "A" antigen were found in a high percentage in young chickens. These data are useful in routine diagnosis for MD, but we must not forget that the final outcome of infection with

this virus depends upon many factors. The virulence of the field virus which might infect the chickens has crucial importance, followed by the age of the chickens and their genetic susceptibility. It is very difficult to perform detailed analyses in the field in our country because of the small number of diagnostic methods available, including experimental conditions. It would be useful to extend the laboratory methods mentioned in this paper in accordance with present day knowledge in this field and to include even more sensitive methods for investigation of cellular immunity.

Acknowledgments

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OTKRIVANJE INFEKCIJE ŽIVINE VIRUSOM MAREKOVE BOLESTI UZ POMOĆ VIŠE LABORATORIJSKIH METODA

MAJA GAGIĆ, S. ĐURIŠIĆ, RUŽICA AŠANIN, i T. PALIĆ

SADRŽAJ

U radu je opisano više laboratorijskih metoda pomoću kojih je moguće dokazati virus Marekove bolesti (MB) kod pilića.

Patogeni virus MB izolovan je na kulturi pačijih fibroblasta a vakcinalni virus na kulturi pilećih fibroblasta. Citopatogeni efekat vakcinalnog virusa bio je intenzivniji i brže nastajao u odnosu na patogeni soj upotrebljen za infekciju pilića.

U serumu inficiranih pilića dokazana su antitela za "A" antigen virusa MB metodom agar gel precipitacije dok u vakcinisanoj grupi nisu ustanovljena antitela za isti virus. U folikulima pera inficiranih pilića virus MB mogao je biti dokazan metodom imunodifuzije u gelu dok vakcinalni virus nije ustanovljen na ovaj način.

Prilikom utvrđivanja infekcije pilića virusom MB koji cirkulišu na farmama pored izolovanja virusa na ćelijskim kulturama bilo bi dobro koristiti i druge laboratorijske metode o čijim prednostima i nedostacima izveštavamo o ovom radu.