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PATHOLOGICAL AND IMMUNOLOGICAL CHANGES IN RABBITS INFECTED WITH INFECTIOUN BOVINE RHINOTRACHEITIS VIRUS (IBR)

(With one Table and 7 Figures)

By

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التغيرات الباثولوجية والمناعية فى الارانب المعدية بفيروس التهاب الأنف والقصبة الهوائيه البقرى المعدى

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أستخدم فى هذه الدراسه اربعون ارنبا نيوزيلندى خاليه من الامراض قسمت الى ثلاث مجاميع: الاولى ٢٤ أرنب حقنت فى الوريد بفيروس التهاب الانف والقصبه الهوائيه البقرى المعدى، والثانيه ١٠ أرانب أعطيت نفس الجرعه فى الأنف، والثالثه شملت ٦ أرانب كضابط. وأظهرت النتائج ان أفضل دراسه مرضيه للفيروس فى الارانب كانت للتى حقنت فى الوريد حيث تأثرت الاعضاء المختلفة بالفيروس بالاضافه الى وجود اجسام مندمجه وكذا تنكرز الطبقه الطلائيه للأوعيه الدمويه، علاوة على ذلك وجدت استجابه مناعيه مصليه وخلويه باستخدام اختبار الاجسام المناعيه المتعادله واختبار عامل منع الهجرة لخلايا الدم البيضاء.

SUMMARY

Forty pathogen free newzealand rabbits were used in this study. They were divided into 3 groups: the first group consisted of 24 infected intravenously with IBR virus. The second of 10 infected intranasally and the third of 6 as uninoculated control. The intravenous route proved to be the best one for studying the pathogenesis of IBR virus infection in rabbits. The virus affects

various organs with intranuclear inclusion bodies as well as endotheliosis. Moreover there is both humoral as well as cell-mediated immune responses as detected by Serum neutralization test (SNT) and leukocytic migration inhibitory factor (MIF) respectively.

Key words: *IBR- Infection of rabbits-Patholoy-Immunoloy.*

INTRODUCTION

IBR primarily affects cattle causing respiratory, genital and nervous manifestations (Kahrs. 1977). Other animals such as goats, swines and water buffaloes are naturally infected. Serological studies indicated that infection could occur in other species (Afshar and Fadjbakhsh, 1970). The virus was not pathogenic for mice and guinea pigs (Merchant & Pacher, 1967; Armstrong et al., 1961). It was pathogenic for rabbits by skin scarification, intracerebral and corneal routes (Persechino et al., 1965) as well as by intratesticular and intradermal routes (Armstrong et al., 1961).

Yet we still need a suitable laboratory animal for studying the pathogenesis as well as the diagnosis of IBR virus infection and evaluation of the vaccines.

MATERIAL and METHODS

I- Material:

1- Rabbits: Fourty rabbits (Newzealand) each of 1.8-2 kg body weight were used. The rabbits were fed a standard diet and sulfaquinoxaline for one week prior to virus infection. Thirty four were used for experimental infection while six left as uninoculated controls.

2- Virus: IBR (Japanese strain-758) was propagated and titrated in vero cell culture according to the method of El-Nakashly et al. (1985).

3- Cell culture: Both bovine and vero cell cultures were propagated and maintained according to Youngner (1954).

II- Methods:

1- Infection of rabbits: The rabbits were divided into two groups: the first of 24 and the second of 10 rabbits. Each animal of the first group was intravenously inoculated with 1 ml. of the virus ($10^5 TCID_{50}$), while each animal of the second group was infected by the intranasal instillation of the same dose of virus. Each animal of the control group received one ml. of strile saline solution. The animals were kept under observation with daily recording of the body temperature. Two animals of the inoculated group

were slaughtered every 48 hours, while from the control group one was sacrified every 4 days.

2- Collection of Samples:

A- Heparinized blood: At predetermined intervals, uncoagulated blood was collected from each animal to be used for separation of leukocytes and mononuclear cells.

B- Serum: In addition, at predetermined intervals, blood was collected from each animal separately, left to clot and serum stored at -20°C till used.

C- Tissue specimens: Tissue specimens were collected from both infected and control animals, prepared for histopathological studies and stained with the following stains:

-hematoxline and eosin (Harris, 1898).

- Lendrum's phloxin tartrazine (Clayden, 1971).

- Crossman's stain (Culling, 1963).

3- Serological tests:

A- Serum neutralization test: According to Hafez and Frey (1973).

B-Leukocyte migration inhibition factor (L.I.F.):

RESULTS

1- Clinical Symptoms: Only a mucopurulent discharge was observed from the nostriles of infected animals during the first week post-infection. As for temperature recording, results are presented in Fig (1).

2- Neutralization test: Results are shown in Fig (2).

3- Leukocytic Migration factor: Results are grouped in Fig (3).

4- Histopathological studies: Results of this investigation are presented in table (1) and Fig. (4).

DISCUSSION

Rabbits intravenously inoculated with IBR virus manifested a rise of temperature for 3 days (Fig. 1) This rise of temperature was attributed to the viraemic period in rabbits (Lupton et al., 1980 and Arab et al., 1984) and cattle (Miller, 1955). In addition there was a mucopurulent nasal discharge in both the infected groups. This was previously mentioned by Lupton and Reed (1979), which reflects the affinity of the virus to the respiratory system.

The immune response of rabbits to IBR virus was studied by conducting the SNT and Leukocytic migration inhibition factor. Results as presented in Figure (2) reveal an increase in antibody titre, reaching a maximum between

20 and 24 days post-infection. The same finding was mentioned by Berrios et al. (1983). Results of the LMIF as detected in Fig. (3) reveal that there was an early response of leukocyte to the virus (by the 7th day post-infection), even before the appearance of a humoral immune response. This test may be useful in case of vaccine evaluation. However Schollenberger and Prandata (1982) recommend both the cellular and humoral immune responses.

Histopathological studies revealed that, IBR virus affected different organs with a cellular destructive power especially the parenchymatous organs. The most characteristic lesions were necrobiotic changes (Liver, Kidney) together with infilteration of monocytes. According to Baker et al. (1960), these changes are greatly specific to IBR virus. They reported that the virus possesses a cytolytic effect on the host cell with release of the virus extracellularly infecting and destroying more cells. Thompson (1984) further added that immune response to the virus leads to morphological and functional alterations damaging the tissues where the virus grows resulting in progressive lesions and clinical disease.

The hyperplasia of the peribronchial lymphoid follicles may be explained as an immune defence mechanism against the virus. This explains also the hyperplastic proliferation of spleen lymphocytes, since the spleen is a site of antibody formation. Lesions in the central nervous system are typical. Lesions in viral affections (Okot Swangamoni and Kaminfola 1973). The presence of cowdry's intranuclear inclusion bodies is a manifestation of cellular infection with herpes viruses.

Lesions in the blood vessels indicate the endotheliotropism of the virus. Once more the hyperplasia of blood vessels could be attributed as a mean of defence mechanism of blood vessels against continuous irritation of the virus.

The previous results support the view that rabbits are susceptible to infection with IBR, producing both clinical and immunological responses. Hence this laboratory animal could be used with success in the evaluationof vaccines against this virus.

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Table. 1: Pathological lesions in various organs from rabbits experimentally infected with IBR virus.

Organ and /or tissue	Main lesion in the examined organ from the respective group		
	I	II	III
Liver	Congested-necrotic changes in the first week, then multifocal to diffuse areas of advanced necrosis-leukocytic infilteration with lymphocytes and round cells-focal hemorhage in the necrotic areas. activation of kupffer cells-the lesions subsided by the third week Fig. (5)	Some cells showed necrosis with lymphocytic infiltration, others dissociation with granularity and vacuolarity of cytoplasm- Activation of kupffer cells. Macroscopically there was congestion.	
Kidney	Congestion with focal depressed areas. In the first week, early diffuse necrobiotic chanes associated with perivascular oedema-By the 2nd week, complete necrosis of tubules, infilteration with lymphocytes and round cells infilteration. Some tubules showed atrophoid, others showed cystic dilatation Fig (6).	Some tubules showed proteinous dystrophy-Congestion of cortical & medullary blood vesseles are observed.	

Content Table 1:

Organ and /or tissue	Main lesion iin the examined organ from the respective roup		
	I	II	III
Lung	Oedema with round cell infilteration activation of septal cells & infilteration of monocytes narrowing of the bronch al lumen-perivascular oedema then narrowing of air spaces with compansatory emphysema in some air spaces. By third week, lestions subside with endotheliosis. Grossly the lung was congested Fig (7).	Lung congested- bronchitis- perivascular cuffing of peribronchial blood vessels.	
Brain	Perivascular oedema- heomorrhage in brain ventricles focal gliosis-intranuclear inclusions in neuron & glial cells- focal encephalomalacia.	Minute focal areas of gliosis-perivascular cuffin- degeneration in some neurons.	
Adrenal gland	Minute necrotic foci in zona fasculata-congestion of blood vessels-then multiple focal to diffuse areas of necrosis in zona fasculata, reticularis & medulla. Later severe necrosis, infilteration of monouclear cells & intranuclear inclusion bodies.		
Spleen	First hyperplasia of lymphoid follicles-then depletion of lymphocytes- activation of reticulo-endothelial system. Finally, reticular tissue replaced white pulp. Grossly it is diffusely enlarged.		
Heart	Hyaline degeneration and congestion of blood vessls, then myomalacia with leukoytic infilteration and perivascular oedema. Finally lesions subside except the mononuclear cell infilteration.	Myomalacia with infilteration of mononuclear cells. Congestion of blood vessls and hemorrhage in endocardium.	
Trachea	Congestion of blood vessels and oedema in lamina propria with infilteration of mononuclear cells.	As in group I.	

I= Intravenous Group. II= Intranasal Group III = Control Group.

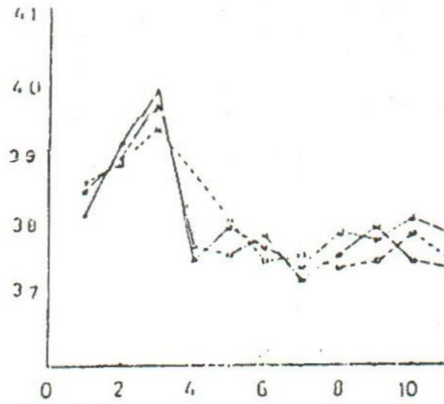

Fig. (1): Temperature degrees of rabbits infected intravenously with IBR virus.

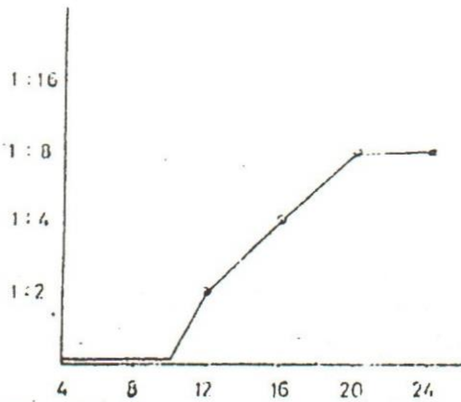

Fig. (3): Neutralizing Ab titres in sera from rabbits intravenously infected with IBR virus.

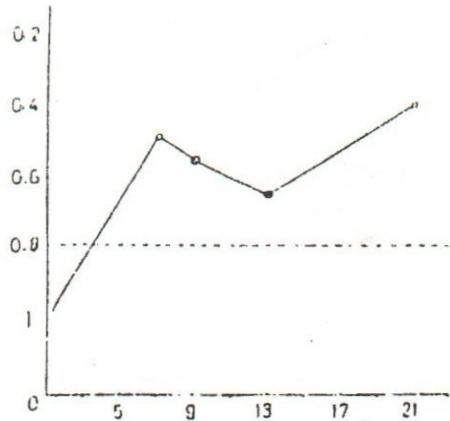

Fig. (4): Migration index of leukocytes from rabbits intravenously infected with IBR virus.

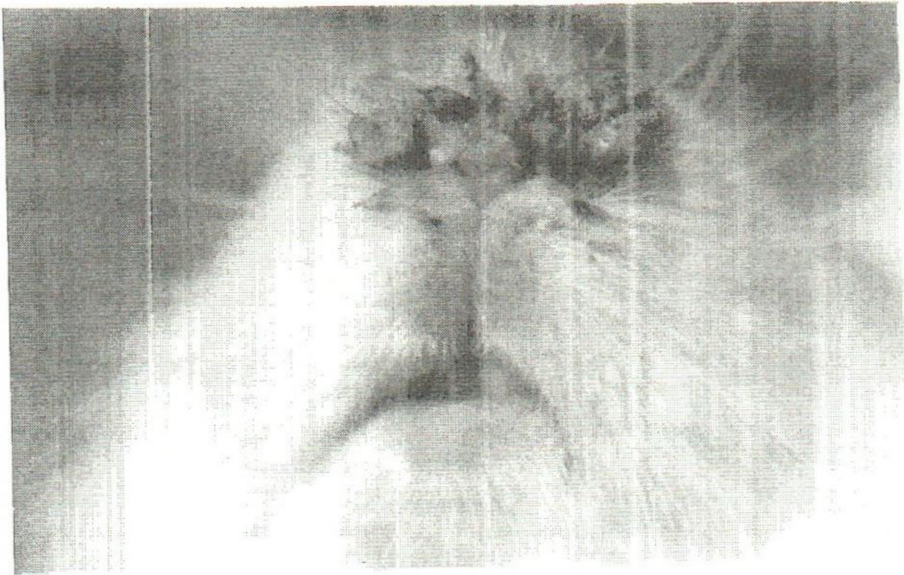

Fig. 2: Mucopurulent discharge from the nontrils of a rabbit after 5 days post intravenous infection with IBR virus.

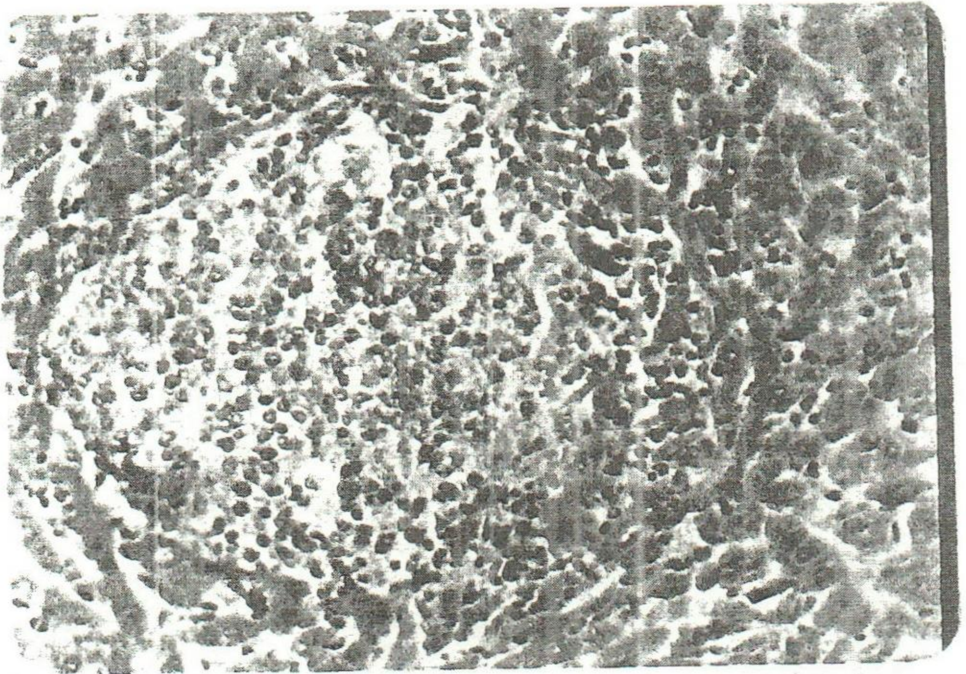

Fig. 5: Focal necortic area in liver represented by remnant of cells and surrounded by inflammatory cells.
H & E X 375.

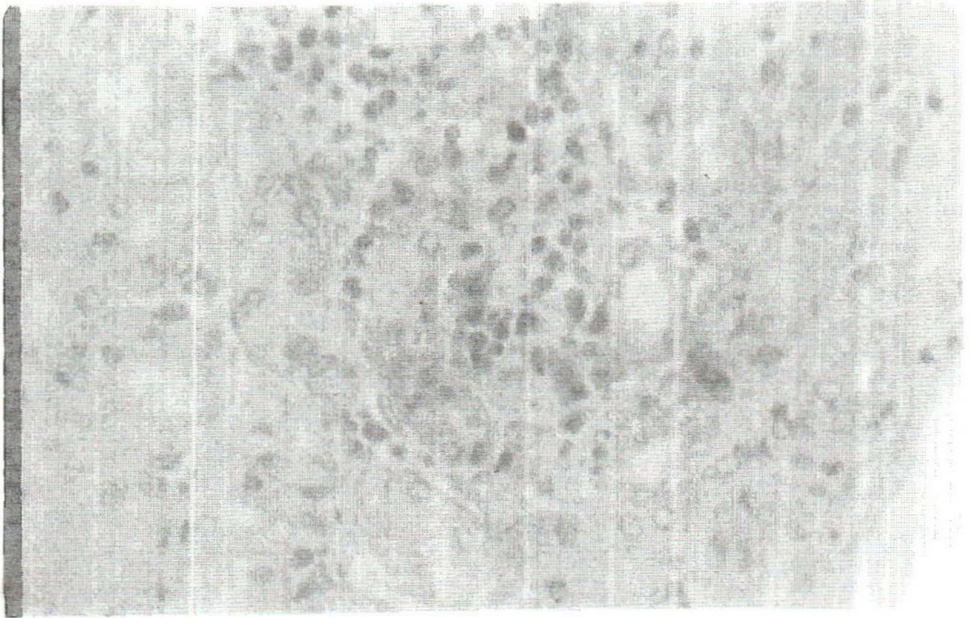

Fig. 6: Necrosis of renal tubules of kidney and infiltration of lymphocyte: and plasma cells. H & E X 600.

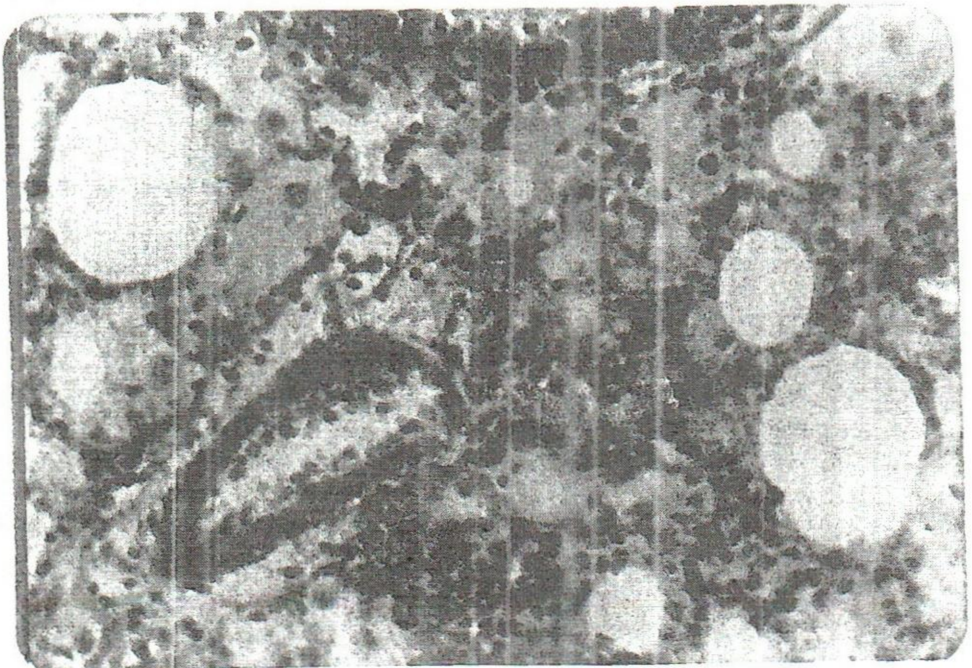

Fig. 7: Lung oedema and interstitial pnumonia characterized by activation of septal cells and round cells infiltration. H & E X 375.