Experimental Infection with bovine parainfluenza type 3 virus in mice

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Summary

Four weeks old mice were used for experimental infection with isolated bovine parainfluenza type 3 virus. Mice were divided into three groups: The first group was infected by intracerebral route, the second group was infected by intranasal route and the third group was a control group. Experimental infection with isolated bovine parainfluenza type 3 virus in mice revealed that the isolated BPIV-3 was unable to kill 4 weeks old mice within 65 days when inoculated by intracerebral route, but isolated BPIV-3 was shown to kill (5 out of 10) 4 weeks old mice within 65 days when inoculated by intranasal route. There was no mortality in the control group within the same period. Two weeks old mice were used for experimental infection with isolated bovine parainfluenza type 3 virus and histopathological examination of infected organs was performed. Experimental infection of 2 weeks old mice by isolated BPIV-3 revealed that isolated BPIV-3 induced hydrocephalus after intracerebral inoculation. Histopathological examination for collected organs of BPIV-3 infected 2 weeks old mice (brain and lung) indicated histopathological changes were detected in comparison to non-infected collected organs. Virus was reisolated from infected brains of experimentally infected mice with locally isolated BPIV-3.

Keywords: Bovine parainfluenza 3, Viruse, Mice.

Introduction

Bovine parainfluenza virus type 3 (BPIV-3) is one of the agents associated with upper and lower respiratory tract of cattle; the virus was first isolated from calves with respiratory disease (1). BPIV-3 is an envelope, non-segmented, negative-sense RNA virus within the genus Respirovirus, member of Paramyx-oviridae family, antigenically related to human parainfluenza virus type 3 (HPIV-3) (2 and 3). The role of this virus in bovine respiratory disease is well-established (4), the virus was found in acute and chronic cases of pneumonia in cattle, but it is most frequently recorded in young animal (5). Most strains of BPIV-3 induced pathogenic effects when inoculated in mice (6). In order to detect pathogenic effects of isolated BPIV-3 in mice, mice were experimentally infected with isolated BPIV-3 and histopathological changes were examined in infected organs (brain and lung).

Materials and Methods

Thirty BALB/c mice 4 weeks of age and 12 BALB/c mice 2 weeks of age were supplied by Iraqi Center for Cancer and Medical Genetics Researches. Isolated bovine parainfluenza type 3 virus ($10^{4.5}$TCDI50/ 50 µl). Secondary embryonic bovine kidney (SEBK) cell culture was prepared in college of Veterinary Medicine - Baghdad University.

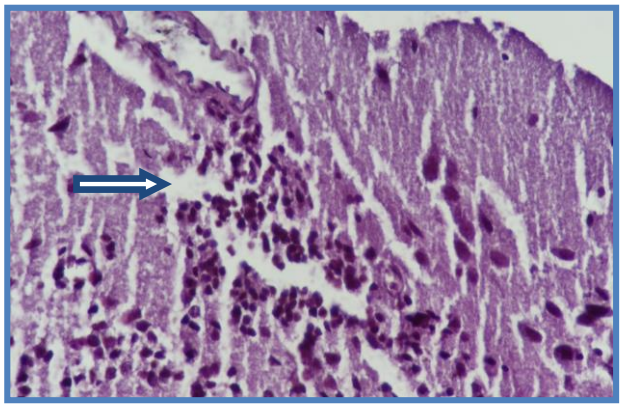
The experimental design was conducted according to (7) as follows: Healthy four weeks old mice were divided into equal 3 groups, first group were inoculated intracer brally with $10^{4.5}$TCDI50/ 50µl of BPIV-3, the second group were inoculated intranasally with $10^{4.5}$TCDI50/ 50 µl of BPIV-3 and the third group were inoculated intranasally and intracerebrally with normal SEBK cell suspension as control. The animals were examined daily. Two other groups of mice include younger mice 2 weeks old was subdivided into 2 groups, first group include 6 mice were inoculated intracerebrally with $10^{4.5}$TCDI50/ 50 µl of isolated virus and the second group, include 6 mice were inoculated intracerebrally with SEBK cell suspension media. After 3-7 day post infection, two mice were killed in each group. Collected samples (Lung, and brain) were used for histopatho-logical examination; samples were fixed in buffered 10% formalin for 24 hrs. and tissues were embedded in low- melting point paraffin then sectioned at 5 *M*m thickness and stained with hematoxylin and eosin (8). Lung and brain were used for virus isolation, which were homogenized with maintenance media and inoculated into SEBK cells.

Results and Discussion

Experimental infection in 4 weeks old mice: The isolated BPIV-3 was unable to be lethal for the 4 weeks old mice in a period of 65 days when these were inoculated intracerebrally. This result disagreed with other studies, which showed that M strain and YN strain were lethal for mice (6) but it agreed with (7) who showed that 910 N strain and other wild strains of BPIV-3 were not lethal for mice.

The isolated BPIV-3 was able to kill (5 out of 10) 4 weeks old mice for a period of 65 days when inoculated by intranasal route. The affected mice showed retardation of growth, red nose and pale lung. Ability of virus to kill mice when inoculated intranasally corresponding to (5), who showed that the virus (BPIV-3) spread primarily by large droplet transmission once inhaled into the respiratory tract, a BPIV-3 would first encounter a mucous layer with a high content of N-acetylneuraminic (sialic) acid, a natural substrate for the neuraminidase activity of the HN glycoprotein in the viral envelope, based on early studies conducted with bovine nasal secretions in vitro it was proposed that the HN molecule in the viral envelope would specifically and sequentially bind to the sialic acid residues in mucus, causing its degradation and effectively allowing the penetration of the virus to subjacent target epithelial cells (9).

Experimental infection in 2 weeks old mice: The isolated BPIV-3 induced hydrocephalus in 2 weeks old mice after intracerebral inoculation, this result was in agreement with (10) who have shown that the 910 N strain induced hydrocephalus in newborn mice after intracerebral inoculation. Histopathological examination of sections from brain and lung tissue of mice revealed the followings: In day 3 post infection: Brain showed odema and congestion of meninges. Lung showed congestion of alveolar cells and infiltration of inflammatory cells. In day 5 post infection: Brain showed odema and congestion of meninges with aggregation of glial cells (Fig. 1), Lung showed odema, congestion of capillary of alveolar cells and infiltration of polymorphonuclear cells in the alveolar cells (Fig. 2).



Figure, 1: Odema, Congestion of Neural Cells and Aggregation of Glial Cells in the Infected Brain, Tissue of Mice within 5 Days P. I. Stained with (H and E) (100X).

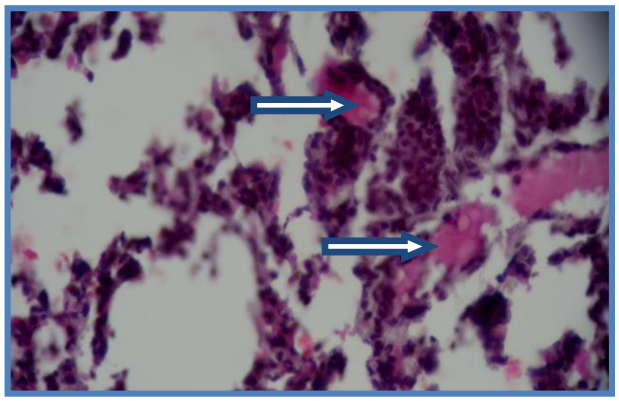
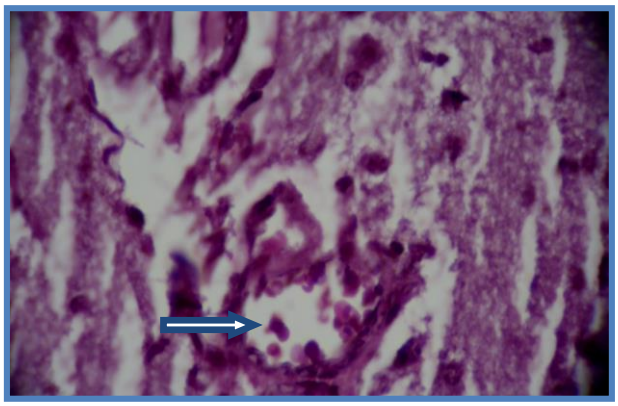
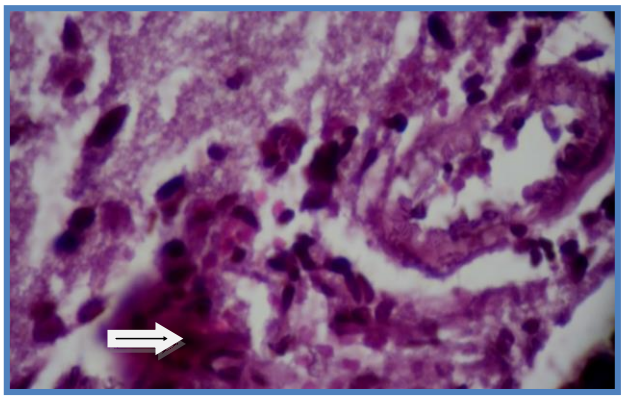


Figure , 2: Odema, Congestion of Capillary of Alveolar Cells and Infiltration of Polymorphonuclear Cells in the Alveolar Cells of Mice Lung after 5 Days of Infection with Isolated BPIV-3 Stained with (H and E) (100X).



Figure, 3: Cerebral Odema, Congestion in the Brain Tissue and Meninges with Vaculation in the Infected Brain Tissue of Mice with Isolated Virus Stained with (H and E) (400X).

In day 7 post infection: Brain showed congestion and perivasular odema with cerebral odema (Fig. 3), Lung showed thickening of alveolar wall, bronchial sloughing, slight infiltration of inflammatory cells, odema, congestion of capillary of alveolar cells and slight thickening of interalveolar septae (interstitial pneumonia) (Fig. 4).



Figure, 4: Odema, Congestion of Capillary Alveoli and Slight Thickening of Alveolar Septae (interstitial pneumonia) in Infected Lung of Mice with Isolated BPIV-3 Stained with (H and E) (400X).

Many histopathological changes were recorded in brain and lung of infected mice with isolated BPIV-3. This is in agreement with (7) who have shown that both 910 N-infected and M-infected newborn mouse brains at 3 days post infection showed ventricular, subependymal and meningeal infiltration of lymphocytes and granulocytes, and degenerative changes in many ventrieular epithelial cells and in some meningeal cells.

By 7 days M-infected brains, but not 910 N-infected brains, revealed diffuse lymphocytic infiltration in the deep parenchyma where viral antigens had been demonstrated. Inflammatory reactive processes were much more evident in 2 week old mouse brains than in newborn mouse brains. Both 910N and M-infected brains showed ependymitis and meningitis manifested by diffuse and perivascular infiltration of lymphoeytes at all stages. The histopathological hallmark of BPIV-3 associated lung lesion characterized by infiltration of inflammatory cells with sloughing of effected cells into lumen of airways with intracytoplasmic inclusion bodies which are most common between 2-7 days post infection (5).

Result of virus isolation from experimentally infected two weeks old mice: BPIV-3 was isolated from the infected brain tissue after two passages in SEBK cell culture however; the distinctive CPE was evident on the first passage of the infected lung tissue in SEBK cell culture in agreement with other studies (7) by using Madin Darby bovine kidney (MDBK) cells.

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الاصابه التجريبية بفايروس نظير الأنفلونزا ألبقري نوع 3 في الفئران

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الخلاصة

إستعملت فئران بعمر أربعة أسابيع لغرض إجراء الاصابه التجريبية بفايروس نظير الأنفلونزا ألبقري نوع 3، قسمت الفئران إلى ثلاث مجاميع الأولى أعطيت الفايروس عن طريق الحقن في الدماغ والأخرى عن طريق الحقن في المنخرين والأخرى تركت كمجموعة سيطرة. لم تظهر الاصابه التجريبية بفايروس نظير الأنفلونزا ألبقري نوع 3 المعزول محليا في الفئران بعمر 4 أسابيع أي هلاك عند حقنها في الدماغ خلال 65 يوم في حين كانت نسبة الهلاك خمسة فئران من عشرة عند حقنها عن طريق المنخرين خلال نفس الفترة في حين لم تظهر أي هلاكات في مجموعة السيطرة وللفترة نفسها. إستعملت فئران بعمراسبوعين لغرض إجراء الاصابه التجريبية بالفايروس عن طريق الدماغ ومن ثم إجراء الفحص النسجي على الأعضاء المصابة. أظهرت الاصابه التجريبية بالفايروس في الفئران بعمر أسبوعين موه الدماغ عند حقنها عن طريق الدماغ كما اظهر الفحص النسجي تغيرات نسجيه مرضيه في الاعضاء المصابه التي تم جمعها (الرئتين والدماغ) مقارنة بالاعضاء غير المصابه التي جمعها من مجموعة السيطره. اعيد عزل الفايروس من ادمغة الفتران المصابه تجريبيا بفايروس نظير الانفلونزا البقري المعزول محليا.

الكلمات المفتاحيه: نظير الانفلونزا البقري نوع 3، فايروس، الفئران.