

معهد انتاج الأمصال والمقاحات بالعباسية - القاهرة .
مدير المعهد : أ. د. / سيد سلامه .

عدوى الاغنام صناعيا بفيروس التهاب الرئوى (PI-3)

٢ - دراسات فيرولوجيه وعلاقتها بالأعراض الاكلينيكية

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أمكن عدوى الحملان " الرحمانى صناعيا بالفيروس المسبب للالتهاب الرئوى المعزول محليا والمسمى (Parainfluenza-3 virus) ولقد ظهرت على الاغنام المحقونه أعراض رشح مخاطي أو رشح مخاطى ممدد سميك ابتداءً من اليوم الثالث بعد الحقن ثم توالى ظهور الأعراض الاكلينيكية والتي كانت تتميز بوجود ارتفاع في درجة الحرارة ، سعال ، عطس مع صعوبة في التنفس .

ولقد أمكن عزل الفيروس من الـ Nasal passages لحدى عشر حيوان بعد ٧٢ ساعة من الحقن ثم ارتفع العدد الى ثلاثة عشر حيوانا بعد خمسة أيام ثم عشرة حيوانات بعد اليوم السابع ومن أربعة أغنام بعد اليوم التاسع . ولكننا لم نتمكن من عزل الفيروس من الحيوانات التي تم فحصها في اليومين الحادى عشر والثالث عشر بعد الحقن .

ولقد أمكن اثبات وجود أجسام مناعيه من النوع الثلاثى ضد فيروس البارافلوزا - ٣ في أمصال اثنى عشر حيوانا فحصت بعد ثلاثة أيام من الحقن . ثم ارتفعت القوة العياريه للأمصال بعد ذلك حتى اليوم الأخير للتجربة .

وبعد ذبح الحيوانات امكن عزل الفيروس من رئة سبعة عشر حيوانا وأيضا من الكبد وغدد الجهاز التنفسي اليمفاويه وأيضا من مسحات مأخوذة من القصبة الهوائية لبعض الحيوانات ($\frac{8}{18}$)

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EXPERIMENTAL VIRAL PNEUMONIA IN SHEEP
II. VIROLOGICAL STUDIES IN RELATION WITH CLINICAL MANIFESTATIONS
(With 5 Tables)

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SUMMARY

Pneumonia could be experimentally induced in "Rahmany" lambs by inoculating the animals with a local isolate of parainfluenza-3 virus (PI-3).

The included lambs developed a mucoid to a mucopurulent nasal discharge on the 3rd day post inoculation (p.i.). The clinical reactions increased afterwards and were characterized by pyrexia, coughing, sneezing and respiratory distress.

The virus was recovered from the nasal passage from the 3rd day and till the 9th day p.i. The animals examined on the 11th and 13th days p.i. were negative for virus isolation.

The HI titre appeared on the 3rd day p.i., then increased progressively till the end of the experiment (13th day p.i.).

In animals sacrificed at different intervals, the virus could be reisolated from pneumonic areas in the lungs of 16 out of 18 lambs and also from the liver, respiratory lymph nodes as well as from the tracheal swabs of some of the sacrificed animals (8 out of 18 swabs).

INTRODUCTION

Pneumoenteritis of animals is a very important economic problem due to the losses and high death rates it causes among young animals and to a lesser extent among adult ones.

Parainfluenza-3 virus (PI-3) plays an important role as one of the causes of respiratory diseases affecting different animal species as well as man (SINGH and BAZ, 1966; HORE and STEVENSON, 1967; STEVENSON and HORE, 1970).

PI-3 virus infection of sheep has been described as asymptomatic (FISHMAN, 1967) or associated with outbreaks of respiratory disease (HORE *et al.* 1968). Responses of sheep to experimental infection has been studied by STEVENSON and HORE (1970). In addition, the same authors reported that calves were susceptible to an ovine PI-3 virus. Accordingly, the possibility of natural transmission between the two species can't be excluded.

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In Egypt, BAZ (1971) isolated PI-3 virus from lambs dying from pneumonia and pneumoenteritis. She added that PI-3 virus either alone or with other viruses and bacteria may play a role in producing respiratory illness.

The purpose of the present work is to investigate the pathogenesis and distribution of the virus in the secretions and internal organs in one of our native sheep breeds El-Rahmany, which was experimentally infected with PI-3 virus. The final goal was to find out the correlation between the virological findings and the clinical manifestations, hoping to determine the optimal time for the collection of appropriate samples needed for PI-3 diagnosis during the course of the disease.

MATERIAL and METHODS

1. Animals: Twenty-one "Rahmany" lambs of 10-11 weeks-old were chosen for this purpose. Before being selected, their sera were tested by means of the haemagglutination-inhibition test (HI) at two occasions 21 days apart, where it proved to be free from PI-3 specific HI antibodies (titre of 5). The animals were divided into seven groups of three lambs each. Six groups were inoculated with PI-3 virus (infected groups), while the seventh was left as a non-infected control group in a separate isolated place.

2. Virus inoculation: Each animal of the infected groups (18 lambs) was given 5 ml. intranasally and 3 ml. intratracheally of the 4th tissue culture passage of PI-3 virus (strain 1413) which contained $10^{6.5}$ TCID₅₀ per ml. Following inoculation, the animals were kept under observation with recording of any clinical manifestations. The used virus was that isolated by BAZ (1971).

3. Sample collection: Nasal and lacrimal swabs as well as blood samples were collected from the infected and control lambs on the following days post inoculation (p.i.): 3rd, 5th, 7th, 9th, 11th and 13th days. In addition, three infected animals were slaughtered at each of these intervals, where tracheal and pharyngeal swabs were aseptically collected from each animal in a separate container. Lung, bronchial, retropharyngeal and mediastinal lymph nodes as well as tonsils, trachea and liver were collected for viral reisolation and quantitative assays.

4. Viral isolation and assay: Reisolation of PI-3 virus was done in embryonic bovine kidney cell culture (EBK) following the technique described by MOHSEN *et al.* (1980). Positive samples were quantitatively titrated and the titre expressed as log₁₀ TCID₅₀ per ml. (REED and MUENCH, 1938).

5. Serological methods: Haemadsorption (HAD) and HI tests were carried out using guinea-pig erythrocytes according to the method described by VOGEL and SHELEKOV (1957).

RESULTS

1. Clinical symptoms: The uninfected control animals did not show any thermal reaction or clinical evidence of respiratory affection throughout the observation period. On the other hand, the infected lambs manifested clinical symptoms which started on the 2nd day p.i. by severe dyspnea followed by other symptoms, then declined showing only slight nasal discharge by the 13th day p.i. (Table 1).

2. Virus isolation and quantitative determination: Results of the reisolation as well as the quantitative assay of PI-3 virus from swabs and samples collected from infected lambs are presented in Tables (2, 3 and 4). These results revealed that the virus was mainly isolated from the nasal

VIRAL PNEUMONIA IN SHEEP

samples, and after slaughtering the lungs were the organs that contained the virus in a consistent manner.

Meanwhile, the virus could not be detected in pharyngeal secretions except from only one sample collected on the 11th day p.i. Concerning the other organs, PI-3 virus was detected in lymph nodes (bronchial, mediastinal and retro-pharyngeal), liver and tonsils. Samples from control uninfected lambs did not contain PI-3 virus.

3. Immune response: Results of this investigation are presented in Table (5). It is clear that there was a rise in the HI antibody titre of the sera of infected groups reaching up to 320. Meanwhile, there was no rise in the titre of sera of non-infected control group (> 5).

DISCUSSION

The clinical manifestations expressed by local "Rahmany" lambs inoculated with ovine PI-3 virus were similar to those described by HORE and STEVENSON (1967, 68, 69), BIBRISTEIN (1971), SINGH *et al.* (1977) and SHARP *et al.* (1978). These signs were clearly seen from the 5th to the 9th day p.i. (Table 1), which were also the peak days for virus dissemination from infected animals.

The present study showed that the maximum period during which the virus could be secreted through the nasal passages was 6 days, from the 3rd to the 9th day p.i. (Table 2 & 4). 70% of the nasal swabs collected during this interval contained the virus with a titre ranging between $\log \geq 2.0$ and 5.5 TCID₅₀ per ml. This raised the question, whether these animals could be considered as virus shedders and thus a source of infection to the surrounding animals. In this respect, FRANK and MARSHALL (1971) cited that the maximum time for virus isolation from nasal secretions of calves was from the 4th to the 9th days, whereas HORE and STEVENSON (1967) could recover the virus from the nasal passages of experimentally infected sheep 24 hours up to 8 days p.i. In our study, the highest isolation percentages were 86, 83, 61 and 44% for the 5th, 7th, 3rd and 9th days p.i. respectively, although the highest titres were on the 5th and 7th days for the same animal (Tables 2 & 4).

Moreover, the virus was detected from the conjunctival secretion of one lamb on the 5th day p.i. However the amount of this excreted virus was relatively small being $\log \geq 2.0$ TCID₅₀ per ml. This result is in agreement with HORE and STEVENSON (1967), where they reisolated the virus from a conjunctival swab collected from one lamb on the 5th day p.i. They added that the same lamb from which the virus was isolated from the conjunctival secretion showed a bilateral ocular discharge on the 5th day p.i. The only explanation for how the virus could be detected in conjunctival secretion is by ascending dissemination via the lacrimal canals.

Absence of the virus from the secretions of infected animals and especially from the nasal passages after the 9th day p.i. and the subsiding of the clinical symptoms could be attributed to the presence of a locally induced immunity (NARIN, 1968) in addition to the classical humoral antibodies which started to increase in amount from the 5th day p.i. (Table 5). Moreover, following the appearance of these specific antibodies, the virus disappears from the blood stream and become localized in the lower respiratory tract (predilection site) mainly the lungs, where gross lesions of proliferative and non-suppurative pneumonia were more prominently observed (STEVENSON and HORE, 1970).

At autopsy, the virus could be isolated from the tracheal and pharyngeal swabs as well as from different organs and tissues (Tables 3 & 4). The relatively high titre and percentage of virus isolation from the tracheal swabs may explain why infection is readily transmitted to healthy animals

through coughing. The following organs gave the respective isolation percentages in a descending order: lungs (88%), bronchial lymph nodes (39%), mediastinal lymph nodes (39%), liver (33%), retropharyngeal lymph nodes (22%) and tonsils (16%). Apart from the lungs which showed the highest virus titre (5.8), the other organs had titres ranging between 2.0 and 4.8. The results of virus isolation from tissues and organs at autopsy are in agreement with those found by WOODS *et al.* (1965), HORE and STEVENSON (1967, 69). The last authors detected the virus at autopsy from tissues and organs of infected lambs beyond the 10th day and BIBERSTEIN (1971) isolated the virus at the 12th day only from lungs.

Although the liver is not a predilection site for PI-3 replication, yet the explanation of detecting the virus in this organ is by reaching it via the lymphatic or blood stream.

Since various agents may produce the same clinical symptoms and morphological lesions as PI-3 infection leading to confusion, thus one should rely on virus isolation and identification together with other helpful techniques as immunofluorescent technique, hematological studies and histopathological studies to reach a specific diagnosis. There was a close relationship between the present results concerning percentage and concentration of the isolated virus in different organs and secretions of infected lambs and the findings of EL-SIRGANY *et al.* (1982) with respect to the histopathological changes, hematological picture (especially those in virus infections) and the FA findings as well as the clinical manifestations.

Thus with marked clinical symptoms, there was high virus titres in secretions and organs, marked specific histopathological changes and hematological findings as well as virus detection by the FA technique, while the reverse is a sign of recovery (EL-SIRGANY *et al.* 1982).

Finally, experiments should be conducted using various concentrations of the virus while extending the observation period beyond 13 days. This may help in detecting whether the animals can completely get rid of the virus or still harbour it for much longer period and be a virus shedder, and thus a possible source of infection to contact susceptible animals.

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VIRAL PNEUMONIA IN SHEEP

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Table (1): Clinical symptoms manifested by lambs experimentally infected with PI-3 virus

Group No.	Day of Sacrifice	Clinical symptoms following PI-3 inoculation
I	3	Pyrexia (up to 39°C), general dullness and dyspnea, nasal discharge and lacrimation.
II	5	Pyrexia (up to 40.1°C), same above symptoms. In addition abdominal respiration was also observed.
III	7	Pyrexia (up to 40.4°C), same above syndromes plus mucopurulent rhinitis and profuse watery lacrimation.
IV	9	Pyrexia (up to 40.3°C), marked depression, severe abdominal respiration, bilateral mucopurulent nasal discharge and lacrimation.
V	11	Pyrexia (39.8°C), slight respiratory syndromes, slight bilateral mucopurulent nasal discharge.
VI	13	Temperature declined to (39.6°C), only slight nasal discharge was observed.
VII	Control	No clinical manifestations were noticed during the observation period.

Table (2): Frequency detection of PI-3 virus from the nasal and lacrimal swabs collected from infected lambs

Group of animals	No. of lambs	Frequency detection at the following days post inoculation											
		3		5		7		9		11		13	
		N	L	N	L	N	L	N	L	N	L	N	L
6	18	11	0	13	1	10	0	4	0	0	0	0	0
		18	18	15	15	12	12	9	9	6	6	3	3
Cumulative isolation													
Percentage (%)		61	0	86	6.6	83	0	44	0	0	0	0	0

N : Nasal swab.

L : Lacrimal swab.

VIRAL PNEUMONIA IN SHEEP

Table (3): Frequency detection of PI-3 virus in tissues and organs collected from infected lambs after slaughtering

Total No. of animals in inoculated groups	Route and dose of inoculated virus	Isolation of the virus from swabs and organ samples collected after slaughtering of the lambs at the various intervals							
		Tr	Ph	R.	B.	M.	Lg	Tn	Lv
18	5 ml I/N	$\frac{8}{18}$	$\frac{1}{18}$	$\frac{4}{18}$	$\frac{7}{18}$	$\frac{7}{18}$	$\frac{16}{18}$	$\frac{3}{18}$	$\frac{6}{18}$
	+								
	3 ml I/Tr								
Cumulative isolation Percentage (%)		44.0	5.5	22	39	39	88	16	33

I/N: intranasal; I/Tr: intratracheal; Tr: Tracheal swab;
 Ph.: Pharyngeal swab; R.: Reropharyngeal lymph nodes;
 B.: Bronchial lymph nodes; M.: Mediastinal lymph nodes;
 Lg.: Lungs; Tn.: Tonsils; Lv.: Liver.

Table 4. Recovery and concentration of the virus in the internal organs and excretions of lambs experimentally inoculated with ovine parainfluenza-3 virus.

No. of lamb	Day of sacrifice	Titre of PI-3 virus isolated from the nasal and lacrimal swabs at the following days post infection												Titre of PI-3 virus isolated from different organs after slaughtering of the animals							
		3		5		7		9		11		13		Trachea	Pharyngeal swabs	Liver	Tonsils	Retropharyngeal L.N.	Broncheal L.N.	Mediastinal L.N.	Lungs
439	3	N	3.2	L	-	N	-	L	-	N	-	L	-	4.7	-	3.2	-	-	3.4	3.0	3.3
584		N	3.6	L	-	N	-	L	-	N	-	L	-	-	-	-	-	2.0	-	2.4	3.7
453		N	4.0	L	-	N	-	L	-	N	-	L	-	3.8	-	-	-	-	-	3.2	3.6
577	5	N	>2.0	L	-	N	3.0	L	-	N	-	L	-	3.4	-	-	-	>2.0	-	-	4.0
529		N	-	L	-	N	3.2	L	-	N	-	L	-	4.2	ND	3.0	-	-	3.6	-	3.5
512		N	>3.0	L	-	N	3.8	L	-	N	-	L	-	2.8	-	-	-	-	-	-	3.7
537	7	N	-	L	-	N	-	L	-	N	3.7	L	-	-	-	-	-	-	4.0	3.2	5.7
586		N	-	L	-	N	3.2	L	-	N	2.0	L	-	3.0	-	2.8	-	-	-	3.6	5.5
434		N	-	L	-	N	3.6	L	-	N	-	L	-	-	-	-	-	2.0	-	-	5.4
518	9	N	>3.0	L	-	N	2.3	L	-	N	3.4	L	-	4.2	ND	-	3.6	-	3.0	-	5.6
583		N	>3.0	L	-	N	>3.0	L	-	N	3.2	L	-	-	-	3.0	-	3.0	-	3.0	5.5
424		N	-	L	-	N	3.0	L	>2.0	N	>3.0	L	-	3.4	-	-	-	-	4.8	4.2	5.8
504	11	N	3.0	L	-	N	4.0	L	-	N	-	L	-	-	-	4.2	3.2	-	-	-	-
567		N	2.0	L	-	N	3.2	L	-	N	3.5	L	-	-	3.5	-	-	-	-	-	3.5
486		N	-	L	-	N	4.1	L	-	N	3.2	L	-	-	-	3.7	-	-	3.3	-	4.2
539	13	N	>3.0	L	-	N	5.7	L	-	N	5.5	L	-	-	-	-	-	-	-	-	3.2
565		N	2.0	L	-	N	3.6	L	-	N	3.0	L	-	-	-	-	-	-	-	-	3.3
423		N	-	L	-	N	-	L	-	N	3.6	L	-	-	-	-	3.2	-	3.2	-	-

See Table (3). ; Titre expressed as log₁₀ TCID₅₀ per ml. ; N: nasal swab. ; L: lacrimal swab. ; ND: Not done.

VIRAL PNEUMONIA IN SHEEP

Table (5): HI antibody titre in sera of lambs experimentally infected with PI-3 virus

Group No.	No. of animals per group	Day of sacrifice	HI titre before virus inoculation	HI titre ^o at the following days post-inoculation					
				3	5	7	9	11	13
I	3	3	> 5	10,20, 20(15) ^o - 20) ^o					
II	3	5	> 5	5,10, 10(10)	10,10, 20(15)				
III	3	7	> 5	10,5, 10(10)	20,5, 20(15)	20,10, 40(25)			
IV	3	9	> 5	20,10, 10(15)	20,10, 20(15-20)	20,20, 20(20)	20,40, 40(30)		
V	3	11	> 5	10,5, 5 (10)	20,10, 10(15)	40,40, 20(30)	40,40, 40(40)	80,80, 80(80)	
VI	3	13	> 5	10,10, 5 (10)	20,20, 10(15-20)	20,20, 20,(20)	40,40, 20,(30)	80,80, 40(40)	320, 160, 80 (190)

^o Reciprocal of the serum dilution.^{oo} Average HI titre of the group.

