A FIELD EXPERIMENT TO ASSESS THE ROLE OF PARAINFLUENZA TYPE 3 VIRUS IN PNEUMONIA IN A FLOCK OF SHEEP IN VICTORIA

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Abstract

A flock of 33 lambs born to ewes with neutralizing antibody to parainfluenza type 3 (PI3) virus was run with a flock of approximately 150 other lambs and their mothers. When bled shortly after birth, 100% of the 33 lambs had maternal antibody to PI3 virus. Four months later, the proportion had fallen to 22%, about which time a natural infection with PI3 virus occurred in the flock as judged by subsequent appearance in the sera of PI3 serum neutralizing antibody.

Microscopic evidence of pneumonia was obtained in lambs 4, 7, and 12 weeks of age, 1–3 months before PI3 virus infection occurred in the flock. Clinical pneumonia was observed in the flock when the lambs were 11 months of age, 7 months after two-thirds of the flock had encountered PI3 infection.

PI3 virus did not appear to be the cause of either the asymptomatic pneumonia of lambs 1 month old or the clinical pneumonia at 11 months of age. There appeared to be a syndrome of asymptomatic chronic pneumonia in this flock which was not caused by PI3 virus, the cause of which was not established.

I. Introduction

A strain of parainfluenza type 3 (PI3) virus was isolated from the lungs of an 8-month-old lamb which died of pneumonia on a research station in Victoria on 20 March 1967 (St. George 1969). Five lambs chosen at random at this time from a flock of approximately 1300 lambs of similar age showed very low titres of serum neutralizing (SN) antibody to this virus and in serum samples collected 49 days later, the average titre was 18-fold greater. However, clinical pneumonia had been in evidence in most of the flock for the previous 2 months. The isolation of PI3 virus, plus the evidence of a rise in titre, pointed to a widespread PI3 infection in the flock within the previous week or two whereas most of the flock had had signs of clinical pneumonia for 2 months. In anticipation that pneumonia would occur in lambs born the following season, an experiment was designed to determine when natural infection with PI3 virus occurred and whether this infection was related to clinical signs of pneumonia.

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II. Materials and Methods

A flock was established comprising 190 maiden ewes, 40 of which were randomly selected to provide the lambs for use in the experiment. All 40 of these ewes were positive for SN antibody to PI3 virus.

The lambs from these ewes were identified at birth by means of numbered ear-tags and the number of the mother of each lamb was recorded. Later, the heads of the lambs were marked to distinguish them visually from the other lambs with which they were running but otherwise they were left with the larger flock. The 33 lambs used for the trial were born between 4 August 1967 and 12 September 1967.

These lambs were bled for serum samples in 1967 at various times after birth then as a group on 19 September, 17 October, 14 November, 12 December and, in 1968, on 3 January, 30 January, 26 February, and 26 March, at which time the trial was concluded and the lambs were incorporated in the main farm flock of approximately 1000 weaned lambs. Nine of the experimental group were identifiable on 16 July 1968 and were bled again. Swabs of nasal mucus were also collected from these lambs at this time.

On each occasion when blood samples were collected, two lambs were killed. Their lungs were examined and pieces of lung were collected with sterile instruments for culture for PI3 virus as described by St. George (1969) and for chlamydiae by the method of Dungworth and Cordy (1962) except that 5-day-old embryonated eggs were used instead of 7-day-old. The lungs were cultured on blood agar for bacteria. Examination for mycoplasmas was performed by Mr. G. S. Cottew of this laboratory and the methods used and identification of the mycoplasmas will be reported elsewhere. Other pieces of lung were placed in buffered formol–saline for histopathological examination.

All the serum samples were examined for PI3 antibody as described by St. George (1969). The serial serum samples of half the lambs were tested for complement fixing (CF) antibody to chlamydiae by the method of Donnelley (1951) except that chlamydia group antigen of Dane (1955) was substituted for influenza antigen.

III. Results

A summary of the principal findings on the lambs killed at approximately monthly intervals is shown in Table 1. No viruses or chlamydiae were isolated and there was no serological evidence of infection with chlamydiae. Microscopic evidence of pneumonia was present in one of the first two lambs killed at 4 weeks of age. The first obvious macroscopic lung lesions were noted in one lamb killed in November 1967 at 12 weeks of age, though some probable lesions 1 or 2 mm in size involving a single lobule had been seen in one of the two lambs killed in September.

The macroscopic lesions consisted of irregularly shaped areas of consolidation, red in colour. The only variation seen in most of the subsequent examinations of other lambs was the size, which ranged from a few millimetres to 4–6 cm. The pattern of the lesions was not constant and they were distributed through the substance of the lung lobes. Some of the lesions were not visible from the surface, particularly in the thicker diaphragmatic lobes. In the lambs killed in July 1968 there was an accumulation of clear mucus in the bronchioles and to a lesser extent in the bronchi. This feature was not evident in the lambs killed at an earlier age. Also, one of the two lambs killed in July 1968 had five thick-walled abscesses in the diaphragmatic lobes varying from 1 to 4 cm in diameter. These contained thin, liquid, yellow pus.

In the histological sections examined from the first two lambs killed at 4 weeks of age, one lamb appeared to have normal lungs (Fig. 1). The second had areas of atelectasis (Fig. 2). The degree of atelectasis varied throughout the lung in a patchy

TABLE 1

MONTHLY EXAMINATION OF A FLOCK OF LAMBS FOR PNEUMONIA AND INFECTION WITH PARAINFLUENZA TYPE 3 VIRUS AND OTHER INFECTIOUS AGENTS

Date killed	Sheep No.	Age (weeks)	Macroscopic lesions		Microscopic lesions of pneumonia	Bacteria	Myco-plasmas	Anti-body to PI3*
			No. of lobes affected	% surface affected				
19 Sept.	66	4	0	0	—	—	—	M
	88	4	2	1	+	—	—	M
17 Oct.	104	7	0	0	+	—	—	M
	27	9	0	0	+	—	—	M
14 Nov.	78	12	0	0	+	—	—	M
	98	12	4	1	+	—	—	—
12 Dec.	129	15	7	25	+	‡	+§	(?)
	70	16	7	10	+	—	—§	—
3 Jan.	3	22	4	1	+	—	—	A
	118	22	4	1	+	—	—	—
30 Jan.	46	23	7	10	+	—	+	A
	69	23	2	5	+	—	+	A
26 Feb.	99	26	2	1	+	—	+	A
	102	26	3	5	+	‡	—	A
26 Mar.	114	30	2	1†	+	—	—	A
	40	32	7	10	+	—	+	A
16 July	130	46	7	10	+	—	+‖	—
	54	47	7	10	+	—	+‖	A

* M, maternal antibody as judged by a declining antibody titre; A, active antibody—titre of antibody has risen after previous decline; ?, the trace of antibody detected only in undiluted serum may have been active or passive antibody; — = negative result.

† Lungworm *Dictyocaulus filaria* also detected. ‡ Gram-negative bacteria detected on these dates. § Single coughs observed. ‖ Pneumonia observed.

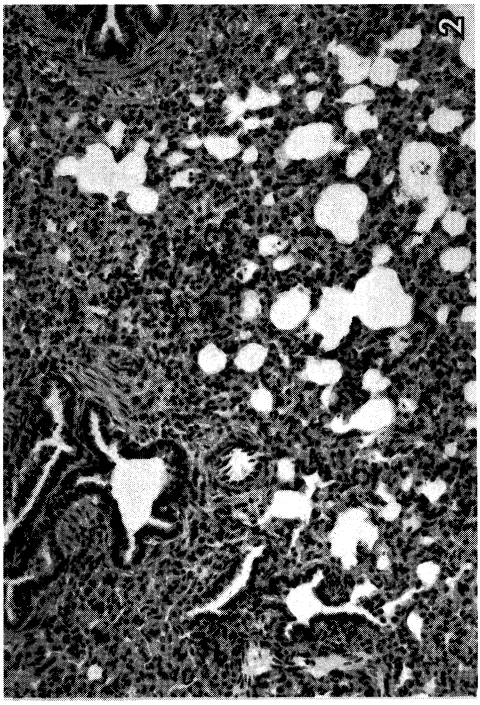

Fig. 1.—Section of lamb No. 66, the first animal killed in the series at 4 weeks of age; the lungs were apparently normal. Haematoxylin and eosin. × 40.

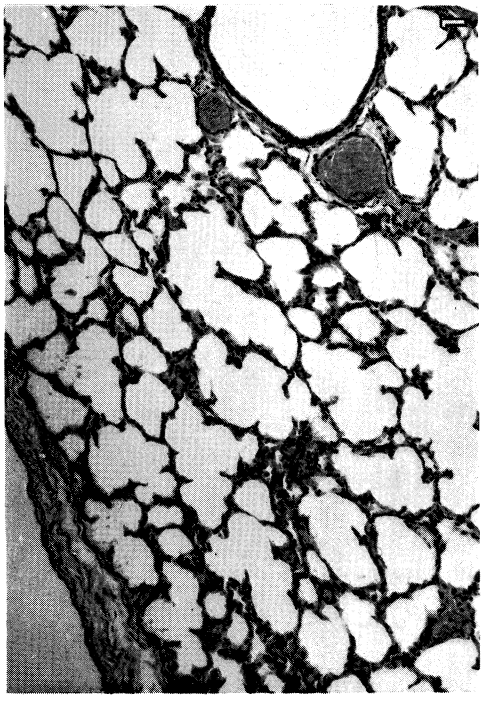

Fig. 2.—A variable degree of atelectasis can be seen in this section; areas of complete collapse are interspersed with areas where the alveolar walls are oedematous. Haematoxylin and eosin. × 40.

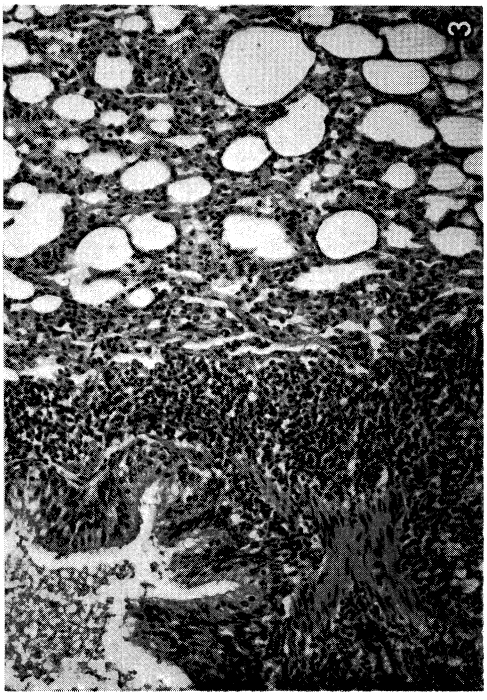

Fig. 3.—This section of lung shows oedematous alveolar walls on the right and a bronchiole containing mucus surrounded by an area of lymphoid hyperplasia on the left. Haematoxylin and eosin. × 40.

manner. In areas of complete collapse there was some infiltration by leucocytes and fibroblasts. In some less completely collapsed areas the alveolar walls were thickened and some alveoli were filled with oedema fluid. There was no perivascular oedema of any significance. The areas of collapse were a feature of each of the sections of lung collected subsequently. Proliferation of peribronchial lymphoid tissue (Fig. 3) also occurred and exudation into the bronchioles occurred in the animals killed later in the series. Abscesses were found only in the lungs of the last lamb killed. No macroscopic evidence of lungworm infection was found. The histopathological changes did not vary much in nature throughout the series, but the extent of the lesions and the proportion of lung tissue damage was greater in the lambs examined later in the experiment.

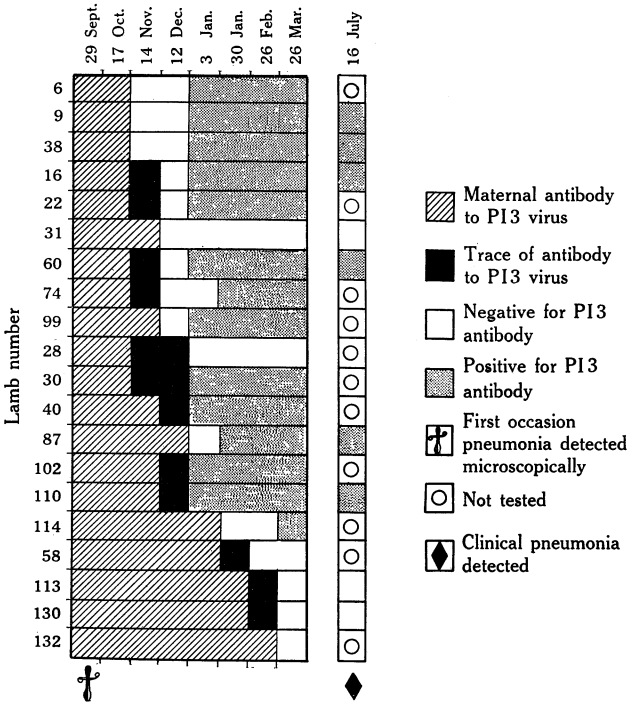

Fig. 4.—Occurrence of maternal and actively produced antibody to parainfluenza 3 antibody in relation to pneumonia detected by histopathological or clinical examination.

The antibody to PI3 shown in Table 1 has been classified as being transferred maternal, or actively produced antibody, on the basis of a change of antibody titres in serial samples. All lambs had antibody to PI3 virus when they were first bled after birth and at the first complete bleeding of the whole lamb flock. Two months later, some of the lambs in the flock had lost their maternal antibody and others lost it progressively. Between December 1967 and March 1968 most of the flock again had PI3 antibody. The *percentage* of serums with antibody to PI3 from those lambs still

alive at successive monthly bleedings are as follows: 19 September, 100%; 17 October, 100%; 14 November, 59%; 12 December, 22%; 3 January, 67%; 31 January, 77%; 26 February, 70%; and 26 March, 73%.

From the serological evidence, infection of the flock with PI3 virus first occurred in December 1967. During the visit on 12 December, mild, single coughs were heard in the flock and this coughing was more noticeable in the week following the sampling.

PI3 virus was not isolated from the lungs of any sheep in the trial though mycoplasmas and bacteria were isolated as shown in Table 1. The first isolation of mycoplasmas and bacteria was made from material collected in December 1967.

A summary of the serological results from those animals not klIled during the trial and of nine of them on 16 July 1968 is shown in Figure 4.

The lambs in the pneumonia trial flock and the larger flock of which they were a part grew well for the first 5 months of life. In December 1967 about 10% of the flock was noticed to have a cough, but the animals which were heard to cough did not appear otherwise to be in poorer health than the balance of the flock. Coughing was not noticed at the examinations made on 31 January, 26 February, or 26 March 1968. General loss of body condition did not occur until March 1967, at the end of a dry summer, and then the loss was not severe.

In July most of the main flock of weaners appeared to be affected with some degree of mucous discharge from the nose and with moist, multiple coughs. The weaners were in poorer condition than would have been expected on the available pasture and no abnormalities were found except in the thoracic cavity. The rectal temperatures were normal. Pneumonia was detected in sheep with no antibody to PI3 and in sheep which had experienced PI3 infection, as indicated by serological evidence months earlier.

IV. Discussion

Though a natural infection with PI3 virus occurred in the flock, clinical pneumonia did not occur until much later than expected when the trial was planned and at a time when the trial flock was not under regular examination.

From the first occasion on which lambs were killed, at 4 weeks of age, there was histological evidence of pneumonia. Macroscopic lesions were visible in lambs 4 and 9 weeks of age and were obvious in lambs 12 weeks and older.

From the serological evidence, two-thirds of the trial flock acquired PI3 SN antibody between 12 December 1967 and 3 January 1968 indicating that the first infection occurred in these lambs, at the earliest, 7–10 days prior to 12 December and, at the latest, 7–10 days prior to 3 January. Single coughs were noticed in this flock in this period, supporting the serological evidence that PI3 infection occurred about this time but otherwise the flock was normal. If the flock had not been under special observation, no particular attention would have been paid to the cough; the mild cough would have been attributed to infection with lungworm. A light infestation of lungworm, *Dictyocaulus filaria*, was found in a lung of one lamb on only one occasion. Infection with this parasite was probably of minor significance in this flock in the period of the trial. Clinical pneumonia with similar signs in sheep with and without PI3 antibody was not evident in the flock until approximately 7 months after PI3 infection had occurred.

There are three findings to consider, namely: the areas of atelectasis detected microscopically from 1 month of age and obvious macroscopically from 1 and 3 months of age; the serological detection of PI3 infection at 4 months; and the appearance of clinical pneumonia at 11 months of age.

The PI3 virus appeared to produce a mild pneumonia which was superimposed for a short time on the existing and continuing pneumonia. The clinical pneumonia that was observed when the lambs were 11 months old could have been the result of another infection superimposed on the existing pneumonia or else the existing pneumonia could have progressed to a stage where it produced clinical effects.

The numbers of lambs killed on each occasion were too small to draw conclusions about the role of bacteria and mycoplasmas. Signifiant numbers of bacteria were isolated from only two of 16 lungs in the trial period and on each occasion this was subsequent to the time when PI3 infection was first detected in the flock. Mycoplasmas were not detected in six sets of lungs examined prior to PI3 infection and were found five out of eight times subsequent to PI3 infection in the period of the trial. Mycoplasmas were found also at the time clinical pneumonia occurred in July 1968.

The failure to isolate PI3 virus from the lungs of any of the lambs which were killed was influenced by the small numbers of lung samples collected during the period when the virus was actively moving through the flock. PI3 virus probably persists for only 7–10 days in the lung before systemic antibody develops, so chance plays a part. The cultural methods as were used on the lungs in this trial were successfully used to isolate a strain of PI3 virus from the lungs of a lamb which died in another flock on the same farm (St. George and Liefman, unpublished data). The serology was done weeks after the time of sampling so that by the time it was known that the virus was infecting the lambs it was too late to try to intensify attempts to isolate it.

This investigation points up the limitation of working with field outbreaks of pneumonia. The role of each of the organisms isolated during the trial is uncertain. In this particular flock a natural infection with PI3 virus caused very little clinical effect. The experiment does indicate the complex nature of sheep pneumonia in Australia and indicates that there are at least two separate pneumonia syndromes in addition to that caused by lung parasites. There is no evidence that any of the various forms of pulmonary adenomatosis described overseas (Stevenson 1969) played a part in the clinical condition studied here.

V. Acknowledgments

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VI. References

Dane, D. S. (1955).—*Med. J. Aust.* **i**, 382.
Donnelley, M. (1951).—*Aust. J. exp. Biol. med. Sci.* **29**, 137.
Dungworth, D. L. and Cordy, D. R. (1962).—*J. comp. Path. Ther.* **72**, 49.
St. George, T. D. (1969).—*Aust. vet. J.* **45**, 321.
Stevenson, R. G. (1969).—*Vet. Bull, Weybridge* **39**, 747.