

## EXPERIMENTAL TRANSMISSION OF JAAGSIEKTE (OVINE PULMONARY ADENOMATOSIS) TO GOATS

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### ABSTRACT

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Jaagsiekte was successfully transmitted to at least 2 out of 6 goats inoculated intratracheally with partially purified jaagsiekte retrovirus. Multiple, small, well circumscribed nodules found in the lungs consisted of typical papilliform proliferations of neoplastic Type II epithelial cells. Histological evidence of a mild interstitial pneumonia in 4 of the experimental animals can probably be attributed to a contaminating lentivirus in the jaagsiekte retrovirus preparation, as suggested by the seroconversion of the animals.

### INTRODUCTION

Early reports on the natural occurrence of jaagsiekte (JS) in goats (Nobel, 1958; Cuba-Caparo, De la Vega & Copaira, 1961; Rajya & Singh, 1964) were controversial because of the paucity of published data and inadequate differentiation between alveolar epithelialization and true adenocarcinomatous lesions (Stamp & Nisbet, 1963; Tustin, 1969). Although later reports (Stefanou, Tsangaris & Lekkas, 1975; Banerjee & Gupta, 1979; Sriraman, Pao & Naidu, 1982) provided more convincing evidence of a very low incidence of the natural disease in goats and Sharp, Angus, Jassim & Scott (1986) have experimentally transmitted the disease to a goat kid, it was though necessary to confirm the susceptibility of goats by experimental transmission.

The experimental transmission of JS to sheep has been widely reported (Tustin, 1969; Wandera, 1971). By concentrating and partially purifying the virus inoculum and inoculating new-born lambs with the inoculum, the efficiency of transmission was greatly increased (Verwoerd, Williamson & De Villiers, 1980; Sharp, Angus, Gray & Scott, 1983). The presence of a contaminating lentivirus in the crude jaagsiekte retrovirus (JSRV) strain used in these studies may have contributed to the enhanced efficiency (Payne, York, De Villiers, Verwoerd, Querat, Barban, Sauze & Vigne, 1986).

The above techniques and materials were used in the present study in which the successful transmission of JS to new-born goats proved the susceptibility of this species to the disease.

### MATERIALS AND METHODS

#### Experimental animals

Six goat kids of indigenous (boerbok) origin were inoculated intratracheally with 4 ml of a JSRV suspension between 2 and 7 days after birth. They were housed as a group under semi-isolated conditions, well separated from other experimental animals, and kept under daily observation.

#### Inoculum

The JSRV suspension was obtained from the lungs of an experimental case of jaagsiekte which formed part of a serial transmission study in sheep. The sheep's lungs were rinsed with 1 500 ml of cold tissue culture medium (Minimal Eagle's Medium) which was then centrifuged for 90 min in a Beckman Ti-15 batch rotor at 100 000 × g. The sediment was resuspended in about 24 ml of PBS and stored before use for 14 weeks at -70 °C. Immediately before inoculation it was thawed out and extracted

once with cold Freon 113 (Dupont). Each kid received  $0,36 \times 10^6$  RDP units of virus (Verwoerd, Payne, York & Myer, 1983). By comparison, the same dose of virus produced advanced jaagsiekte lesions in newborn lambs within 2½ months (Verwoerd, Tustin & Payne, 1985).

#### Preparation of lung samples

Necropsies were performed and lung specimens were fixed in buffered formalin and further processed for histological examination, using standard techniques. For transmission electron microscopy (TEM), samples were prepared as described previously (Payne, Verwoerd & Garnett, 1983) and viewed with a Joel transmission electron microscope.

#### Serology

Serum was collected from each animal before inoculation and from 4 others at the time of slaughtering. Sera were tested for antibodies to the p28 and p16 antigens of the South African isolate of lentivirus by means of an immunoblot technique described previously (Payne *et al.*, 1986).

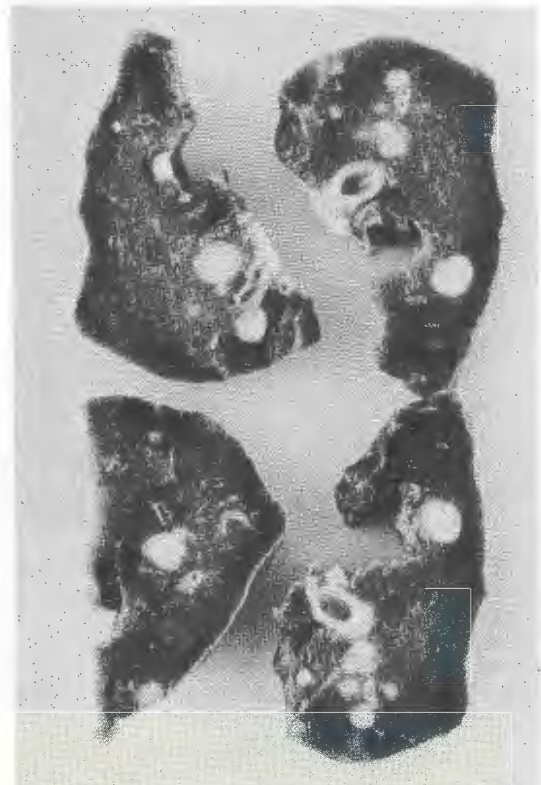


FIG. 1 Multiple, discrete, white nodules 2-7 mm in diameter, of jaagsiekte lesions in the lungs of Goat 2 after formalin fixation

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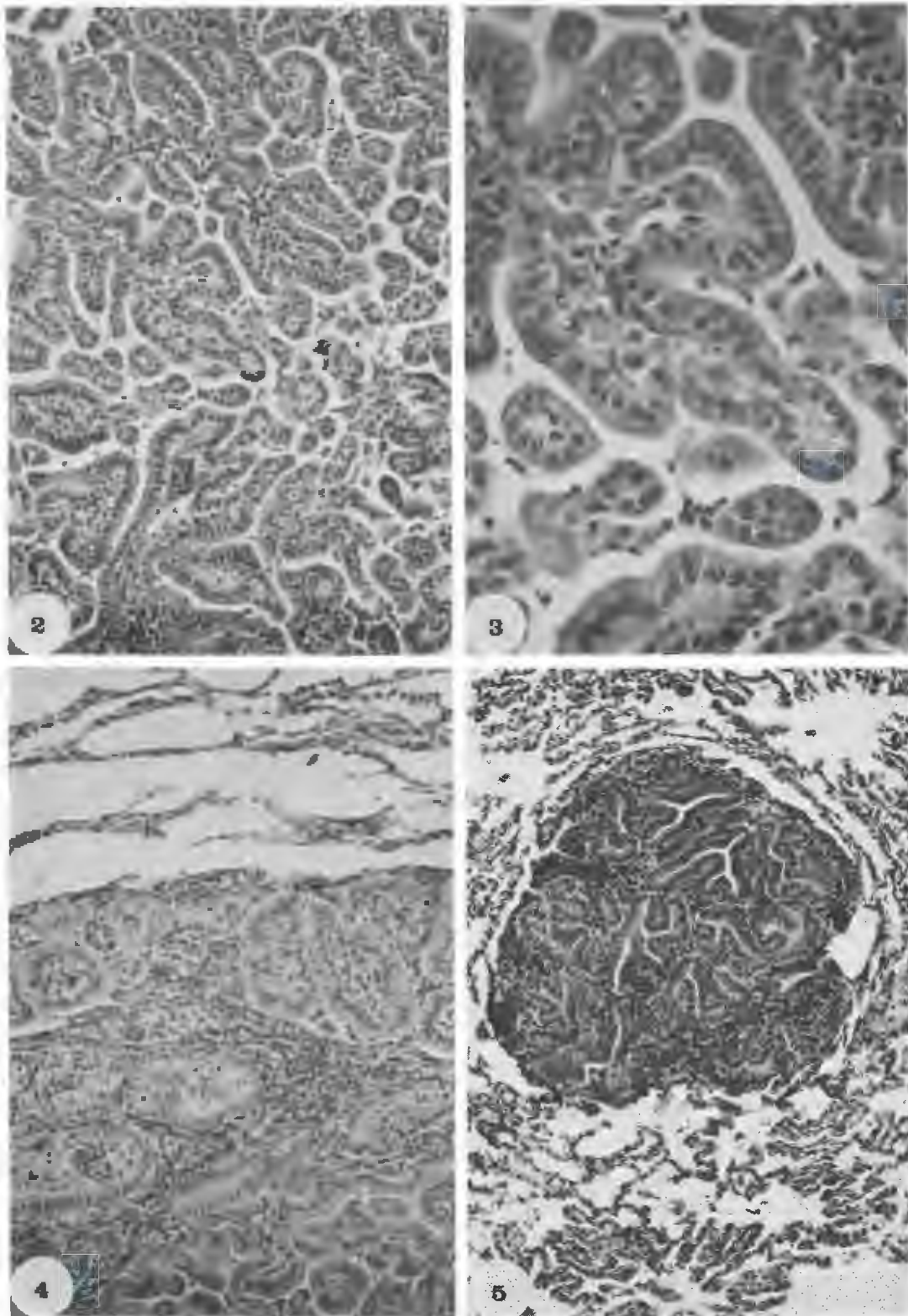


FIG. 2 Goat 3: Bronchiolo-alveolar adenocarcinoma identical with that of ovine jaagsiekte. HE  $\times$  120

FIG. 3 Well-differentiated cuboidal to columnar neoplastic epithelial cells supported by a delicate fibrous connective tissue stroma in the lungs of Goat 3. HE  $\times$  300

FIG. 4 The edge of one of the tumour nodules in Goat 3. Many of the lesions were infiltrated by neutrophils (smaller dark-staining nuclei) with no surrounding fibrous capsule. HE  $\times$  120

FIG. 5 A section through a small intact tumour nodule in Goat 2. HE  $\times$  120

## RESULTS

### *Clinical signs*

One of the 6 kids (Goat 1, see Table 1) died at the age of 5 months without developing any respiratory symp-

oms. Death was caused by acute coccidiosis. Another (Goat 2) developed nervous signs after 9 months. Necropsy revealed an acute meningitis but also multiple solid nodules in the lung. A 3rd animal (Goat 3) showed mild dyspnoea and coughing, suggesting possible jaag-

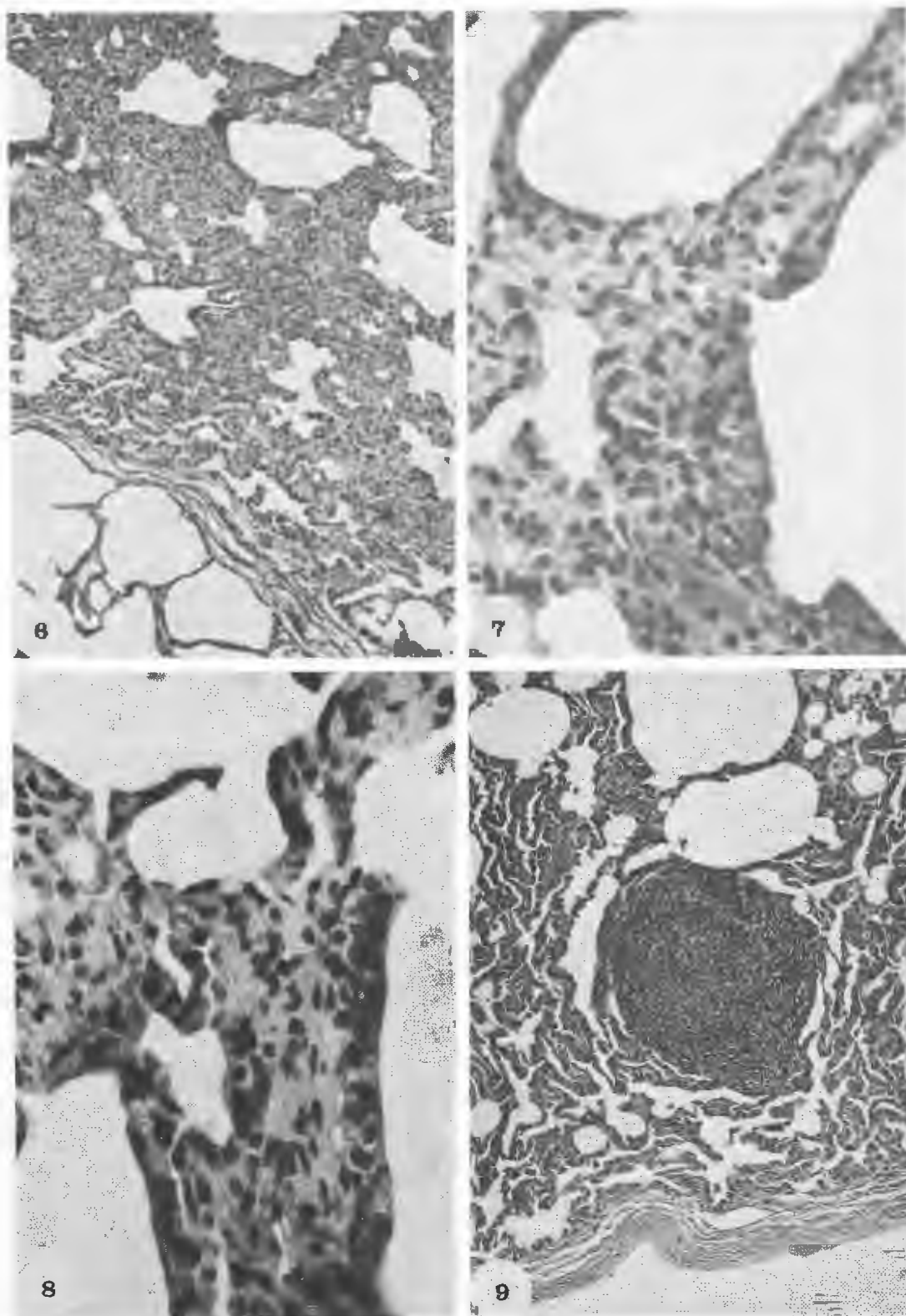


FIG. 6 A lobule in the lung of Goat 5 exhibiting interstitial pneumonia adjacent to a normal lobule. HE  $\times$  120

FIG. 7 Interstitial pneumonia in the lungs of Goat 2 manifested by a thickening of alveolar walls caused by an infiltration principally of macrophages and lymphocytes and a reduction in the size of alveolar spaces. HE  $\times$  480

FIG. 8 "Epithelialization" or pneumocyte Type II proliferation present in some of the areas exhibiting interstitial pneumonia, represented here also by a thickening of alveolar walls by macrophage and lymphocyte infiltration and connective tissue proliferation of Goat 5. HE  $\times$  480

FIG. 9 A section through a nodule of lymphoid hyperplasia in the lungs of Goat 6. HE  $\times$  120

siekte, at the age of 13 months. The other 3 did not develop any clinical signs before they were slaughtered.

#### Pathology

A summary of the most salient features of the pathology is presented in Table 1.

In the lungs of Goats 2, 3 and 5, macroscopically visible lesions resembling jaagsiekte were evident. These were most obvious in Goat 3 and comprised milia-ry, discrete, firm, grey-white opaque nodules varying in diameter from 1–10 mm which were most numerous

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TABLE 1 Summary of the pathological findings in 6 experimental goats after intratracheal inoculation

Goat No.	Age at inoculation (days)	Interval between inoculation and death (days)	Macroscopical pathology of the lung	Microscopical pathology of the lung	Remarks
1	2	145	No lesions evident	Not done	Died from coccidiosis
2	3	281	Miliary firm, grey-white, opaque, glistening nodules, varying in diameter from 2-7 mm present in all lobes	Multiple discrete foci of jaagsiekte present, as well as an interstitial pneumonia, with some perivascular and peribronchiolar lymphoid hyperplasia	Slaughtered <i>in extremis</i> as a result of acute meningitis
3	2	391	Nodules resembling those in Goat 2 present. Sizes vary between 1-10 mm. Most numerous in caudal lobes	Multiple foci of jaagsiekte resembling those in Goat 2. Mild interstitial pneumonia present in parts of lung	—
4	7	404	Single subpleural nodule 1,5 mm in diameter present, resembling those in Goat 2	Nodule noticed macroscopically comprising lymphoid hyperplasia	Negative for jaagsiekte
5	7	434	Single nodule, 2 mm in diameter present, resembling those in Goat 2	Fairly severe interstitial pneumonia present, with lymphoid hyperplasia and mild epithelialization. Nodule not examined histologically	Jaagsiekte not confirmed histologically
6	3	1 070	One irregular, sunken, consolidated, hyperaemic area, about 50 × 50 mm in extent, at junction of right cranial and middle lobes. Numerous firm, white foci, 1-3 mm in diameter, distributed through both lungs, especially subpleurally	Widespread, chronic, interstitial pneumonia, in parts accompanied by atelectasis. Scattered foci of lymphoid hyperplasia	Negative for jaagsiekte

TABLE 2 Immune blot results showing the cross reaction between the different goat sera and SA-lentivirus antigens before and after inoculation

Goat No.	Sera before inoculation <sup>1</sup>		Sera at slaughter <sup>1</sup>	
	p28	p16	p28	p16
1	NT	NT	NT	NT
2	-	-	NT	NT
3	-	-	-	-
4	-	-	+	F
5	-	-	+	-
6	-	-	+	+

<sup>1</sup> Sera were diluted 1/10

- = no detectable cross reaction

F = faint detectable band in the p28 or p16 region

+ = band detected with the same intensity as the positive control

NT = not tested

in the upper 2/3 of the caudal lobes and lower half of the middle lobes. Very few were present in the cranial lobes. The nodules could easily be detached or "shelled-out" from the normal surrounding lung tissue. The appearance of the lesions in Goats 2 and 5 very closely resembled those in the previous case. In Goat 2 they were relatively numerous, varied in diameter from 2-7 mm and were more or less evenly distributed throughout all lobes of the lungs (Fig. 1), while in Goat 5 only a single subpleural nodule about 2 mm in diameter was noticed.

In the lungs of Goat 4 a subpleural, grey-white nodule of lymphoid hyperplasia about 1,5 mm in diameter was observed. Goat 6, which was slaughtered in apparent good health 1070 days after inoculation, showed an irregularly shaped area at the junction of the right cranial and middle lobes, which was about 50 × 50 mm in extent and was sunken, red and of increased consistency. In addition, in this animal there were also fairly numerous small (1-3 mm in diameter) firm, dense, white glist-

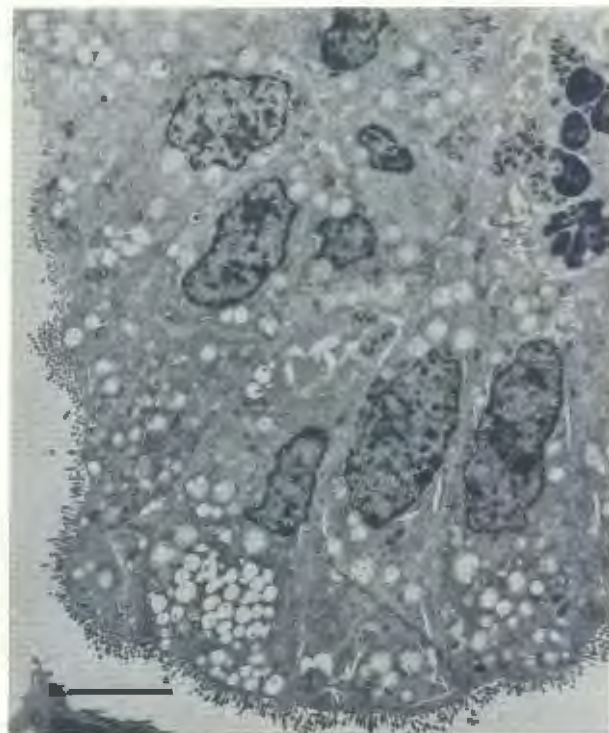


FIG. 10 Columnar tumour cells lining the alveolar lumen. Bar = 5 μm

tening foci of lymphoid hyperplasia scattered throughout the lung parenchyma, most, however, being located subpleurally.

Lesions of intestinal coccidiosis were present in Goat 1 which were responsible for the death of this animal.

No other pathological changes considered to be of significance were observed macroscopically in any of the animals.

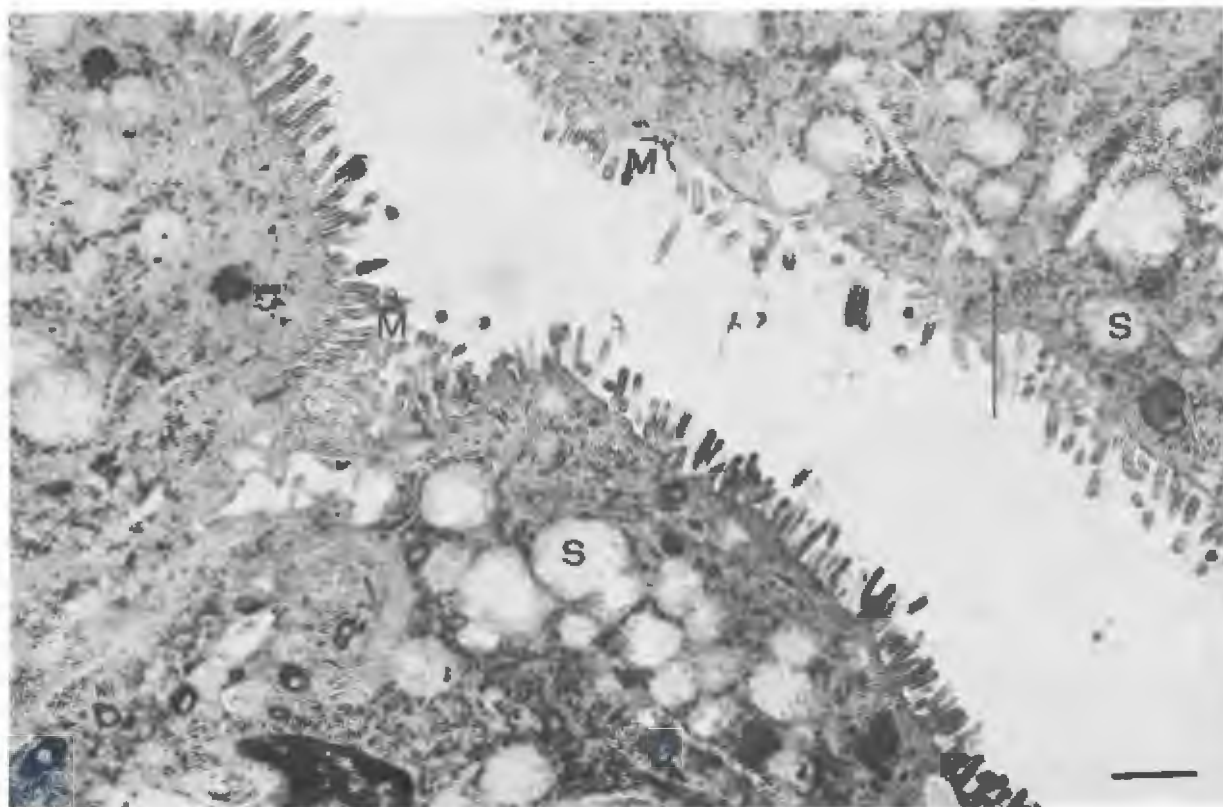


FIG. 11 Secretory granules (S) in tumour cells. Note the microvilli (M) on the surface of the tumour cells and junctional complexes (arrow). Bar = 1  $\mu$ m

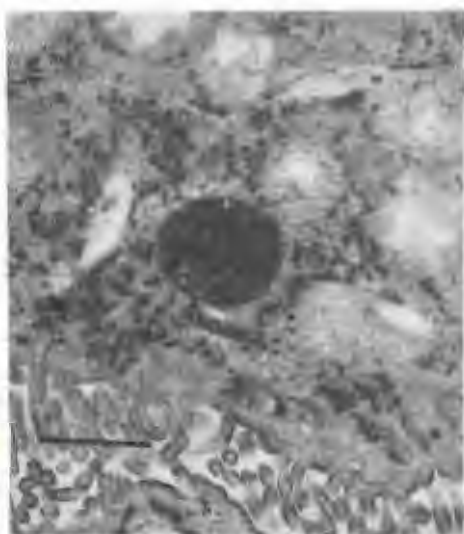


FIG. 12 Multivesicular body in a tumour cell. Bar = 1  $\mu$ m

Histologically the nodules seen macroscopically in Goats 2 and 3 consisted of lesions typical of those of ovine jaagsiekte or bronchiolo-alveolar adenocarcinoma (Fig. 2 & 3). The neoplastic epithelial cells were well differentiated, being mainly one layer thick, while the cords were separated from each other by relatively delicate fibrous connective tissue. In some of the lesions, mild perivascular and peribronchiolar lymphoid hyperplasia was present, and in most a neutrophil infiltration into the lumina formed by the tumour cells was quite pronounced (Fig. 4). The lesions were well circumscribed but were not encapsulated (Fig. 5).

In Goats 2, 3, 5 and 6 an interstitial pneumonia was noticed on microscopical examination. The lungs of Goat 1 were not examined histologically. The pneumonia varied in degree, being most pronounced in Goat 5 and least severe in Goat 3. It consisted primarily of a thickening of the alveolar walls caused by an infiltration particularly of macrophages but also of lymphocytes and mild fibrous connective tissue proliferation (Fig. 6 & 7). In areas where these changes were marked, not all lobules being equally affected, there was a reduction in the number and size of alveoli. In some alveoli evidence of partial "epithelialisation" or hyperplasia of type II epithelial cells was observed, but this was not a striking phenomenon (Fig. 8). Perivascular, peribronchial and peribronchiolar lymphoid hyperplasia was present but not always very obvious except in Goat 6 where it was very marked and accounted for the numerous white foci seen macroscopically (Fig. 9). Exudation or infiltration of cells into the alveoli or bronchial lumina was not a feature of the lesion.

The single nodule encountered in the lungs of Goat 4 proved to be one of lymphoid hyperplasia. No other lesions were noticed on microscopical examination of the lungs of this animal.

#### *Electron microscopy*

The lesions found in Goat 3 were examined with TEM. Columnar tumour cells lined the alveolar lumen and in numerous areas papillae formation was observed (Fig. 10). The nuclei were usually situated towards the base of the cell and were regular in shape. The tumour cells in the lesion were epithelial in character, and resembled type II pneumocytes. There were well-defined junctional complexes connecting the cells and the apical surfaces of the tumour cells were covered with abundant

microvilli. Most tumour cells contained pleomorphic secretory granules (Fig. 11) which were usually located in the apical region of the cells. There were also smaller granules along the basal lamina. The granules varied from electron-dense to electron-lucent, but no myelinoid bodies were observed. Multivesicular bodies were present in many of the tumour cells (Fig. 12) and glycogen also in limited quantities. Mitochondria were fragile and ill-defined. This is possibly a preparation artifact, as cytoplasmic clefts were also observed. The alveolar lumina were mostly clear but in rare areas they were filled with electron-dense secretions. No viral particles were observed in the sections.

### Serology

None of the sera collected before inoculation contained antibodies against lentiviral antigens. Seroconversion, however, was observed in 3 of the 4 animals tested at the time of slaughtering (Table 2). The highest concentration of antibodies was found in Goat 5, which also had the most severe interstitial pneumonia. No seroconversion could be demonstrated in Goat 3, which had the most pronounced JS lesions.

### DISCUSSION

The successful experimental transmission of jaagsiekte to new-born kids clearly proves the susceptibility of goats to this disease. However, the fact that a viral dose that produced extensive lesions in more than 90 % of new-born lambs after 2–3 months (Verwoerd *et al.*, 1985) only produced small circumscribed lesions in at least 2 out of 5 goats after 9–13 months suggests that goats are much less susceptible than sheep. This is supported not only by the results of Sharp *et al.* (1986), which indicated that transmission of jaagsiekte to goats is less efficient and much slower than is the case in sheep, but also by previous reports of a very low incidence of the natural disease in goats. For example, 3 out of 1 410 goats slaughtered in Greece (Stefanou *et al.*, 1975) and 18 out of 3 956 goats in India (Rajya & Singh, 1964) had a low incidence of the disease. It may also be significant that the 2 goats that developed jaagsiekte lesions were inoculated 2 and 3 days after birth, respectively, whereas 2 of the 3 that did not develop lesions were inoculated only at the age of 7 days. It was found in sheep that the efficiency of transmission rapidly diminishes after birth (Verwoerd *et al.*, 1985). The nodular character of the lesions in the experimental disease in the goat described by Sharp *et al.* (1986) is similar to that present in the cases reported here.

The presence of lentivirus in the inoculum did not seem to influence the development of lesions, since no evidence of seroconversion and only very mild interstitial pneumonia was found in Goat 3, the animal which had the most pronounced jaagsiekte lesions. The relationship, if any, between these 2 sheep retroviruses which are so commonly found together still needs clarification.

The fact that jaagsiekte retrovirus infection in goats results in the development of a neoplasm closely resembling that caused by the same virus in sheep, that the same (or very similar) disease occurs naturally in goats

in some countries and the tumour is a bronchiolo-alveolar adenocarcinoma (Stünzi, Head & Nielsen, 1974) and not an adenoma lend support to the contention that the disease should be called jaagsiekte rather than ovine pulmonary adenomatosis or ovine pulmonary carcinomatosis.

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