

## Detection by immunoperoxidase technique of parainfluenza type-3 virus and respiratory syncytial virus antigens in naturally occurring pneumonia in lambs

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### SUMMARY

Using an avidin-biotin peroxidase staining method, parainfluenza type 3 virus (PIV-3) and respiratory syncytial virus (RSV) antigens were demonstrated in pneumonic lamb lungs. PIV-3 and RSV antigens were detected in 8 (5,8 %) and 5 (3,6 %) of 137 pneumonic lung samples, respectively. Both antigens were exclusively seen in the cytoplasm of bronchial and bronchiolar epithelium in addition to bronchial gland epithelium. PIV-3 antibody showed positive reaction in alveolar epithelium in contrast to RSV antibody. This study displayed that both viruses included in naturally occurring ovine pneumonia and immunoperoxidase technique may be useful for differentiation of PIV-3 and RSV infections in lambs.

**Key words:** Parainfluenza type-3 virus, respiratory syncytial virus, lamb, pneumonia.

**Doğal koyun pnömonilerinde respiratuvar sinsityal virus ve parainfluenza tip-3 virus antijenlerinin immunoperoksidaz teknikle belirlenmesi**

### ÖZET

Bu çalışmada, koyunlarda pnömoni olgularında parainfluenza type 3 (PI-3) ve respirator sinsityal virus (RSV) antijenleri avidin-biotin peroksidaz metot kullanılarak tespit edildi. 137 akciğer örneğinde, PI-3 ve RSV antijenleri sırasıyla 8 (%5,8) ve 5 (%3,6) olguda belirlendi. Her iki antijen de özellikle bronş ve bronşiyol epitellerinde ve bronş bez epitel hücrelerinin sitoplazmalarında gözlemlendi. Ayrıca PI-3 antikor, alveol epitel hücrelerinde de reaksiyon verirken, RSV antikor ile alveol epitellerinde negatif sonuç alındı. Bu çalışma kuzu pnömonilerinde her iki virusun varlığını ve kullanılan yöntemin her iki virustan kaynaklanan pnömoni olgularının ayırımında yararlı olabileceğini göstermiştir.

**Anahtar kelimeler:** Parainfluenza tip-3 virus, respirator sinsityal virus, kuzu, pnömoni.

### INTRODUCTION

Respiratory tract diseases in sheep cause major economic losses in sheep industry in several countries. It's known that some viruses with bovine origin cause experimental or natural respiratory tract infections in sheep (10). Sheep are especially susceptible to parainfluenza type-3 virus (PIV-3) and bovine respiratory syncytial virus (RSV). Antibodies to both viruses have also been demonstrated in cattle and sheep (5, 7, 11-13, 18, 23). Although isolation of PIV-3 is done in cells culture of bovine origin, RSV is a labile virus that grows slowly in cells culture; therefore, virus isolation is hard to perform (6, 23). Moreover, especially the RSV has rarely been isolated from natural cases of the diseases in sheep (12) and a strain of bovine RSV can cause severe pathological changes in the lungs of lambs without apparent clinical signs (24). Because pathological changes in lungs may be similar for both PIV-3 and RSV infections, histological examination of hematoxylin and eosin-stained sections alone is not sufficient for diagnosis (10). Immunoperoxidase staining of paraffin sections facilitates permanent and clear demonstration of antigen component and lesions. As a result, immunoperoxidase detection of PIV-3 and RSV would provide a diagnostic method (3, 4, 15, 19, 20, 22). The detailed immunohistochemical studies associated with PIV-3 and RSV have been documented in cattle (6, 3, 15, 21, 28). However, studies in sheep are scant and have included almost experimental RSV infections (1, 4, 19, 20, 27). The presence of both infections in sheep in Turkey has been detected by serological studies (5, 7, 23). The aim of the present report was to determine the presence of PIV-3 virus and bovine

RSV in the lung sections of slaughtered lambs with naturally occurring lower respiratory disease.

### MATERIAL AND METHOD

#### Lung material and tissue processing

Between February and May 2000, the lungs of the 3010 slaughtered lambs, eight and twelve-month-old were collected from Van abattoir, Turkey. Pneumonic lung materials were obtained from 137 lambs. The distribution and gross appearance of the pneumonic areas were recorded. Tissue samples were fixed in neutral buffered 10 % formalin and sections from paraffin-embedded blocks were stained by hematoxylin and eosin.

#### Immunohistochemistry

Rabbit hyperimmune sera were prepared against PIV-3 and RSV with bovine origin obtained from Institute for Animal Science and Health (ID DLO, Holland). Immunization and immunoperoxidase staining procedures (avidin-biotin peroxidase complex method=ABPC) were performed as described previously (14) with some modifications.

### RESULTS

Most lesions had lobular pattern and consisted of consolidated, red to grayish-white lung tissue, especially involving the first cranial lobe of the right lung. Mucopurulent exudate was seen in the small bronchi and bronchioli. The demarcation line between pneumonic lobes

and unaffected areas was well defined. Emphysema was detected in the various portions of these lungs.

Histologically, two main groups were recorded as proliferative and exudative pneumonias. Proliferative and exudative pneumonias were detected alone in 10 and 7 cases respectively whereas bronchointerstitial pneumonias with mostly mixed proliferative and exudative components were observed in 120 cases since exudative type pneumonias had actually proliferative characteristics. The characteristic findings of proliferative lesions were marked peribronchial and perivascular lymphoreticular hyperplasia, and hyperplasia of the bronch-bronchial epithelial cells. Interstitial changes characterized by mononuclear cells causing thickening of alveolar septa were present in the lungs. In some cases, fibromuscular hyperplasia and an increase in the number of type II cells and intra-alveolar macrophages and obliterative bronchitis were also detected. Exudative lesions showed changes characterized by infiltrations with neutrophil leucocytes. Large amounts of exudates containing numerous neutrophils, cell debris, mucus, fibrin, erythrocytes, macrophages and necrotic cells were present in the airways. In many cases, edematous distention of the interlobular septa with inflammatory cells was seen. The syncytial cells typical of the PIV-3 and RSV infections were not observed in any cases. In three cases, many alveoli and bronchial lumina were filled with oat-shaped clustered inflammatory cells having elongated or streaming nuclei referring to pasteurellosis. Besides in one case, many eosinophilic intracytoplasmic inclusion bodies were detected in the hyperplastic bronchial epithelium. Pulmonary adenomatosis and verminous pneumonia, often accompanied with atypical pneumonia, were also detected in 6 and 1 cases respectively.

In immunohistochemical examination of the sections, strong reactions specific to PIV-3 and RSV were detected in 8 (5,8%) and 5 (3,6%) of 137 pneumonic lungs, respectively. Positive immunostaining was almost observed in bronchointerstitial pneumonias with mostly mixed proliferative and exudative components. There was no difference between staining characteristics of PIV-3 and RSV antigens. Both PIV-3 and RSV antigens were exclusively seen in bronchial epithelial cells (Fig. 1a and b) as well as bronchiolar and bronchial gland epithelium (Fig. 2). PIV-3 antibody also displayed positive immunostaining in the alveolar epithelium in contrast to RSV antibody (Fig. 3) and none of them was demonstrated in the bronchus-associated lymphoid cells (Fig. 1). Immunostaining for both antigens was predominately intracytoplasmic although intense staining was observed frequently in cell margins of the bronchial epithelium (Fig. 1). Intranuclear staining was not detected for both viruses. The exudates and some exfoliated cells within the bronchiolar and alveolar lumina were intensely immunostained. Despite treatment with normal goat serum, some nonspecific staining of connective tissue was seen. However, this staining could be differentiated from specific staining.

## DISCUSSION

In the present study the gross and histopathological findings observed in lambs were completely agreed with those obtained by Hazirolu *et al.* (16) in Turkey. Similar

histological lesions associated with PIV-3 and RSV have also been observed in experimental studies (8, 9, 17, 19, 20, 22, 24, 26, 27). It is unclear to what extent the lesions described are due to PIV-3 or RSV alone, due to other pathogens, or are due to additive or synergistic action of combined pathogens. The lesions observed in both infections are comparable and, PIV-3 or RSV and other pathogens are often simultaneous agents included in enzootic pneumonias of lambs and calves (3, 4, 10, 25). However, we were not able to detect concurrently both viruses in the same case. In many instances, there has been evidence of concurrent infection by other pulmonary pathogens including adenovirus, *Pasteurella haemolytica*, *P. multocida*, and *Mycoplasma* sp. (10). Respiratory viruses including PIV-3 and RSV increase the susceptibility of sheep to secondary *P. haemolytica* infections. The concept of viral-bacterial synergistic interaction has been documented in PIV-3 or RSV with *P. haemolytica* in the pneumonic lung (2, 10, 25). Although there were many alveoli and bronchial lumina filled with oat-shaped inflammatory cells referring to pneumonic pasteurellosis in three cases, we could not detect viral antigens in these cases. PIV-3 and RSV antigen distribution had a close correlation with lesions, and staining patterns were similar to immunostained tissues from calves and lambs with induced and naturally occurring PIV-3 and RSV diseases (1, 3, 4, 6, 15, 19, 20, 21, 22, 28). It is impossible to differentiate gross or histologic lesions of PIV-3 and RSV infections. In comparing PIV-3 and RSV infections in cattle, it appears that RSV is more likely to cause severe respiratory disease by itself and that in fatal cases there is a reasonable chance of findings intracytoplasmic inclusion bodies in the exaggerated syncytial giant cell formation. Once the giant cell and inclusion body stage has passed, it is not possible to distinguish the lesions (10). Therefore, immunoperoxidase technique may be applicable to differentiation of PIV-3 and RSV infections in sheep. In the present study, one case showed the intracytoplasmic inclusion bodies in the hyperplastic bronchial epithelia and there was not giant cell formation in any case. Some authors have been reported that syncytia are not prevalent in tissues from sheep infected with PIV-3 (9) and RSV (8, 27). Moreover, in the case including inclusion bodies positive immunostaining were not seen for both PIV-3 and RSV.

The staining with polyclonal antibodies in calves experimentally infected with PIV-3 is diffusely cytoplasmic with accentuation of the alveolar and bronchial epithelial cell surface (15). There is a reduction in RSV tropism for alveolar epithelia compared to bronchial and bronchiolar epithelia in lambs (20). Moreover, a particular cell type preferentially infected by RSV would not be justified, however, the greatest proportion of infected cells are bronchiolar epithelium and type I pneumocytes (22). In positive cases of the present study, PIV-3 antigens were detected mostly in the bronchiolar epithelium and occasionally in the alveolar epithelium whereas RSV antigen only in bronchiolar epithelium. Because of the limited number of field cases naturally infected with PIV-3 and RSV, it is not possible to explain this difference in sites of detection of both antigens.

Immunohistochemical techniques for demonstration of PIV-3 and RSV in tissues from animals with respiratory disease can be a rapid and reliable means. The

accomplishment of this procedure is dependent upon fixation techniques and the quality of antisera directed against these viral antigens because the fixation may destroy immunogenic epitopes (15). In prolonged postinoculation periods, the number of positive cells detected by immunohistochemistry decreases and it is also sometimes difficult to differentiate between positive and negative signals (20). Moreover, viral antigens may have been cleared from the lungs or the presence of blocking antibodies within the respiratory tract may have caused failure of the antigens detection (6). In this study, the failure of detection of both antigens in many cases may be coincided with a marked serologic response and viruses may have been cleared or masked by immunoglobulins. We also speculated negative results that the prepared antibodies were with different origin thus may have failed to detect different epitopes of antigens. The exact reasons for the poor results obtained with this method during the current study remain unknown.

The serological surveys reveal that both PIV-3 and RSV may be wide spread in sheep in Turkey (5, 7, 23). However, in the present study, the number of positively detected cases was low on contrary to the expectation. This study demonstrated that PIV-3 and RSV should be considered in naturally occurring ovine pneumonia and immunoperoxidase technique can help differential diagnosis of infections associated with both viruses in sheep. Furthermore, to define exactly the presence of both viruses in ovine pneumonia, detailed immunohistochemical studies by more specific antibodies are needed.

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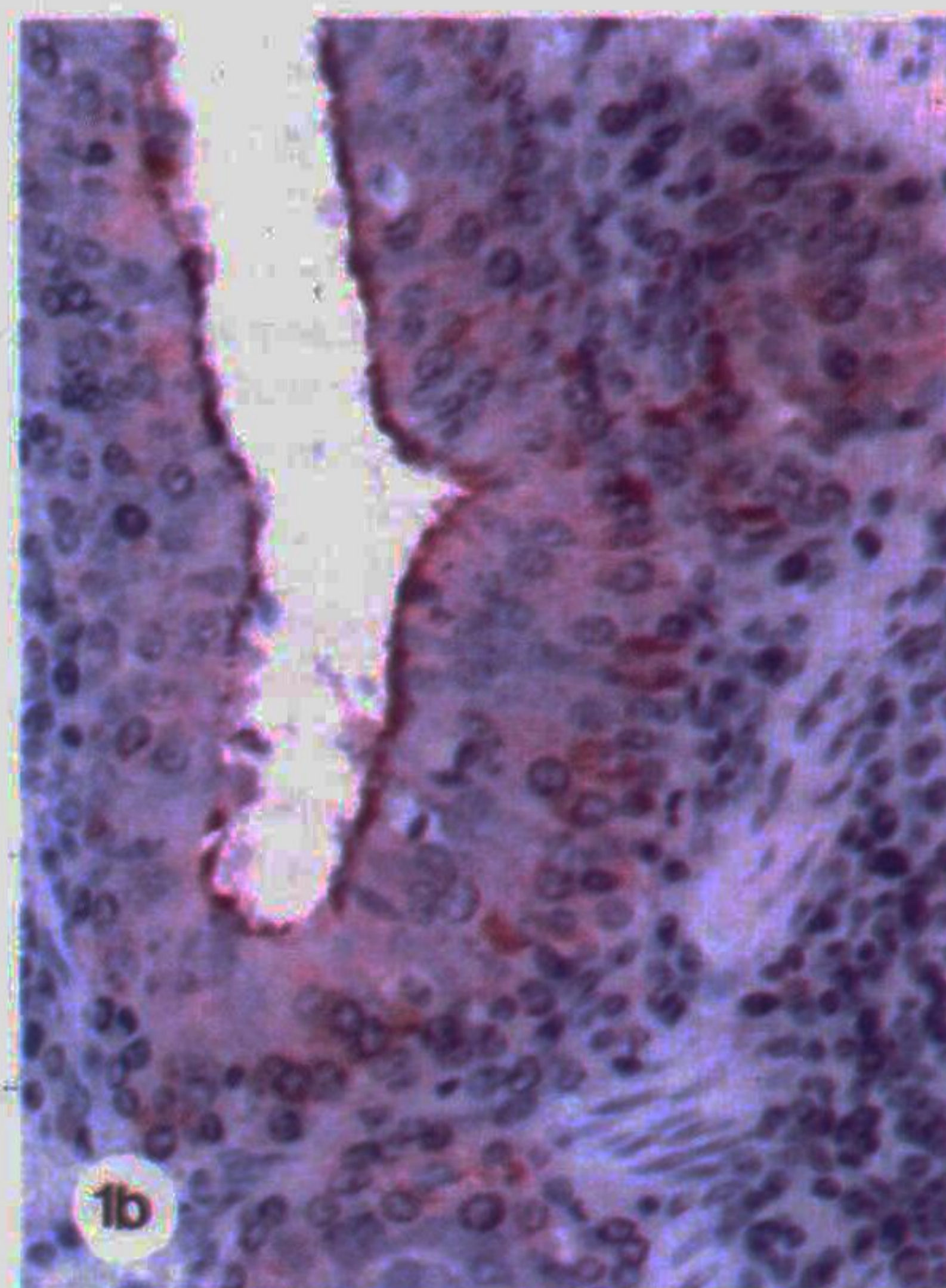
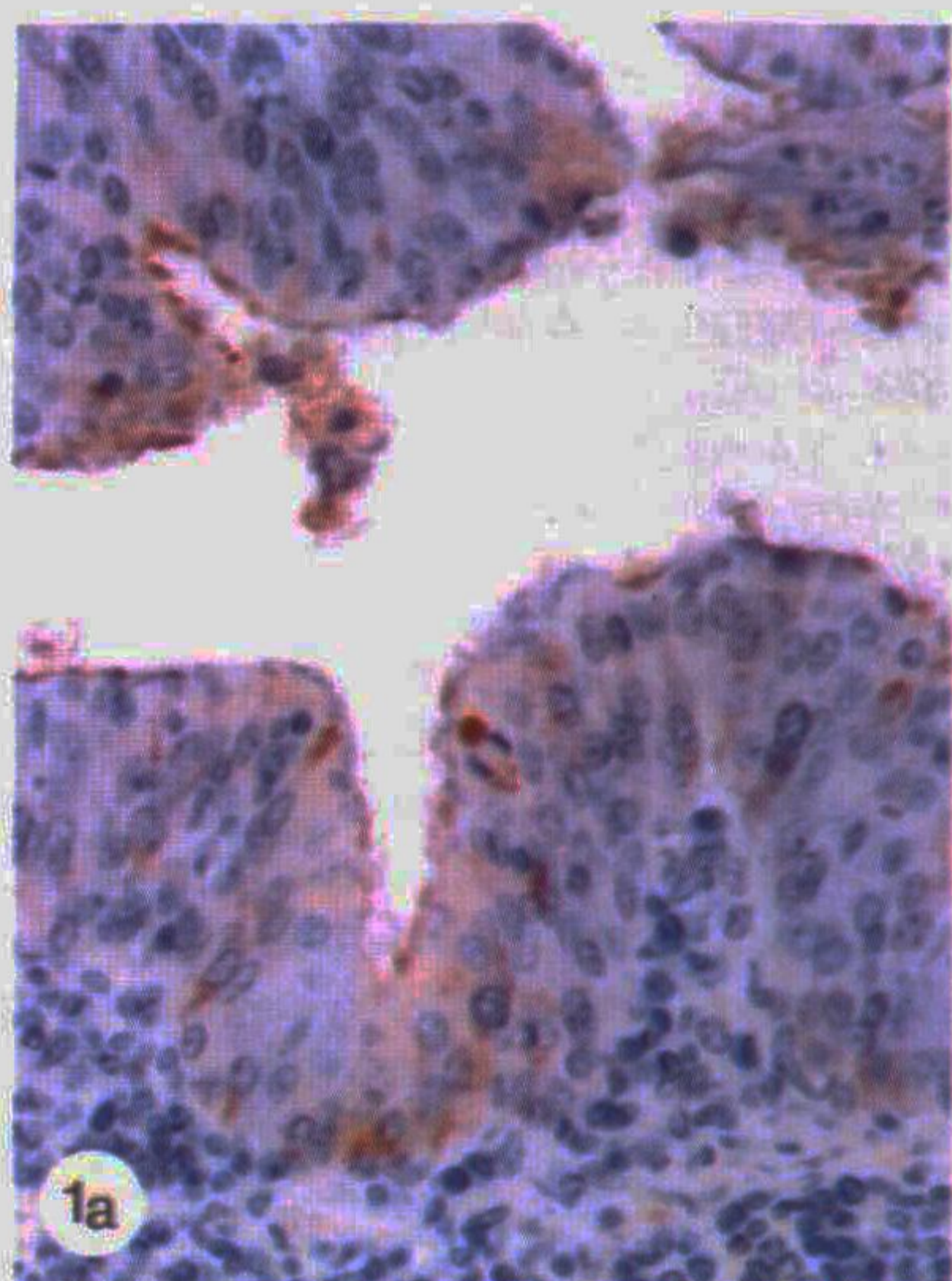


Figure 1. Positive immunostaining for PIV-3 (1a) and RSV (1b) antigens in the cytoplasm and on the surface of the bronchiolar epithelium. Note lack of the immunoreactivity in the bronchus-associated lymphoid tissue (L). ABPC, Mayer's hematoxylin counterstain, X 600.

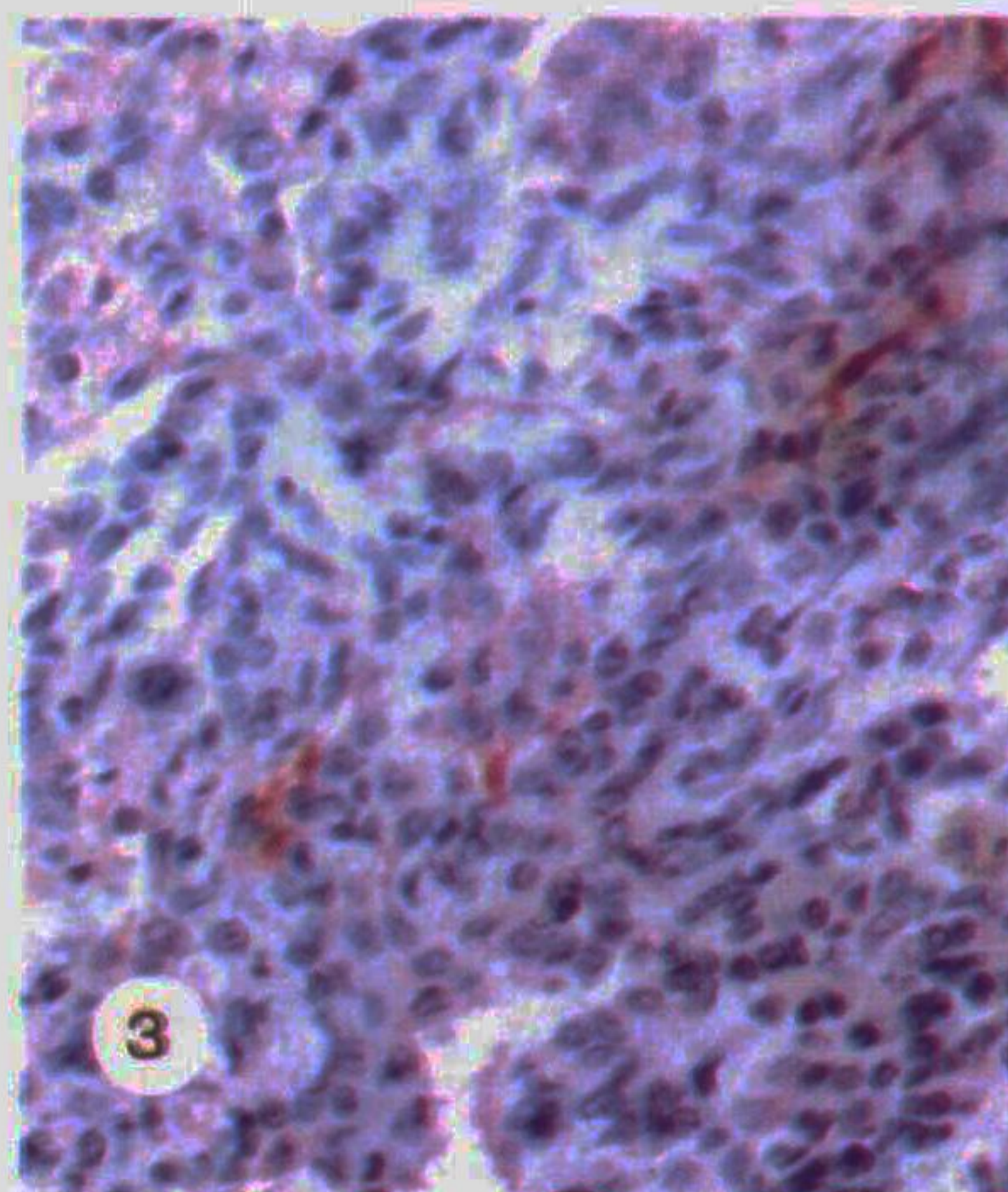
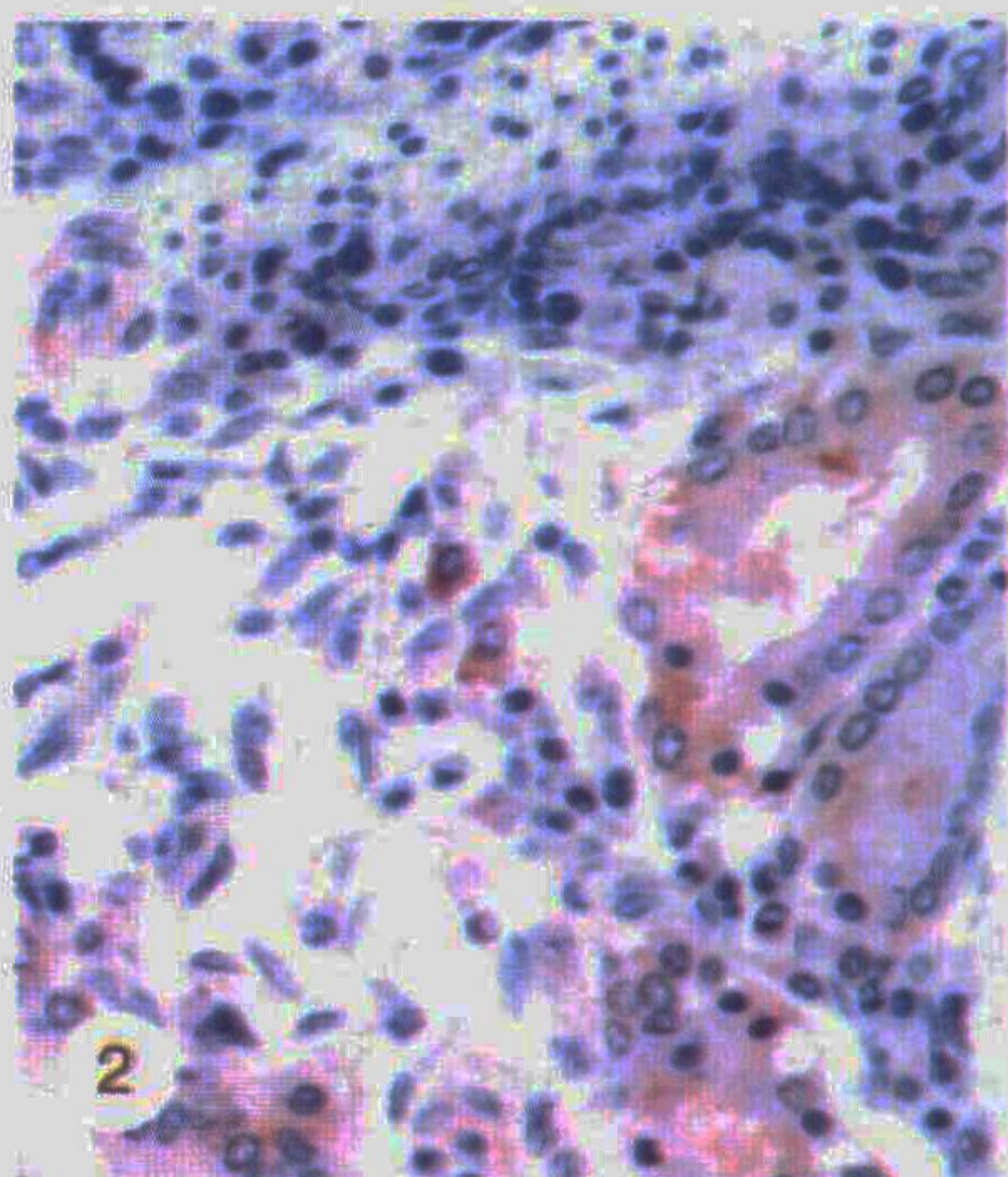


Figure 2- Positive immunostaining in the bronchiolar gland epithelium and macrophages for PIV-3. ABPC, Mayer's hematoxylin counterstain. X 600.

Figure 3- PIV-3 positive reactions in consolidated alveoli. ABPC, Mayer's hematoxylin counterstain. X 400.