An outbreak of acute infectious bursal disease in poultry: ultrastructural studies on bursae from clinical cases

D Suresh Christopher, J Md Minnie Mathan, A B Peter, S P Anbumani, J Doraiswami, M P Rajendran and M Murugan

Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu 632 402

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ABSTRACT

Ultrathin sections of bursae from chicks affected with infectious bursal disease (IBD) were studied under transmission electron microscope (TEM). Crystalline array of viral particles in the cytoplasm of mononuclear cells, intracellular edema, fatty degeneration and margination of chromatin material were frequently observed. Icosahedral viral particles were seen either free or bound inside phagolysosomes.

Key words: IBDV-Bursae-ultrastructure, Infectious bursal disease, Poultry

Very virulent form of infectious bursal disease (vvIBD) occurred in the poultry pockets of Tamil Nadu during March 1993 causing >80% mortality in grower chicks (Suresh Christopher et al. 1996) as against the classical form of IBD which caused <25% mortality (Purushothaman et al. 1988, Johnson and Chandramohan, 1992).

Transmission electron microscopy is considered as an important aid in diagnosis of avian viral disease (McFerran et al. 1978). Electron microscopic studies on the pathogenesis of classical form of IBD in India was reported by Pathak et al. (1988) and Dash et al. (1989). Munova et al. (1992) reported on the ultrastructural changes caused by a highly virulent virus in Japan. In this paper, the results of ultrastructural studies on bursal tissues collected from clinical cases of vvIBD are presented.

MATERIALS AND METHODS

The morbidity for ultrastructural studies were collected from bursal tissues of 6-week-old affected poultry. These specimens were positive for IBD virus antigen by AGID (OIE Manual 1992), RPHA and IFAT (Scott 1991). IBDV serotype I standard antigen (Farager 52/70) and standard antiserum obtained from Central Veterinary Laboratory (CVL), Weybridge, UK, were used, and the isolate developed lines of identity with IBDV reference serotype I antiserum as described elsewhere (Suresh Christopher et al. 1996). These specimens were also routinely processed for histopathological studies.

Bursal tissue samples (5) were fixed in 2.5% glutaraldehyde, post-fixed in osmium tetroxide and processed for embedding in Araldite. One micron survey sections were stained with toluidine blue (for light microscopy) and ultrathin sections cut on LKB UM4 Ultra microtome were stained with aqueous uranyl acetate and lead citrate and examined with an electron microscope. The object size was determined using the magnification formula (Cottrell 1978).

RESULTS AND DISCUSSION

In 5 µ paraffin-embedded and H&E stained bursal sections necrosis and severe depletion of lymphoid follicles with haemorrhages, infiltration of heterophils and mononuclear cells, cystic cavities in medullary areas of follicles and inter-follicular edema were observed as described classically by Henry et al. (1980).

The toluidine blue stained 1µ sections revealed paucity of lymphocytes in the follicles which contained many macrophages with intracytoplasmic inclusions. These cells with intracytoplasmic inclusions, under transmission electron microscope (TEM), contained crystalline array of icosahedral viral particles of 50-55 nm diameter. At the early phase of the infection the cytoplasm showed numerous large droplet fatty degeneration, which is characteristic of more slowly developing toxic or viral diseases as against the small droplet fatty degeneration which is typical of acute metabolic diseases; the fatty degeneration of tissues other than liver, kidney and heart (in that order) is less common (Cheville 1989). The nucleus showed margination of chromatin material. Both the
Figs 1-2. 1. Macrophage with crystalline non-membrane bound viral particles adjacent to the nucleus. Cytoplasm shows lysosomes adjacent to the viral particles, lipid droplets (x 16000). 2. Autophagosome with membrane bound viral particles, electron dense structures and cytoplasmic fragments (x 16200).

rough and smooth endoplasmic reticulum were found dilated. Few viral particles were seen in small crystalline arrays without a segregating membrane (Fig. 1). The presence of non-membrane bound viral clusters is an indication of early phase of infection in a cell as per Kaufer and Weiss (1976) who reported that these non-membrane bound forms of viral clusters are detectable only in the early phase of infection, i.e. by 6 h post-infection (pi) following intrabursal application of a highly pathogenic strain of IBDV.

In bursal cells other than macrophages, viral particles could be seen in membrane-bound autophagosomes which also contained cellular membranes and cytoplasmic particles with lipid droplets or myelin-like figures, markedly electron dense particles and cellular fragments (Fig. 2). With further multiplication of viral particles, severe cell changes could be seen with mitochondrial swelling, dilatation of endoplasmic reticulum and nuclear lysis. At this stage large number of viral particles were seen diffusely dispersed in the cytoplasm or in membrane-bound vacuoles.

The cells at the terminal stage of infection showed lysis of cells with release of viral particles discharged into the intercellular spaces. The cellular substructures were not clearly seen and only lysosomes were recognizable.

This appearance is the typical change reported by others (Kaufer and Weiss 1976, Panisup et al. 1988, Dash et al. 1991). The appearance of myelene-like structures in the cytoplasm has previously been reported only for a highly pathogenic strain of IBDV (Kaufer and Weiss 1976). Similar observations in the present experiment probably indicate the significance of these structures in infections with highly virulent strains of IBDV. Our observations also concur with the ultrastructural changes reported for a highly pathogenic strain of IBDV in Japan (Nunoya et al. 1992).

REFERENCES


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