Ostertagia ostertagi immunology and implications for diagnosis

Genevieve D'Amours of SVS Laboratories discusses the ins and outs of the abomasal parasite *Ostertagia ostertagi*, including diagnostic options.

***OSTERTAGIA* IS A** gastrointestinal parasite of cattle that causes significant economic losses to the dairy and beef industries. It is a nematode that dwells in the mucus of the abomasal glands and induces a mucosal inflammatory reaction and hyperplasia and metaplasia of the mucus neck cells (Figure 1).

These changes lead to an increase in pH and decreased conversion of pepsinogen to pepsin, which causes decreased protein absorption, intestinal bacteria overgrowth and diarrhoea.

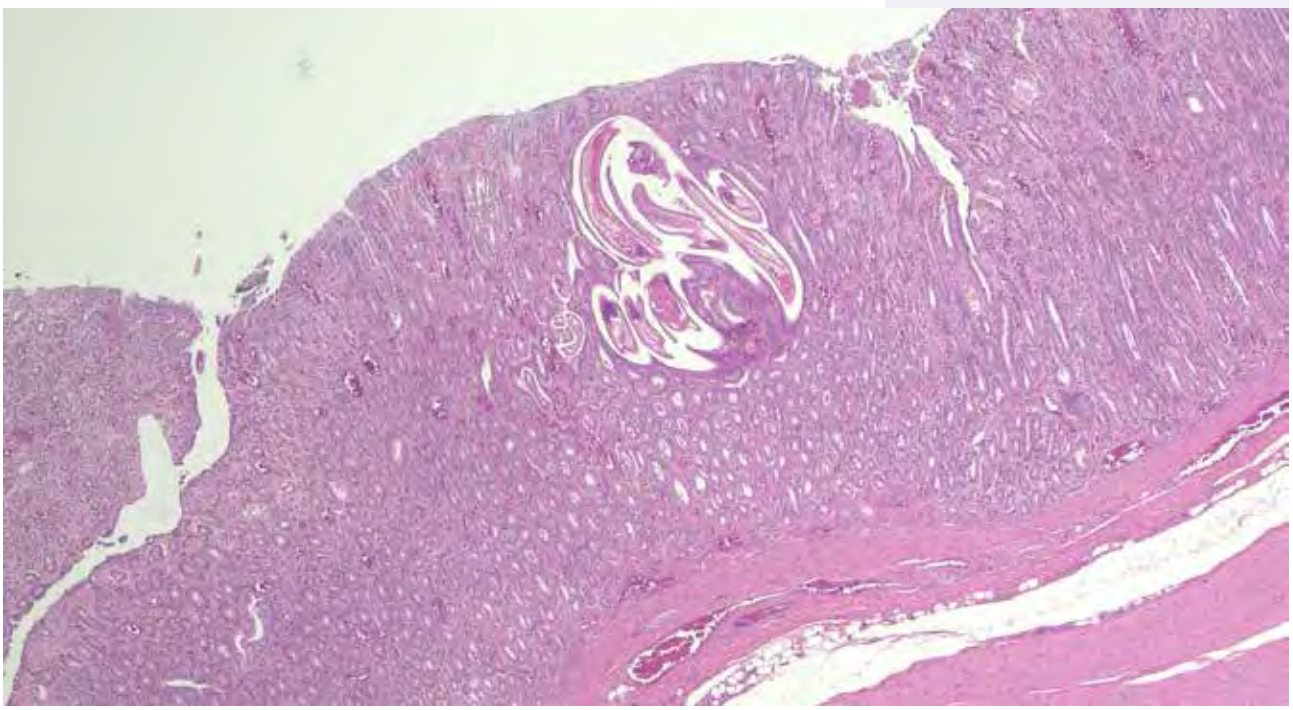
Ostertagia eggs are shed in faeces and develop into free-living L1 and L2 larvae on the pasture. The larvae then develop into the infective L3 stage that, when ingested by a suitable host, develops into L4, L5 and eventually adult nematodes. While *Ostertagia* can become patent within 21 days of ingestion, stage four larvae can undergo maturation arrest in the mucosa and re-emerge en masse at a later date. This phenomenon is more common when L3 larvae are exposed to cool temperatures and in regions with regular dry seasons.

Ostertagia infection can result in two syndromes. Type I disease affects calves in the summer and autumn and is characterised by diarrhoea and ill-thrift. In type II disease, the arrested larvae mature suddenly, causing severe damage to the abomasum as they emerge in nine-month-old to one-year-old or older cattle. This can result in sudden severe illness and even death. Type II disease is uncommon in New Zealand.

IMMUNITY

The innate immune response is involved initially, with neutrophils being present

FIGURE 1: Abomasal mucosa with mucus neck cell metaplasia and hyperplasia and nematodes.



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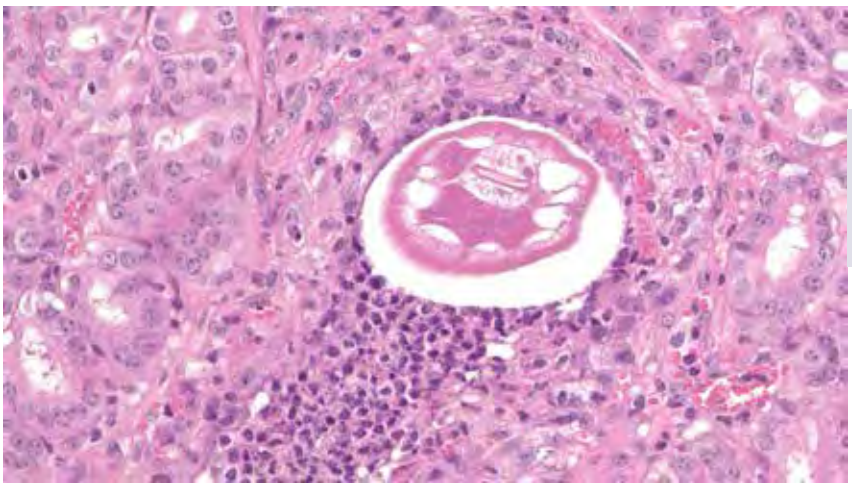


FIGURE 2: *Ostertagia* larvae surrounded by degenerate neutrophils.

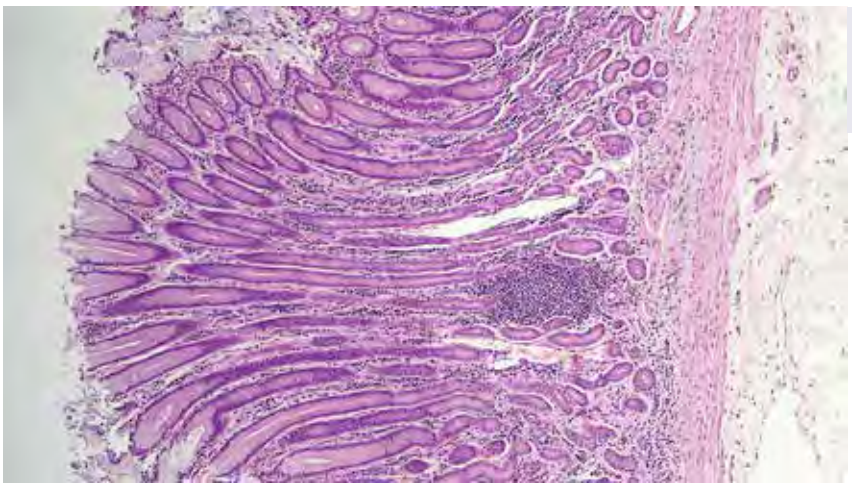


FIGURE 3: Lymphoid nodule within abomasal mucosa.

in the abomasum soon after infection. Neutrophils are recruited to the site quickly after infection (Figure 2). Neutrophils have been shown to be able to release neutrophil extracellular traps (NETs). These consist of a mesh of chromatin strands and protein granules containing myeloperoxidase and neutrophil elastase, which can trap and kill some microbes. *Ostertagia* extract and live L4 larvae have been shown to also induce formation of NETs in vitro (Mendez et al, 2018).

Infections with *Ostertagia* also illicit a humoral response, with immunoglobulins being detected in the serum and mucus of infected animals. *Ostertagia* antigens are presented to the draining lymph nodes soon after infection, and calves quickly mount a response resulting in lymphadenopathy and increased numbers of parasite-specific lymphocytes within the lymph nodes and abomasal mucosa (Figure 3). Immunity to parasites typically involves a Th2 response mediated by the release of several interleukins (IL4, IL5, IL10 and IL13) and IgE, which attract mast cells, eosinophils and basophils. Histologically, there are increased numbers of eosinophils, mast cells and globule leukocytes, which are thought to originate from mast cells, within the abomasal mucosa (Figures 4, 5). *Ostertagia* infections also trigger a Th1 response, which is usually most effective against intracellular pathogens, with upregulation of interferon gamma (IFN-γ).

Compared to other parasites, the immune response to *Ostertagia* is usually slow and incomplete. Protective immunity, which is characterised by resistance to reinfection, can take 18 months or more of continuous exposure to develop. Parasite burdens can be seen in animals older than one year and after stressful periods such as parturition and early lactation.

The mechanisms contributing to resistance to reinfection are still poorly understood, but decreased worm fecundity is thought to be an early manifestation of protective immunity. In calves it is thought that increased IgA levels in the abomasal mucosa may regulate worm fecundity. In sheep, the presence of IgA directed against carbohydrate larval surface antigen (CarLA) has been associated with immunity to parasites, with resulting decreased faecal egg counts (FECs) and increased weight gains. Salivary anti-CarLA IgA assays are being used to select sheep with increased resistance to parasites for breeding. A study looking at anti-CarLA salivary IgA in first season grazing heifers found that IgA levels were negatively correlated with FECs but not with pepsinogen levels, total serum *Ostertagia* immunoglobulins or daily weight gain, and therefore concluded that salivary IgA levels did

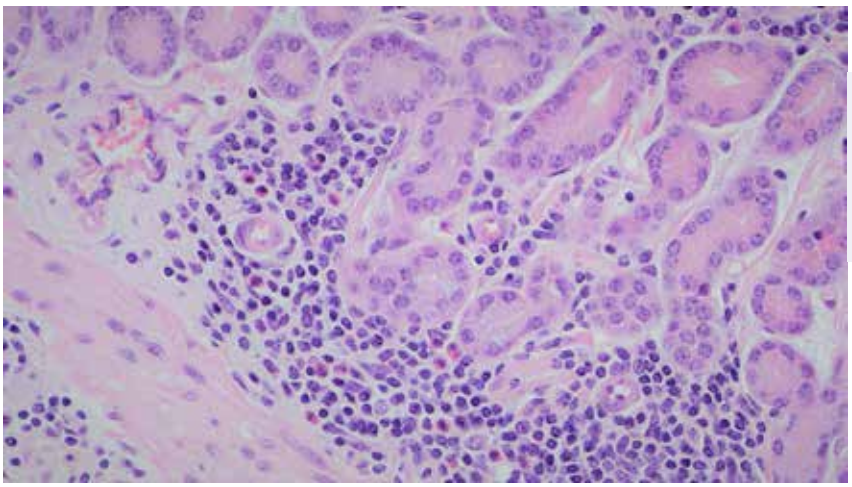
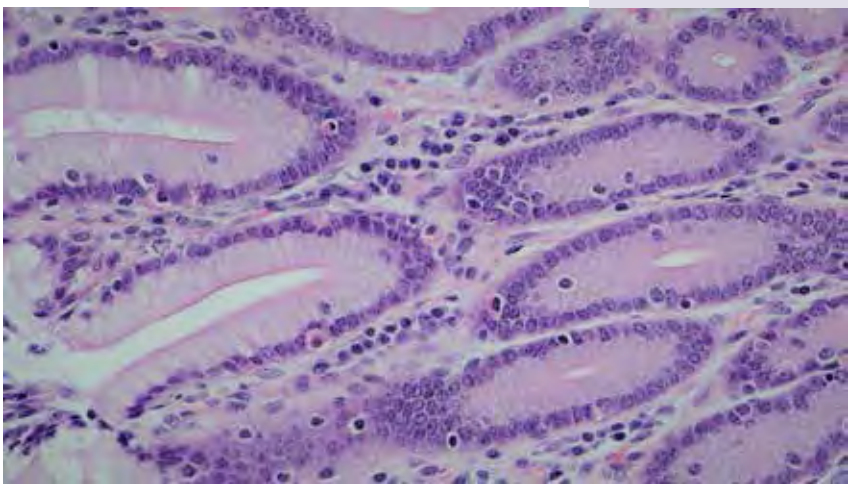


FIGURE 4: Infiltration of lymphocytes and eosinophils within abomasal lamina propria.

FIGURE 5: Globule leukocytes within abomasal epithelium.



not differentiate between immunity or exposure to the parasites (Merlin et al, 2017). More research is needed to elucidate the mechanisms of protective immunity in cattle.

DIAGNOSTIC OPTIONS FOR GASTROINTESTINAL NEMATODES

FECs remain the most widely used tool for the detection of gastrointestinal parasites in cattle and other animals. Faecal larval cultures can then assess which types of parasite are present and what percentage of the nematodes are *Ostertagia*. However, FECs will not always correlate to abomasal damage, especially if large numbers of immature larvae are present. This can be a significant issue in type II ostertagiosis where there are large numbers of hypobiotic larvae.

Serum pepsinogen can be used to detect abomasal damage caused by *Ostertagia*, as the increased abomasal pH decreases conversion of pepsinogen to pepsin and increases serum pepsinogen. This test is a good indicator of *Ostertagia* burden in young animals but not in adult cattle. Other diseases causing abomasal damage or increases in pH, including abomasal displacement, can also increase pepsinogen.

In first-season grazing calves, sampling seven animals from groups of up to 40 animals is considered adequate to interpret mean serum pepsinogen (Charlier et al, 2011).

Serological assays have been developed to look at antibody levels against *Ostertagia* crude worm extract in serum, milk and bulk tank milk (BTM)samples. In previous studies, antibody levels measured by BTM enzyme-linked immunosorbent assay (ELISA) have been negatively correlated to milk yield, and have a positive association with increased milk yield following anthelminthic treatment. However, most of these studies were performed in Europe and North America, where animals are housed for a significant proportion of the year.

In France, response to treatment with anthelminthic was correlated with a longer grazing period, percentage of animals with positive FECs, and BTM *Ostertagia* ELISA, but not pepsinogen levels or individual serum ELISA (Ravimet, 2014). In Argentina, where animals are grazed year round and may be more representative of the New Zealand systems, parasite burden in the first month post-partum is high. In this region, milk production response to anthelminthic treatment in the first month post-partum was correlated to FECs but not with pepsinogen or milk *Ostertagia* ELISA (Mejía, 2011). This study also found that there was no correlation between FEC and individual milk ELISA or pepsinogen. This suggests that BTM ELISA may provide a good indication of parasite exposure of the group, especially in situations where

parasite exposure may be low, and the response of a group to treatment.

However, individual humoral response measured by serum or milk ELISA does not appear to correlate with patent infections and response to treatment.

Control of ostertagiosis is still dependent on the use of anthelminthic. With growing concerns for anthelminthic resistance, targeted treatment has been suggested as a method to mitigate the risks. However, this requires selecting animals most likely to respond to treatment, which may not always be straightforward given the assay limitations. In young animals, FECs and serum pepsinogens are the best options. In adult animals, however, BTM *Ostertagia* ELISA will give a general indication of herd exposure, and FEC in times of stress may help identify the most affected animals. VS

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