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Review

The pathogenesis of proventricular dilatation disease

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Abstract

Bornaviruses cause neurologic diseases in several species of birds, especially parrots, waterfowl and finches. The characteristic lesions observed in these birds include encephalitis and gross dilatation of the anterior stomach — the proventriculus. The disease is thus known as proventricular dilatation disease (PDD). PDD is characterized by extreme proventricular dilatation, blockage of the passage of digesta and consequent death by starvation. There are few clinical resemblances between this and the bornaviral encephalitides observed in mammals. Nevertheless, there are common virus-induced pathogenic pathways shared across this disease spectrum that are explored in this review. Additionally, a review of the literature relating to gastroparesis in humans and the control of gastric mobility in mammals and birds points to several plausible mechanisms by which bornaviral infection may result in extreme proventricular dilatation.

Keywords: Proventricular dilatation disease, parrot bornavirus, achalasia, myenteric plexus, vagus nerve.

Introduction

Naturally occurring bornavirus infections cause a lethal meningoencephalitis in horses and sheep in central Europe (Metzler *et al.*, 1976; Richt *et al.*, 2000). A similar disease can be induced by experimental infection in laboratory rats and mice (Narayan *et al.*, 1983a, b; Kao *et al.*, 1984). The nature of the rodent disease depends upon their age. Adult rats develop lethal encephalitis, whereas neonatal rats develop behavioral abnormalities as a result of defects in neural development (Narayan *et al.*, 1983a; Hornig *et al.*, 1999, 2001; Lin *et al.*, 2013). Experimental bornavirus-associated neurologic disease has also been reported in cats (Lundgren *et al.*, 1997). Recently, a squirrel bornavirus was shown to cause lethal encephalitis in humans (Hoffmann *et al.*, 2015).

Bornavirus-infected birds have a very different clinical presentation that involves the gastrointestinal (GI) tract in addition to more typical neurologic deficits. Infection by psittacine bornaviruses results in impairment of GI motility leading to proventricular dilatation disease (PDD) in parrots (Honkavuori *et al.*, 2008; Kistler *et al.*, 2008; Payne *et al.*, 2012; Rubbenstroth *et al.*, 2014b). As in mammals, not all infected birds develop immediate clinical disease. Many bornavirus-infected birds may remain apparently healthy for years and then die unpredictably from PDD (Hoppes *et al.*, 2010). Experience has also shown that bornavirus infections can manifest themselves in ways other than classical PDD. These include blindness, sudden death due to cardiac failure as a result of severe myocarditis, or non-specific neurologic signs, including ataxia and torticollis (Vice, 1992; Weissenbock *et al.*, 2009; Rubbenstroth *et al.*, 2014b).

Modern molecular techniques have provided insights into the pathogenesis of bornaviral disease in infected mammals although few such studies have been undertaken in birds.

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Nevertheless, based on mammalian examples, it is possible to make rational hypotheses regarding the pathogenesis of bornaviral disease in birds, and to exclude some of the less plausible mechanisms of PDD.

Bornaviruses

Bornaviruses (order Mononegavirales family *Bornaviridae*), are enveloped particles with a non-segmented negative strand RNA genome. Their 9 kb RNA genome encodes five structural proteins, an RNA-binding nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), an envelope glycoprotein (G) and the large RNA-dependent RNA-polymerase (L). The open reading frame of a sixth protein, called X, overlaps the P open reading frame.

Bornaviruses replicate in the nucleus of the infected cell and employ the RNA splicing machinery for gene expression (Ludwig, 2008). Bornaviruses are highly cell-associated and in cultured cells few infectious particles are released (Gonzalez-Dunia *et al.*, 1998; Tomonaga *et al.*, 2002). Instead, Bornaviruses are probably often transmitted between cells in the form of a ribonucleoprotein complex (RNP) (Honda and Tomonaga, 2013). This complex consists of RNA plus oligomeric N proteins together with P and L proteins.

Overall genome organization is well conserved among the bornaviruses. The majority of genome diversity is found in the region between the open-reading frame for the N and X proteins. At this time it is not known how, or if, the diversity of the N–X intergenic regions impacts disease expression in natural infections.

Bornaviral diversity

Until 2008, the only known member of the family *Bornaviridae* was Borna disease virus (BoDV). Currently there are two named viruses (BoDV-1 and BoDV-2) included in the species *Mammalian 1 bornavirus* (Kuhn *et al.*, 2015). BoDV-1 infects mammals such as horses and sheep, as well as its natural reservoir host, the bicolored white-toothed shrew (*Crocidura leucodon*). BoDV-1 has also been reported to be present in wild birds (Malkinson *et al.*, 1993; Berg *et al.*, 2001) although this has yet to be independently confirmed (Durrwald *et al.*, 2007; Rubbenstroth *et al.*, 2013). Although an RNA virus, isolates of BoDV-1 show less than 5% diversity in nucleotides and 3% diversity in amino acid sequences (Lipkin *et al.*, 2011). BoDV-2 was isolated from a horse. A new mammalian bornavirus, variegated squirrel bornavirus 1 (VSBV-1) has recently been associated with fatal encephalitis in human beings (Hoffmann *et al.*, 2015).

In 2008, multiple bornaviruses were identified in parrots (Honkavuori *et al.*, 2008; Kistler *et al.*, 2008). Following initial reports, a variety of wild and captive birds were surveyed, resulting in the identification of additional avian bornaviruses, and a revised nomenclature and phylogeny of the bornaviruses (Fig. 1) was proposed (Kuhn *et al.*, 2015). Currently, within the family *Bornaviridae* there is a single genus (*Bornavirus*) and six named species containing 14 named viruses. Five additional named viruses have not been assigned a species (Kuhn *et al.*, 2015). Two species, *Psittaciform 1 bornavirus* and *Psittaciform 2 bornavirus* (PaBV-1 and PaBV-2) contain viruses isolated from a wide variety of captive parrots. Many isolates were from birds with PDD, although healthy parrots are also infected (Hoppes *et al.*, 2013). The wild bird hosts for these viruses are not currently known. One species, *Aquatic bird bornavirus 1* (ABBV-1), contains viruses common to wild aquatic birds, including geese, swans and ducks (Delnatte *et al.*, 2011; Guo *et al.*, 2013). ABBV-1 appears to be widespread across North America and Europe (Thomsen *et al.*, 2015). The first ABBV-1 isolates were from Canada geese with PDD, but surveys of hunter-harvested geese, swans and ducks suggest that in some flocks, up to 50% of 'healthy' birds are infected (Payne *et al.*, 2012). Three bornaviral species have been isolated from passeriform birds, including captive canaries (CnBV-1 and -2) and estrildid finches (EsBV-1) (Weissenbock *et al.*, 2009; Rubbenstroth *et al.*, 2013, 2014b). Additional bornaviruses also have been detected in African snakes (*Elapid 1 bornavirus*) (Stenglein *et al.*, 2014). Their pathologic significance is unknown (Kuhn *et al.*, 2015). Finally, attesting to the ancient origins of bornaviruses, endogenous bornaviral sequences have been identified in many mammals, including primates (Horie *et al.*, 2010, 2013)

Pathogenesis of bornavirus-induced diseases in birds

The finding that bornaviruses cause PDD in parrots and other birds has revealed several new aspects of their complex pathogenicity. The spectrum of bornaviral disease symptoms in these birds can be diverse (Gray *et al.*, 2010; Mirhosseini *et al.*, 2011; Payne *et al.*, 2011; Piepenbring *et al.*, 2012, 2016). Many, but not all, infected birds develop non-purulent inflammatory lesions in the brain, spinal cord, major nerves and autonomic ganglia that resemble the typical bornaviral lesions seen in mammals (Hoppes *et al.*, 2013). It is unclear whether this encephalitis precedes centrifugal spread of the virus through the cranial nerves and the spinal cord or the reverse. The most significant of the affected cranial nerves are the optic nerve and the vagus nerve. Spread through the optic nerve may lead to retinitis and blindness (Krey *et al.*, 1979; Steinmetz *et al.*, 2008). Spread along the vagus nerve may lead to proventricular dilatation. Spread through the spinal cord may result in ataxia and paralysis. Notwithstanding this, not all birds with proventricular dilatation develop clinical encephalitis. Some, perhaps many, bornavirus-infected birds fail to develop clinical disease and may survive as healthy carriers for many years (Lierz *et al.*, 2009; Heffels-Redmann *et al.*, 2012; Hoppes *et al.*, 2013). This is especially true of waterbirds where clinical disease outbreaks appears to be random and sporadic despite a relatively high prevalence of infection (Delnatte *et al.*, 2011).

Clinical disease

Parrots suffering from PDD present with severe dilatation of their proventriculus and commonly die as a result of starvation.

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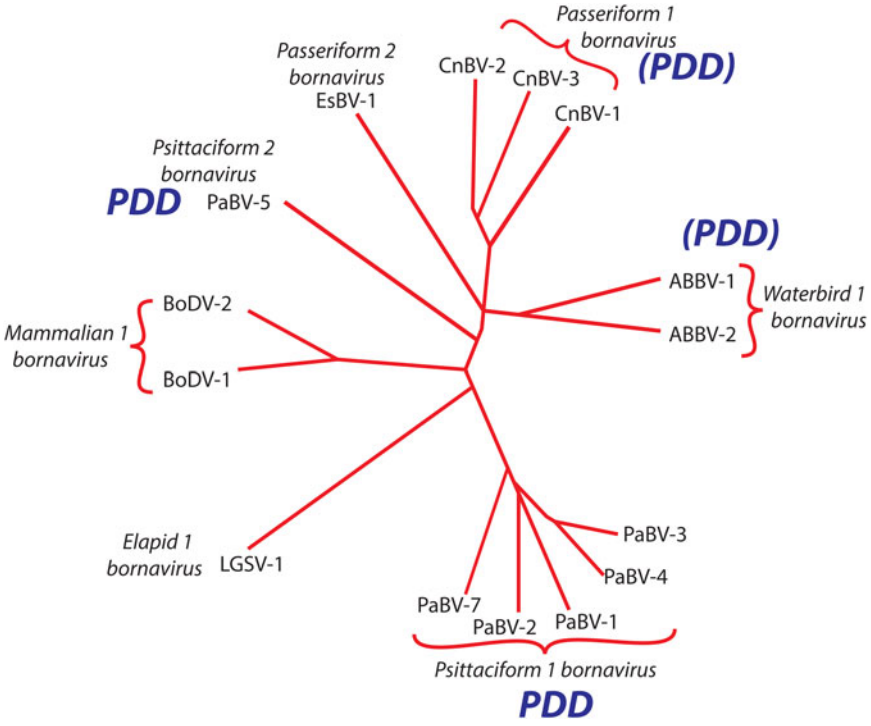

Fig. 1. The current phylogeny and nomenclature of the bornaviruses. The species known to produce PDD are noted. ABBV-2 and Passeriform 2 bornavirus have yet to be associated with proventricular dilatation.

Clinical signs range from weight loss, crop stasis, proventricular and intestinal dilatation, regurgitation, maldigestion, passage of undigested food in feces, and eventually starvation and death (Vice, 1992; Hoppes *et al.*, 2013). Signs reflecting central nervous system lesions may also be present, such as tremors, ataxia, seizures and blindness. Secondary bacterial or fungal infections of the non-motile proventriculus can result in death by sepsis. Affected birds may show both neurological and GI signs (Berhane *et al.*, 2001).

A natural outbreak of PDD in captive parrots following exposure to a bird infected with PaBV-2, has been described by Kistler *et al.* (2010). They reported that in unweaned chicks, proventricular dilatation developed as early as 2–4 weeks after exposure. The age of the host greatly affected the rate of progression of bornavirus infection. In very young birds, nervous system signs appeared within 48 h of feed refusal, and death occurred within 3 days. Older birds became sick much more slowly. Experimental disease induced in cockatiels and conures generally follows a course similar to that seen in older birds (Gray *et al.*, 2010; Piepenbring *et al.*, 2012). The shortest incubation period we have observed following experimental challenge with PaBV-2 has been 44 days (Hameed, unpublished observation). It should also be pointed out that, in our experience, psittacine bornaviruses may readily infect other species such as lovebirds (*Agapornis roseicollis*) or quaker parrots (*Myiopsitta monachus*) but these rarely develop clinical PDD (Tizard *et al.*, unpublished observation).

When canaries (*Serinus canaria*) are experimentally infected with CnBV-1 or CnBV-2, some develop very mild microscopic changes, (mononuclear infiltrations in the cerebrum and peripheral ganglia) but no clinical disease or macroscopic lesions (Rubbenstroth *et al.*, 2013, 2014a). Some naturally infected birds developed a ganglioneuritis in the proventriculus and ventriculus (gizzard) leading to proventricular dilatation. Infections involving the optic tract may lead to retinitis and blindness. Infected birds may become depressed, ataxic or convulsive (Weissenbock *et al.*, 2009; Rubbenstroth *et al.*, 2013). A different species of avian bornavirus (EsBV-1) has been isolated from unhealthy estrildid finches in Germany (Rubbenstroth *et al.*, 2014b). It is not known whether this virus is pathogenic. In canaries as in parrots, seroconversion does not correlate with virus tissue burden or viral shedding (Rubbenstroth *et al.*, 2014a).

In 1991, typical PDD was described in Canada geese (*Branta canadensis*). The birds developed an encephalitis, ganglioneuritis and severe proventricular food impaction (Daoust *et al.*, 1991). Studies by Delnatte *et al.* demonstrated ABBV-1 in the tissues from these specific birds (Delnatte *et al.*, 2011). A survey of multiple waterfowl by Delnatte *et al.* found that this bornavirus is widespread in asymptomatic free-ranging waterfowl in Canada (Delnatte *et al.*, 2014a). Birds shedding viral RNA were more likely to have antibodies to ABBV-1 and tended to have higher antibody levels than those that were not shedding (Delnatte *et al.*, 2014b). Nevertheless many antibody-positive birds did not shed the virus. Delnatte *et al.* described the pathology of ABBV-1-infected waterfowl (Delnatte *et al.*, 2013). Many infected birds presented with proventricular impaction or a clinical history that included weakness and neurologic disease. Neurologic abnormalities included somnolence, weakness, and lethargy, suspected blindness, lameness, torticollis, ataxia, inability to stand or fly, hypermetria, head tremors, stargazing and

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opisthotonus. Approximately 50–75% of the birds were in poor body condition. Histopathological lesions included gliosis and lymphoplasmacytic perivascular cuffing in the brain (97%), spinal cord (50%), peripheral nerves (55.5%) and the myenteric ganglia or nerves (62%) (Delnatte *et al.*, 2013). It should however be pointed out that experimental confirmation of bornaviral causation of these lesions is still lacking.

Encephalitis

Bornaviral RNA, detected by a reverse-transcriptase polymerase chain reaction (RT–PCR), is present in the brain of all cases of PDD in parrots (Hoppes *et al.*, 2013). Ouyang *et al.* detected the virus in 13/13 cases of PDD in psittacines. Viral N-protein was present in the cerebrum, cerebellum and spinal cord. The lesions in the cerebellum were associated with interruptions in the Purkinje cell layer. (Notably, Purkinje cells are also lost in bornaviral infections of neonatal rats (Eisenman *et al.*, 1999). The lesions in the cerebrum were associated with moderate perivascular cuffing and focal gliosis (Ouyang *et al.*, 2009). Raghav *et al.* investigated 16 cases of PDD and found PaBV in neurons and glial cells throughout the brain as well as in Purkinje cells in the cerebellum, neurons, ependymal cells and glia in the spinal cord and neurons in the gut ganglia and the pericardial ganglia (Raghav *et al.*, 2010). Similar results have been reported by Wunschmann *et al.* (2011). Brain lesions may be found in bornavirus-infected parrots not demonstrating clinical PDD and these have been assumed to represent relatively early infections (Lierz *et al.*, 2009).

Other lesions

The presence of bornaviral RNA in organs other than the brain is variable. For example, Delnatte *et al.* reported that the parrot tissues with the highest detection frequency of viral RNA were in order, proventriculus (100%), kidney (71%), colon (50%), cerebrum and cerebellum (43%) (Delnatte *et al.*, 2013). Skeletal muscle consistently had low amounts of viral RNA. Raghav *et al.* also studied this tissue distribution and found that many more tissues were positive by RT–PCR than by histopathology or immunohistochemistry (Raghav *et al.*, 2010). They found that viral antigen was most consistently detected in brain, spinal cord, pancreas, adrenal and kidney. Several other investigators have also pointed out the broad tissue tropism of the parrot bornaviruses (Lierz *et al.*, 2009; Rinder *et al.*, 2009; Wunschmann *et al.*, 2011). (These findings may be directly relevant to the pathogenesis of PDD. A positive proventriculus in the presence of a negative brain suggests that the virus may not spread from brain to proventriculus but vice versa. A similar observation has been noted in a bornavirus-infected canary (Rubbenstroth *et al.*, 2014a).)

Berhane *et al.* necropsied 14 parrots that, in addition to the usual lesions in the brain and proventriculus, had a diffused lymphoplasmacytic neuritis in the peripheral nerves, including the sciatic, brachial and vagus as well as the dorsal root ganglia (Berhane *et al.*, 2001). The histological lesions included mild, diffuse or focal mononuclear cell infiltration; presence of focal clusters of lymphoplasmacytic infiltrates; axonal swelling and myelin degeneration; and the presence of perivascular cuffs.

The pathogenesis of bornaviral encephalitis has been comprehensively analyzed in adult rats (Narayan *et al.*, 1983b; Carbone *et al.*, 1988). In these experimental hosts, BoDV-1 shows tropism for three sites in the brain, the dentate gyrus of the hippocampus, the Purkinje cell layer of the cerebellum, and scattered neurons in the cerebrum. The targeted cells all carry receptors for the excitatory neurotransmitter, glutamate (Ovanesov *et al.*, 2007). The lesions in rats, as in birds, are characterized by perivascular cuffing with lymphocytes and monocytes (but rarely plasma cells or B cells). In rats, the predominant infiltrating cells are CD8+ T cells with some CD4+ T cells and natural killer (NK) cells (Hatalski *et al.*, 1998). Administration of immunosuppressive agents such as cyclophosphamide, cyclosporine or anti-T cell serum prevented the development of disease (Stitz *et al.*, 1992, 2002). A similar effect has been observed in cyclosporine-treated PaBV-infected cockatiels (Hameed *et al.*, unpublished observation). It is therefore believed that the neurologic dysfunction results from a T-cell-mediated immune attack on bornavirus-infected neurons. The target antigen appears to be the bornaviral N-protein (Planz *et al.*, 2001). Additionally, raised levels of glutamate as a result of astrocyte dysfunction may cause neuronal death through excitotoxicity (Zhang *et al.*, 2014). Activated microglia release pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β further disrupting brain functions (Sauder and de la Torre, 1999; Tizard *et al.*, 2016). The encephalitis in bornavirus-infected birds may have a similar pathogenesis.

Blindness

Blindness, resulting from a severe retinitis, is a consistent feature of bornaviral disease in horses, sheep and rodents. BoDV-1 spreads from the brain along the optic nerve into the retina. Blockage of the optic nerves by xenon coagulation prevented development of retinopathy and viral invasion in rabbits (Krey *et al.*, 1979). The retina of infected mammals contains numerous infected cells and on occasion is infiltrated with lymphocytes. This eventually results in complete functional loss of the retina. Cellular infiltrates are also found within the optic nerve (Steinmetz *et al.*, 2008). Narayan attributed bornaviral retinitis in rats to attack by cytotoxic T cells (Narayan *et al.*, 1983b). Immunosuppressive treatment of BoDV-infected rabbits delayed the onset of retinal lesions and reduced their severity (Krey *et al.*, 1981). Stahl *et al.* demonstrated that the retinal T cell infiltration in Lewis rats consisted of αβ TCR+, CD4+ and CD8+ cells (Stahl *et al.*, 2003). Cytokine genes being transcribed in these retinas included IL-1β, IL-6, interferon (IFN)-γ and TNF-α (Sauder and de la Torre, 1999).

Bornavirus-infected canaries also develop a chorioretinitis (Rubbenstroth *et al.*, 2014b) while blindness is a feature of bornaviral infections in parrots (Steinmetz *et al.*, 2008; Delnatte *et al.*, 2013). ABBV-2 has been detected within the retinas of

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infected ducks and ABBV-1 in the retinas of gulls (Guo *et al.*, 2014, 2015). It is probable that the pathogenesis of the retinitis in birds is similar to that in mammals.

Proventricular dilatation

It is not known whether the early lesions of avian bornavirus infections first occur in the brain, and the virus spreads centrifugally, eventually passing down the spinal cord and affecting major nerve trunks such as the sciatic, brachial and vagus or vice versa. (This centrifugal spread has been demonstrated in BoDV-1-infected rats using green fluorescent protein-labeled virus (Ackermann *et al.*, 2010), but there is no similar experimental evidence in birds.) The vagus nerve plays a key role in the dissemination and pathogenesis of avian bornaviral disease because the major visceral organs invaded by this virus are those directly innervated by the vagus, especially the anterior gut, the heart, the adrenal gland and the kidneys (Berhane *et al.*, 2001; Raghav *et al.*, 2010; Delnatte *et al.*, 2014a) (Fig. 2).

A grossly distended proventriculus is the defining characteristic of PDD in psittacine birds (Clark, 1984; Hoppes *et al.*, 2013). Dilatation of the crop, esophagus and occasionally the ventriculus and duodenum may also occur. In typical cases, the wall of the proventriculus is thin and atrophic and may be translucent as a result of severe muscle atrophy (Fig. 3). It may be full of food such as seeds and sometimes contains fluid (Hoppes *et al.*, 2013). Histologically, damage can be detected within the gastric plexus, the duodenal myenteric plexus and the celiac ganglion. The neurons in these ganglia appear to be 'destroyed' and are replaced by a lymphoplasmacytic infiltration with some monocytes (Mannl *et al.*, 1987; Berhane *et al.*, 2001). In addition to the ganglia, the branches of the myenteric plexus that innervate the muscular layer also contain many lymphocytes. Mononuclear cell infiltrates can also be found in the connective tissue between the muscle fibers. Viral antigen may be readily found within surviving neurons by immunohistochemistry (Ouyang *et al.*, 2009).

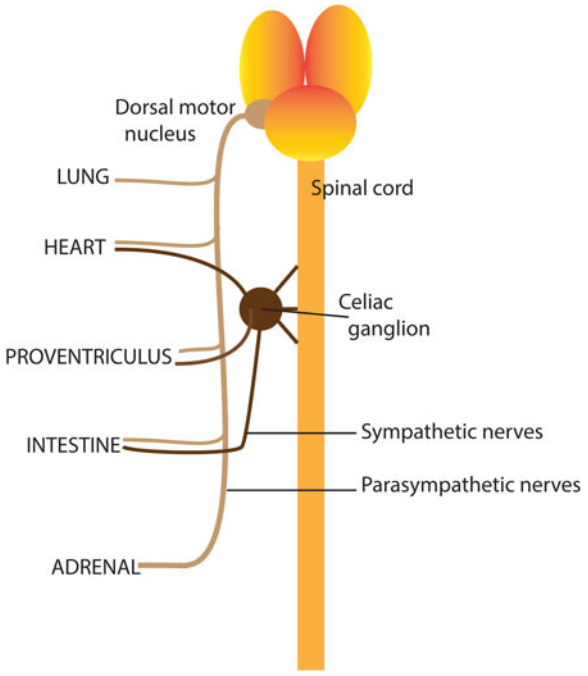

Fig. 2. The innervation of avian visceral organs is mediated through the parasympathetic vagus nerve and the sympathetic nerves from the celiac ganglion. The major organs infected with bornaviruses are those innervated through the vagus (cranial nerve X).

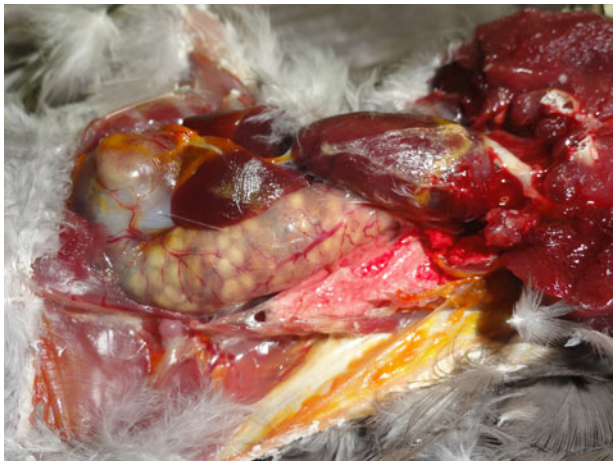

Fig. 3. A typical example of proventricular dilatation. The proventriculus is thin walled and translucent so that the ingested seeds within it are clearly visible. This lesion was induced by experimental infection of a cockatiel with PaBV-2.

The enteric nervous system (ENS)

Gut motility depends upon the relaxation and contraction of enteric smooth muscle cells. These cells generally cycle between relaxation and contraction in slow waves. These slow waves originate in the interstitial cells of Cajal (ICC) (Sanders *et al.*, 2006). For a smooth muscle cell to fully contract, however, it needs an additional stimulus provided by nervous signals. The propulsive and secretory activities of the GI tract are thus controlled by both the central (extrinsic) and enteric (intrinsic) nervous systems. While the general structure of the ENS is similar along the length of the GI tract, there are significant regional differences in the relative importance of intrinsic and extrinsic regulation. For example, the functions of esophageal striated muscle are controlled by neurons originating in the brainstem (Furness, 2008). The mammalian stomach is controlled by central neurons acting through enteric neurons. If, however, these neurons are surgically disconnected, stomach peristalsis is uninterrupted. The motility of the large and small intestines is regulated almost exclusively by enteric neurons (Furness, 2008).

The presence of food in the gut is the most obvious stimulus for gut motility. Food intake triggers the intrinsic propulsive contractions that constitute peristalsis. Mechanical stimulation from the food bolus triggers localized contraction and relaxation

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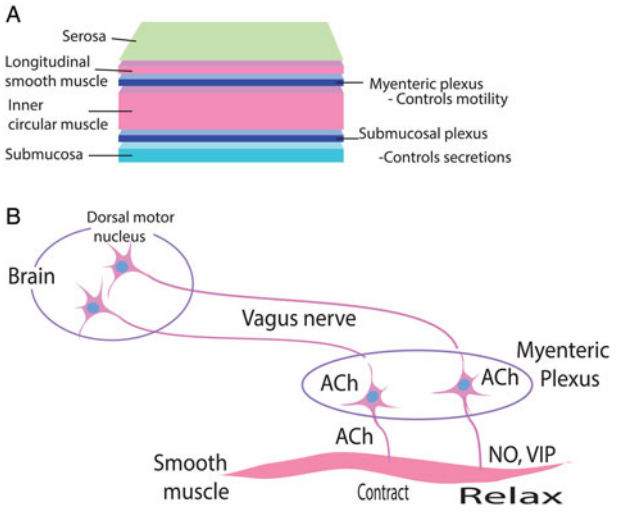

Fig. 4. (a) The arrangement of the enteric ganglia. The outer myenteric plexus (also called Auerbach's plexus) controls muscle function and hence motility. The inner submucosal plexus serves to mainly control gut secretory activity. (b) The brain communicates with the enteric nervous system through a chain of two neurons. The pre-ganglionic nerves originate in the dorsal motor nucleus and end at synapses in the enteric ganglia. The post-ganglionic neurons within the ganglia then innervate the enteric smooth muscle. The cholinergic post-ganglionic nerves mediate smooth muscle contraction. The nitrergic post-ganglionic nerves mediate relaxation.

through autonomic reflexes or local hormone release (Olsson and Holmgren, 2011). The gut also receives extrinsic signals. In the anterior gut, these signals originate in the brainstem and are transmitted through the vagus nerve. In the posterior gut, the extrinsic signals originate in the spinal cord and are mediated through splanchnic nerves (Nilsson, 2011).

The ENS is a network of neurons and glial cells that regulate intestinal motility, secretion and absorption (Wood, 2008). The ENS functions independently of the brain and consists of a network of highly interconnected neurons that secrete diverse neurotransmitters (Brookes, 2001a). Anatomically, the ENS consists of two prominent concentric layers of enteric plexuses (Fig. 4a). The outer myenteric plexus is responsible for maintaining smooth muscle tone and the steady rhythm of gut contractility and peristalsis, while the inner submucosal plexus mainly controls mucosal functions such as secretion (Goldstein and Nagy, 2008). Each plexus consists of a network of nerve clusters (ganglia) accompanied by large numbers of glial cells (Gabella, 1981). Their precise location varies between bird species. Thus, in pigeons and ducks the myenteric plexus is located between the outer longitudinal and inner circular muscle layer of the proventriculus. In the chicken, the myenteric plexus is located within the circular muscle layer (Kuder *et al.*, 2003).

The extrinsic innervation of the anterior intestinal tract in birds is similar to that in mammals in that it is mediated primarily by cranial fibers in the vagus nerve (Nilsson, 2011). These are mainly excitatory although vagal inhibitory pathways are also present. Other extrinsic nerves, such as the nerve of Remak (a nerve trunk running along the intestine) and spinal fibers in the splanchnic and pelvic nerves, innervate the post-ventricular gut (Nilsson, 2011). While the majority of enteric neurons in the bird are nitrergic they also produce the neuropeptide, vasoactive intestinal peptide (VIP), while others produce glutamate (Boros *et al.*, 1994; Li *et al.*, 1994; Balaskas *et al.*, 1995; Martinez *et al.*, 2000; Mirabella *et al.*, 2003). Adrenergic fibers also innervate the myenteric plexus (Olsson and Holmgren, 2011).

Neurons

Nitrergic neurons play an essential role in normal peristalsis as they trigger smooth muscle relaxation. Thus, the resting esophagus is normally constricted. During swallowing these neurons release nitric oxide (NO), resulting in smooth muscle relaxation and temporary esophageal dilatation (Olsson and Holmgren, 2011). Once a food bolus has passed, the smooth muscle behind it contracts under the influence of cholinergic (acetylcholine (ACh)-producing) neurons. Hence, peristalsis is the result of coordinated contraction and relaxation mediated by these two classes of neurons (Furness, 2008).

In mammals, the muscles of the distal esophagus and the cardia of the stomach are innervated by pre-ganglionic fibers from the vagus nerve (Furness, 2008). The cell bodies for these fibers are located in the dorsal motor nucleus of the medulla oblongata (Fig. 4b). These pre-ganglionic fibers innervate neurons within the myenteric plexus through the release of ACh (Olsson and Holmgren, 2011). The esophageal wall is subsequently innervated by post-ganglionic neurons from the myenteric plexus. The excitatory neurons release ACh, while the inhibitory neurons release NO, ATP and VIP. Additionally, some vagal afferent neurons are glutaminergic and interact with neighboring enteric neurons via glutamate (Berthoud, 2008). Glutamine synthetase is a specific marker for enteric glial cells and glutamate is known to be dysregulated in bornaviral infections (Billaud *et al.*, 2000; Zhang *et al.*, 2014). Gastric smooth muscle also relaxes under vagal stimulation (Curro *et al.*, 2008).

T cells

ACh is produced, not only by cholinergic neurons but also by T cells. ACh-synthesizing T cells can therefore relay signals in the vagus nerve (Rosas-Ballina *et al.*, 2011). For example, a signal from the pre-ganglionic vagal nerves inhibits TNF-α production in the spleen. This requires ACh signaling through a receptor on macrophages. However, splenic nerves do not produce ACh. Instead, the action potentials from the nerve stimulate T cells to produce ACh. Loss of these T cells may block ACh signaling to the gut and result in excessive relaxation. Conversely, activation of T cells may result in ACh-mediated smooth-muscle contraction (Rosas-Ballina and Tracey, 2009).

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Glial cells

Enteric glial cells are present within the myenteric plexus where they vastly outnumber enteric neurons (Gabella, 1981). They also actively control intestinal functions. For example, the enteric glia generate neurotransmitters and possess neurotransmitter receptors. These glia play a role in maintaining the integrity of the mucosal barrier. More importantly, they serve as a link between the nervous and immune systems in the gut (Ruhl, 2005). They can synthesize cytokines, capture and present antigens and participate in neuroinflammatory processes (Ruhl, 2005). While most enteric glia are found within the myenteric and submucosal plexuses, they are also present within the mucosa and make close contacts with the epithelial cell layer (Gabella, 1981). There are probably functional differences between these populations (Endo and Kobayashi, 1987).

Enteric glia synthesize neurotransmitters and regulate the levels of γ-amino butyric acid (GABA) in the gut wall (Jessen *et al.*, 1983; Fletcher *et al.*, 2002). Bornavirus infection in rats is known to disrupt GABA production in the brain (Scordel *et al.*, 2015). These glia also detoxify neuronally released glutamate (Jessen and Mirsky, 1983). Should the glia be destroyed, the increase in glutamate could potentially kill nearby neurons through excitoxicity (Ovanesov *et al.*, 2007). It is also possible that enteric glia synthetize NO and VIP (Cabarrocas *et al.*, 2003). If these glia or their associated neurons are destroyed, this would prevent gut relaxation and promote subsequent contraction of the gut wall (Aube *et al.*, 2006). GABAergic cells in the gut modulate both motor and secretory activity as well as GI inflammation (Auteri *et al.*, 2015). In mammals, GABA increases gastric tone by directly modulating intrinsic cholinergic neurons so a loss of such cells could promote gastric relaxation (Rotondo *et al.*, 2010). GABAergic neurons are widely distributed in the chicken gizzard and the duodenum (Saffrey *et al.*, 1983; Gabriel *et al.*, 1990). RNA-seq studies on PaBV-infected astrocytes have shown a massive increase in expression of the receptor for glial cell line-derived neurotrophic factor (GFRA1) (Guo, unpublished observations). This receptor controls the development of enteric glial cells (Lui *et al.*, 2002). Given the susceptibility of microglia and astrocytes to bornavirus infection in rodents, it is likely that destruction or even infection of enteric glial cells may play an important role in the pathogenesis of PDD.

Interstitial cells of Cajal

Embedded in the myenteric plexus and both muscle layers of the GI tract, are networks consisting of the ICC. These networks generate the spontaneous pacemaker activity that controls GI motility (Sanders *et al.*, 2006). In their absence, slow wave activity is not generated and gastric motility is disrupted (Thomsen *et al.*, 1998; O'Grady *et al.*, 2014). ICC are present in the GI tract of birds (Reynhout and Duke, 1999). They are found in the myenteric plexus in the lower GI tract (duodenum, ileum, cecum and rectum). They are absent from the turkey gizzard although the first ultrastructural observations on ICCs were made in the gizzard of a lovebird (*Urolonchа domestica*) (Imaizumi and Hama, 1969). Loss of ICCs in human beings is associated with a reduced current density leading to failures in gastric motility including delayed gastric emptying, diabetic gastroparesis and Chagas disease (Huizinga and Chen, 2014; O'Grady *et al.*, 2014; Bashashati and McCallum, 2015). It is not known if the development of PDD is associated with a loss of proventricular ICC, but this deserves further investigation.

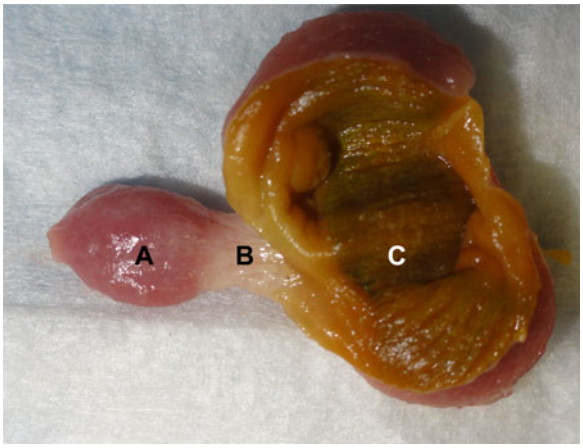

Fig. 5. The anterior stomach in a normal cockatiel. (a) Proventriculus, (b) isthmus, (c) ventriculus.

Proventricular dilatation

Proventricular motility is regulated by signals via the vagus from the brainstem, from enteric neurons (ENS), and from hormones. All these signals must be orchestrated if food is to be propelled smoothly into the ventriculus.

The proventriculus of parrots is relatively small when compared to carnivorous or fish-eating birds (Langlois, 2003). It has relatively little longitudinal muscle, so that the myenteric plexus is located close to the serosa. Unlike the esophagus, the proventriculus lacks longitudinal folds, but its interior is covered with numerous papillae. The gastric glands open at the tips of these papillae. These glands produce hydrochloric acid and pepsinogen (Langlois, 2003). The transition between the proventriculus and ventriculus is marked by a well-defined intermediate zone or isthmus (Fig. 5). In the absence of longitudinal folds, the ability of the normal proventriculus to stretch might be considerably constrained. It does need to contract to force food into the ventriculus. Food is passed backwards and forwards between the proventriculus and ventriculus several times in order to maximize digestion by reducing food particle size.

Dilatation of the proventriculus may result from three possible events (Fig. 6). First, it may result from excessive relaxation/failure of contraction of the proventricular smooth muscle fibers causing them to become flaccid. The resulting failure of peristalsis within the proventriculus could then result

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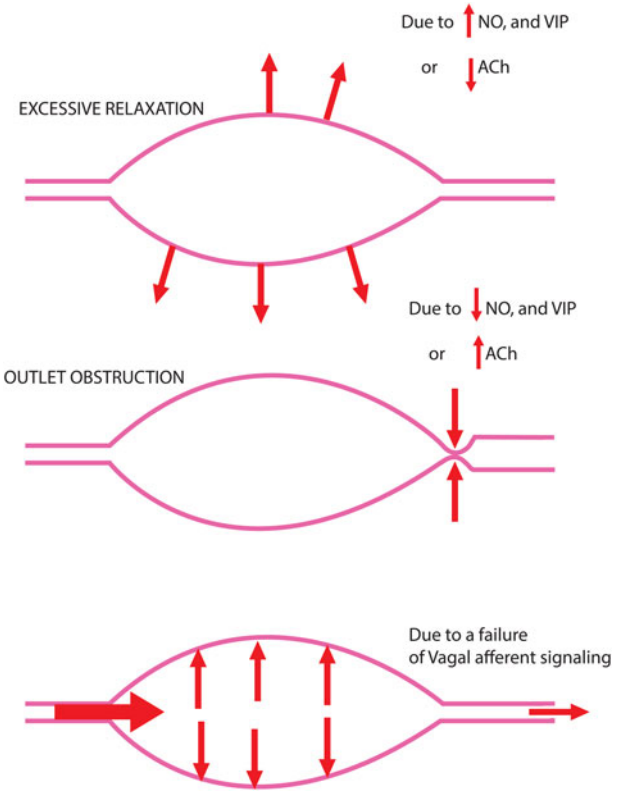

Fig. 6. Three possible mechanisms by which the proventriculus could be forced to dilate.

in the gradual accumulation of ingested food within the flaccid area. Second, proventricular dilatation may result from constriction/obstruction of the intermediate zone that separates the proventriculus from the ventriculus (gizzard) and thus blocks the flow of ingesta. As a result, upstream ingesta will accumulate in a manner similar to the effect of standing on a hosepipe. The upstream region will therefore dilate secondary to downstream blockage. Third, bornavirus infection may result in a failure of satiation signals to reach the brain from the proventriculus. As a result, the bird may continue to eat despite having a constricted proventriculus and so result in proventricular distension. These three hypotheses are by no means mutually exclusive.

Excessive proventricular relaxation

Given the location of the lesions of PDD, it seems clear that they are associated with the vagus nerve. While the vagus contains both excitatory (cholinergic) and inhibitory (adrenergic) nerve fibers, it also contains large numbers of non-adrenergic non-cholinergic (NANC) neurons that trigger smooth muscle relaxation (Martinez *et al.*, 2000; Langlois, 2003). These neurons release three neurotransmitters, NO, VIP and ATP. NO and ATP mediate rapid, short-lived muscle relaxation, while VIP causes slow, sustained relaxation of smooth muscle. Thus, VIP is responsible for long-duration relaxation of the proximal stomach in mammals (Curro *et al.*, 2008). NO, in contrast, causes a very short-lived relaxation as a result of its short half-life. Thus, while NO and ATP are essential for normal stomach motility, prolonged relaxation requires the presence of VIP.

The innervation of the proventriculus has been well described in chickens (Martinez *et al.*, 2000). As in other regions of the GI tract, it has both myenteric and submucosal plexuses. Within the myenteric plexus there is a subset of neurons that contain the neural isoform of nitric oxide synthase (nNOS) and VIP (Huang *et al.*, 1993). Gastric inhibitory peptide, a peptide that inhibits gastric motility, is also present in vascular fibers in contact with blood vessels and in the glandular epithelium (Thomas *et al.*, 1979). Proventricular neurons do not appear to contain somatostatin, bombesin, met-encephalin, serotonin, substance P, galanin or S-100 protein (Martinez *et al.*, 2000).

NO is thus the key neurotransmitter that mediates relaxation of the proventricular smooth muscle, while VIP is produced in parallel with nNOS. Thus, it is possible that PDD could result from excessive or prolonged activities of the NANC neurons in the myenteric plexus leading to the release of NO and VIP. Given however the destructive nature of the lesions within the bornaviral-infected myenteric plexus, it is unlikely that excessive neuronal activity is a major contributor to the dilatation process.

It could be argued that the flaccidity observed in PDD could also result from selective destruction of the cholinergic neurons within the proventricular myenteric plexus. Bornaviral encephalitis in Lewis rats has been shown to be associated with a reduction in the cholinergic activity of the brain (Gies *et al.*, 1998, 2001). This reduction is due to a loss of choline acetyltransferase from the cerebral cortex and hippocampus. This also reflects the loss of neurons in these areas. This reduction can be detected in the pre-encephalitic stage prior to T cell infiltration into the brains (Gies *et al.*, 1998). Given the relatively low numbers of cholinergic neurons in the avian proventriculus (Mirabella *et al.*, 2003), it is unlikely that this is the most significant cause of dilatation. It should also be pointed out that the ganglionic lesions in PDD do not show selective neuronal destruction – they show destruction of all ganglionic neurons (Mannl *et al.*, 1987).

Proventricular outlet obstruction

Liquids within the proventriculus normally would be expected to drain into the ventriculus even if the proventricular walls are flaccid (Langlois, 2003). The fact that some PDD cases have a fluid-filled organ indicates that in such cases downstream flow must be completely blocked as a result of outlet obstruction.

A complex cycle of gastric motility (including the proventriculus) in the chicken and turkey is coordinated by a 'pacemaker' located within the myenteric plexus of the isthmus (Chaplin and Duke, 1990; Hall and Duke, 2000) (Figs. 5 and 7). The sequence of the gastroduodenal cycle is normally, in order, ventricular thin muscle contraction, duodenal contraction, ventricular thick muscle contraction and finally proventricular contraction (Hall and Duke, 2000). Should this sequence become uncoordinated, perhaps by damage to the driving

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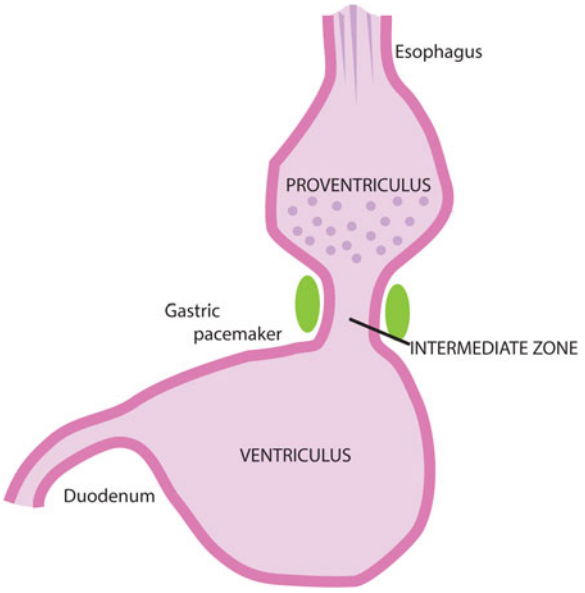

Fig. 7. The location of the gastric 'pacemaker' within the intermediate zone (isthmus) dividing the proventriculus and ventriculus. Ablation of nerve cells in this region leads to proventricular dilatation.

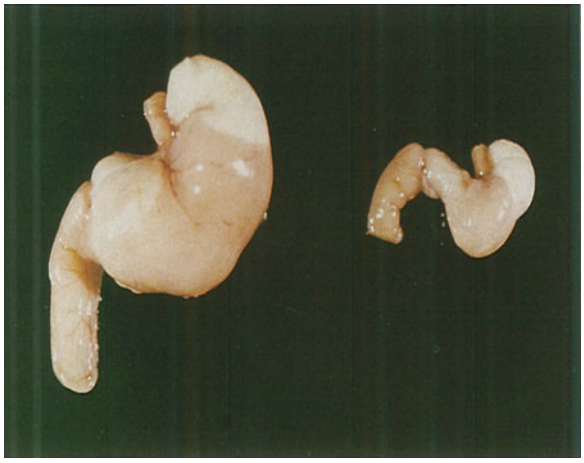

Fig. 8. Photographs of the stomachs of a homozygous mutant nNOS-knockout mouse (Left) and that of an age and size-matched wild-type mouse (Right), immediately following removal (with permission Cell Press). From Huang *et al.* (1993).

pacemaker cells located within the isthmus, then the passage of food from the proventriculus will slow significantly. Complete ablation of this network by application of 1% benzalkonium chloride to the isthmus significantly slows contractions in the ventriculus and duodenum, and almost completely abolishes proventricular contractions after 5 days (Hall and Duke, 2000). As a result of this ablation, the proventriculus and isthmus dilate (Chaplin and Duke, 1990). Note that this dilatation does not develop instantly. Rather, prolonged incoordination of the gastroduodenal contraction sequence leads to a chronic failure to completely empty the proventriculus and eventual gross dilatation.

Additionally, any disturbance in nitrergic signaling from NANC neurons in the esophagus and stomach can result in achalasia, gastroparesis and slow transit disorder (Lies *et al.*, 2014). For example, nNOS-knockout mice develop dilation of the stomach and hypertrophy of the pyloric sphincter (Huang *et al.*, 1993). The stomachs from homozygous nNOS-knockout mice ranged from 1.5 to 3 times normal in linear dimensions corresponding to a 3–27-fold increase in volume (Fig. 8). As the animals aged, the stomachs lost their interior folds and underwent thinning of the walls (Huang *et al.*, 1993). This dilatation was clearly the result of gastric outlet obstruction. Ablation of enteric plexuses in a mouse model is accompanied by a decline in the expression of neuronal VIP- and NO-production and therefore results in local smooth muscle contraction and blockage (Aube *et al.*, 2006). This hypothesis is consistent with the apparent complete destruction of neurons within the myenteric plexus in cases of PDD (Mannl *et al.*, 1987).

It should also be pointed out that in some cases, PDD-associated dilatation may extend further along the GI tract and result in thinning and dilatation of the duodenum. This may result from downstream blockage within the small intestine, perhaps associated with interference/absence of enteric neuronal reflexes.

Failure to regulate proventricular filling

The vagus nerve probably plays a key role in the dissemination and pathogenesis of avian bornaviral disease. It links the brain and proventriculus such that bornaviruses may readily spread from brain to the gut via the intra-axonal route (Langlois, 2003). Nonetheless, it is clear that proventricular dilatation cannot simply be a result of vagal blockage/damage. Bilateral vagotomy at the level of the proventriculus in chickens or turkeys neither fails to alter food intake, nor does it result in the development of proventricular dilatation but merely in a slight slowing of the passage of food through the anterior GI tract (Savory and Hodgkiss, 1984; Hall and Duke, 2000). Complete sectioning of the vagus and two gastric branches of the sympathetic nerve have no effect on the initiation of gastric contractions. The significant lesions that result in PDD almost certainly occur within the neurons of the myenteric plexus (Goldstein and Nagy, 2008).

The proventriculus serves as a reservoir that regulates the flow of food into the ventriculus and duodenum (Langlois, 2003). It relaxes as it fills, thus keeping the intragastric pressure low even when it contains large amounts of food. This is called receptive relaxation and is mediated by a vagal reflex (Curro *et al.*, 2008). This relaxation is maintained by another reflex acting through mechanoreceptors in the stomach wall. This is called adaptive relaxation and involves both intrinsic and extrinsic vagal pathways (Curro *et al.*, 2008).

In the proximal stomach of mammals, the hormones cholecystokinin (CCK), gastrin and secretin inhibit contractility, decrease intragastric pressure and slow gastric emptying (Thomas *et al.*, 1979). Gastric inhibitory polypeptide, and VIP

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also slow gastric emptying because they inhibit proximal gastric contractions. CCK regulates food intake and delays gastric emptying. Such a delay could result in gastric distention as the animal continues to eat. Distention signals are carried to the brain by the vagus nerve and the integrity of the vagus nerve is essential for CKK-mediated distention (Shillabeer and Davison, 1987). Satiety signals also are generated by luminal nutrients and bacteria that signal through enteroendocrine cells (Bohorquez *et al.*, 2015). These cells communicate with enteric nerve cells through a cytoplasmic process called a neuropod (Bohorquez *et al.*, 2015). This allows food and the microbiota to interact with the nervous system (and probably serves as a portal of entry of viruses into the body). Vagal stimulation as a result of gastric distention also signals through the solitary tract nucleus to cause upregulation of the melanocortin pathway which then suppresses appetite (Tome *et al.*, 2009). It is perhaps relevant to note that the uncontrolled appetite and resulting obesity in experimental Borna disease in some mice appears to be due to destruction of the melanocortin-receptor neurons (Herden *et al.*, 2000).

Thus a failure to transmit or receive satiety signals could result in unregulated filling of the proventriculus and its subsequent dilatation. This pathway is unlikely to be the primary cause of the uncontrolled proventricular dilatation in birds, as observation suggests that they cease eating early in the disease process (Hoppes *et al.*, 2013). In addition, bilateral vagotomy in chickens and turkeys appears to cause no more than a short-term suppression of food intake (Savory and Hodgkiss, 1984).

Lead poisoning

Proventricular dilatation is a common sequel to lead poisoning in waterfowl (Kubota *et al.*, 1994). Studies on its mechanism of action may be relevant to the pathogenesis of PDD. Lead causes inhibition of the proventricular smooth muscle contractions induced by either vagal stimulation or externally applied ACh in a chick smooth muscle preparation (Kubota *et al.*, 1994). The vagally evoked contractions are much more sensitive to lead than the responses to ACh. The inhibitory effect is greatest when the frequency of stimulation or the dose of ACh is reduced. Thus, the lead model suggests that the proventricular dilatation in lead poisoning results from both pre- and post-synaptic interference with vagal signaling. Toxic neuropathies are a common effect of chronic lead poisoning (Thomson and Parry, 2006). Lead inhibits the release of ACh at the neuromuscular junction as well as in sympathetic ganglia. As little as 4 ppm of lead can lead to the development of vagal lesions and block vagal transmission in ducks (Kubota *et al.*, 1994).

Human models of dilatation

Idiopathic achalasia

Much more is known regarding the pathogenesis of human esophageal or gastric dilatation than in birds. The most important proximal cause of achalasia is gastroesophageal obstruction. Surgically induced obstruction results in esophageal aperistalsis and achalasia. There have been claims of possible associations between infections such as measles or varicella zoster and achalasia, but there is no consistent causal association between specific viral infections and disease onset. An autoimmune etiology has also been proposed (Park and Vaezi, 2005). The loss of innervation of the achalasic esophagus may be due to defects in extrinsic or intrinsic neuroregulation. Extrinsic causes of achalasia may result from lesions in the brain stem neurons, resulting in vagus nerve abnormalities. However, as vagotomy in humans, as in birds, does not result in achalasia or PDD, such extrinsic abnormalities are likely to be relatively unimportant. Intrinsic causes are of much greater significance (Park and Vaezi, 2005).

Idiopathic achalasia in people is characterized by a loss of inhibitory nitrergic neurons in the esophageal myenteric plexus. This results in gross dilatation of the esophagus (megaesophagus). The myenteric plexus in the affected esophagus is surrounded by a lymphocytic inflammatory infiltrate consisting mainly of CD8+ T cells. There is a total loss of neurons and a significant loss of nerve fibers (Raymond *et al.*, 1999). Thus, this disease bears a resemblance to PDD. VIP-containing neurons, normally present in the esophageal myenteric plexus, are absent in patients with achalasia and nitrergic neurons are also reduced or absent in these cases (Aggestrup *et al.*, 1983; De Giorgio *et al.*, 1999). Mearin *et al.* have also demonstrated a complete absence of NO synthase activity at the gastroesophageal junction in patients with achalasia. As a result, there is a failure of relaxation at this site (Mearin *et al.*, 1993). The number of nitrergic neurons is also significantly reduced, most likely due to a loss of NANC neurons. This loss is associated with lymphocytic infiltration of the myenteric ganglia (Paterson, 2001). The predominant infiltrating cells within the plexus are T cells with some eosinophils and smaller numbers of plasma cells and mast cells (Raymond *et al.*, 1999; Paterson, 2001). Nevertheless the relationship between lymphocytic infiltration and destruction and loss of nitrergic neurons remains unclear as causation has not been proven. It should also be noted that VIP exerts immunosuppressive effects on the functions of neutrophils and eosinophils by inhibiting NF-κB pathways (Smalley *et al.*, 2009). VIP inhibits LPS-induced inflammatory pathways in monocytes and macrophages and reduces their ROS production. VIP also causes dendritic cells to develop an inhibitory phenotype and secrete IL-10 and TGFβ (Smalley *et al.*, 2009). Thus, a loss of VIP-ergic neurons could permit inflammation to develop within the ganglia as a result rather than a cause of neuronal cell death.

Some patients with achalasia have autoantibodies against the myenteric plexus and inflammatory T cell infiltrates may be present within the myenteric plexus. Case control studies using immunofluorescence assays suggest that there is a higher prevalence of such antibodies in achalasia patients than in healthy controls (Park and Vaezi, 2005). However many achalasia patients lack these autoantibodies and very few have antibodies specifically directed against nitrergic neurons (Moses *et al.*, 2003).

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Infantile pyloric stenosis

An inherited disorder, infantile pyloric stenosis affects 0.3% of newborns (Spicer, 1982). Pyloric obstruction develops in these infants at about 3 weeks of age and results in gross distention of the stomach. Histochemical staining of tissues from affected infants showed that NADPH-diaphorase was absent from the enteric neurons that activate the circular muscle of the pylorus (Vanderwinden *et al.*, 1992). As a result these cells cannot generate NO, the pyloric muscles cannot relax, resulting in pylorospasm and gastric outlet obstruction.

Gastric ileus

Gastric ileus following abdominal surgery is thought to be initiated by autonomic reflexes that impair gastric motor function by inhibiting ICC (O'Grady *et al.*, 2014). However, ileus may also occur in response to an endotoxemia (Buchholz *et al.*, 2009). Thus bacterial lipopolysaccharides (LPS) reduce intestinal motility by inhibiting ACh evoked contractions in intestinal smooth muscles. This reduction in motility is mediated through TLR4 by an NF-κB-mediated pathway. LPS does not induce gastric ileus in TLR4-deficient mice (Buchholz *et al.*, 2009). Mice treated with LPS show a delay in gastric emptying associated with higher levels of NF-κB. Activation and translocation of NF-κB in myenteric plexus cells have been reported in LPS-treated mice. This results in the generation of ROS, increased IL-6 and TNF-α and overproduction of NO by way of leukocyte-iNOS production (Hernandez *et al.*, 2011). This overproduction of NO may account for the gastric relaxation that occurs in ileus. Alternatively, immune cell-derived cytokines TNF-α, IFN-γ and IL-1β may modulate motility either directly or indirectly through ICC (Bashashati and McCallum, 2015). It is interesting to note that the N-protein of BoDV-1 possesses an amino acid sequence that inhibits the NF-κB pathway (Makino *et al.*, 2015). However, the results of the study by Hernandez *et al.* suggest that BoDV N-protein may counteract the effects of LPS on the gut (Hernandez *et al.*, 2011). PDD is unlikely to be mediated by this TLR4–NF-κB-mediated pathway.

Diabetic gastroparesis

Another human condition that may be of relevance to PDD is diabetic gastroparesis, a complication of long-standing diabetes mellitus. It is defined as the over-slow emptying of solid food from the stomach (Horvath *et al.*, 2014). Patients have a mild lymphocytic infiltration in the myenteric plexus (CD45- and CD68-positive cells). This results in damage or loss to the ICC, a decrease in the number of nitrergic neurons and a reduction in the intraneuronal levels of NO (Huizinga and Chen, 2014; Bashashati and McCallum, 2015). The nitrergic neurons play a key role in regulation of motility and control the appropriate relaxation of sphincters.

Chagas disease

Megaesophagus commonly develops in chronic Chagas disease in response to infection with *Trypanosoma cruzi* (Nascimento *et al.*, 2013). The esophagus is grossly dilated and there is significant hypertrophy of the muscular wall. There is a reduction in the number of neurons in the enteric plexuses together with a ganglionitis. The ganglia are infiltrated with CD3+ CD4+ T cells, CD8+ cytotoxic T cells, B cells and NK cells. Studies on the neurotransmitters in affected tissues show an increase in the neurotransmitter substance P and a decrease in VIP. The loss of VIP correlates with denervation (Nascimento *et al.*, 2013). However, VIP is immunosuppressive (Smalley *et al.*, 2009). Thus a loss of VIP-producing neurons could permit excessive T-cell activity within the ganglia. Consequently, destruction of the colonic ganglia results in loss of intrinsic control leading to lethal Chagas disease (Furness, 2008).

The gut–brain axis

It would be naïve to suppose that the gut microbiota plays no role in the pathogenesis of PDD. Early life changes in the intestinal microbiota affect neurodevelopment (Borre *et al.*, 2014). The gut–brain axis is a complex communication network between the two systems that modulates both the GI tract and the central nervous system (CNS). The vagus nerve, a nerve readily invaded by bornaviruses and central to the pathogenesis of PDD, can receive signals from the microbiota and pass them to the brain and vice versa (McVey Neufeld *et al.*, 2015). Relevant to this review is the existence of an inflammatory reflex that links inflammation in the GI tract to the brain by way of the vagus nerve (Tracey, 2016). Thus cytokines as well as pathogens and tissue damage signal to the brain via the vagus. The brain stem nuclei then return the signal via the vagal afferents to the spleen where they modulate splenic nerve activity and activate T cells causing them to produce ACh. This reflex also suppresses TNF, IL-1 and HMGB-1 production and hence is anti-inflammatory (Tracey, 2016). Should the reflex arc be blocked by neuronal destruction in the brain stem nuclei, then ACh production would be reduced and intestinal smooth muscle relaxation enhanced. Certain probiotics also affect the brain through vagal signaling (Perez-Burgos *et al.*, 2013). In experimental autoimmune encephalomyelitis induced by T cells, there is evidence for the simultaneous presence of intestinal barrier dysfunction. This dysfunction precedes the development of neurologic disease (Nouri *et al.*, 2014). Administration of encephalitogenic T cells to healthy mice also leads to intestinal barrier changes. So there is an association between brain disease and gut permeability. It has been suggested that the common mechanism involves dysfunction of the intercellular junctions between adjacent enterocytes.

The microbiome also influences brain development and function and thus can affect behavior (Sampson and Mazmanian, 2015). Mice given the probiotic *Lactobacillus rhamnosus* display a

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decrease in anxiety behavior. This effect is ameliorated by vagotomy. Thus microbial signals are directed to the CNS by the vagus (Bravo *et al.*, 2011). The great variations in the development and severity of PDD lesions in different birds may well be determined in part by the microbiota within the dilated proventriculus.

Bornaviral enteric lesions in other species

BoDV-1 infected mammals may also develop enteric lesions. For example, horses show GI signs in addition to their encephalitis (Bode *et al.*, 1994; Ludwig and Bode, 2000; Richt *et al.*, 2000; Pfannkuche *et al.*, 2008). Recurrent colic or constipation are common during the prodromal phase of equine Borna disease. Thus, enteric neurons may well be a target in horses. Enteric neurons differ in the neurotransmitters they release (Brookes, 2001b). ACh is released from excitatory motor neurons, from secretory neurons, from some interneurons and from intrinsic primary afferent neurons (McConalogue and Furness, 1994). A rat-adapted bornavirus isolate that originated in a horse has been studied by Pfannkuche *et al.* They infected 4-week-old Lewis rats by the intracerebral route and sacrificed them 4–14 weeks post-infection. The rats did not show intestinal dysfunction but BoDV-positive neurons were found in the submucosal and myenteric plexus of their proximal colons. The virus was not detected in the submucosa. In the myenteric plexus, BoDV appeared to target neurons that were reactive for choline acyltransferase (Pfannkuche *et al.*, 2008).

Cholinergic primary afferent neurons contain the calcium-binding protein calbindin D-28k (CALB) (Eisenman *et al.*, 1999). CALB is present in 96% of the submucosal and 67% of the myenteric neurons. The number of CALB immunoreactive neurons is significantly higher in the myenteric plexus of bornavirus-infected rats compared to controls. Thus BoDV infection is specific to a subpopulation of cholinergic enteric neurons that may serve as a reservoir site for BoDV (Eisenman *et al.*, 1999). Cholinergic neurons are a main target for BoDV and the number of cholinergic neurons decreases in the brain during BoDV infection (Gies *et al.*, 2001). There is a selective loss of CALB-positive neurons in the hippocampus in BoDV infections (Mayer *et al.*, 2005), even though BoDV may upregulate CALB in the myenteric plexus. Thus, in infected rats, 29% of enteric neurons express CALB, while in control rats only 15% of neurons are CALB+. BoDV upregulates CALB expression, and CALB effectively protects many cell types against apoptotic death (Christakos *et al.*, 2003).

Autoimmunity

In 2006, Rossi *et al.* suggested that PDD results from an autoimmune response to brain gangliosides (Rossi *et al.*, 2008). They proposed that its pathogenesis was similar to that of Guillain–Barre syndrome in mammals. The discovery of avian bornaviruses in 2008 resulted in these authors modifying their

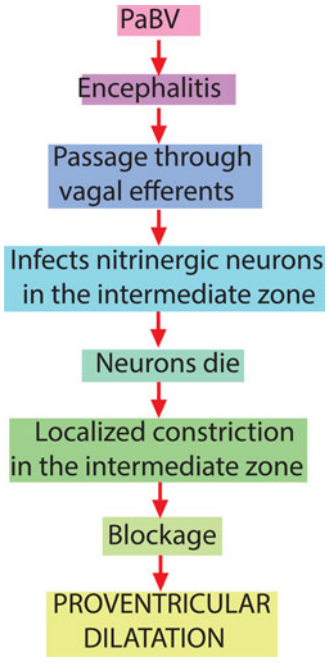

Fig. 9. A postulated pathway for the induction of proventricular dilatation in birds infected with avian bornaviruses.

hypothesis to suggest that the bornavirus triggered the production of anti-ganglioside production. While initially attractive, insufficient data have been published to date to confirm the hypothesis. Their original studies suggested that immunization of birds with gangliosides result in the development of PDD-like lesions. We have repeated their studies in both chickens and Quaker parrots and both fail to generate any lesions resembling PDD (Leal *et al.*, unpublished observations).

Guillain–Barre syndrome has been described in chickens and does not resemble PDD in psittacines. The chickens develop a spontaneous paralysis within 12 days. Animals display hind limb weakness, difficulty in standing or walking, wing- and head-droop and difficulty feeding. Li *et al.* described lesions that were largely associated with degeneration of the sciatic nerve (Li *et al.*, 1996). In only one of Li's cases was there a mild mononuclear infiltration in the nerve, but otherwise lymphocyte infiltration was absent. Some nerve fibers underwent demyelination and Wallerian-like degeneration. Rossi *et al.* have also suggested that the presence of Campylobacter in the bird intestine is necessary for the development of PDD (Ang *et al.*, 2010). However, our studies on the psittacine gut microflora have failed to detect Campylobacter in the feces of Scarlet macaws or cockatiels suffering from PDD (Tizard I, unpublished observations).

We have however, tested sera from parrots infected with bornaviruses for the presence of autoantibodies to brain antigens. Of 12 sera examined, three contained antibodies reactive with normal brain tissue by Western blotting. Two birds had antibodies to a 40 kDa protein, while one bird had antibodies to myelin basic protein. The presence of these antibodies was not clinically significant, a situation resembling that in human bornaviral disease. One of the human patients that developed

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fatal encephalitis caused by VSBV-1 also had anti-Yo (Purkinje cell) autoantibodies in their cerebrospinal fluid (Hoffmann *et al.*, 2015; Jarius and Wildemann, 2015). The authors suggested that this was an epiphenomenon resulting from viral neuronal lysis. It is also of interest to note that anti-neuronal autoantibodies have been detected in human cases of idiopathic achalasia (Moses *et al.*, 2003). However, there is no correlation between intensity of staining and disease severity or duration. Moses *et al.* concluded that this is a consequence and not a causative factor (Moses *et al.*, 2003). Storch *et al.* also detected autoantibodies against the myenteric plexus in human achalasia patients (Storch *et al.*, 1995). While they found a higher prevalence of these antibodies in achalasia patients than in healthy patients, they too could not demonstrate an etiological role.

Conclusions

Human achalasia likely results from loss of inhibitory nitrergic neurons. Proventricular dilation in lead-poisoned birds also results from a loss of nitrergic neurons. Knocking out nNOS in enteric neurons of mice results in gastric dilatation. In addition, from our knowledge of the innervation of the proventriculus and the ventriculus, the major neuronal population within the myenteric ganglia are nitrergic cells. Based on this, a coherent case may be made that proventricular dilatation in bornavirus-infected birds is mainly a consequence of the loss of nitrergic neurons in the esophagus/isthmus (Fig. 9). Upregulation of GFRA1 may also contribute to this process. As a result of segmental constriction and chronic blockage, food accumulates within the proventriculus and forces that organ to dilate. Excessive dilatation is unlikely to result in the accumulation of fluid within the esophagus and would require that cholinergic neurons be destroyed or nitrergic neurons overstimulated. Based on our knowledge of similar dilatation syndromes this is unlikely. A failure of satiation signals to provoke excess proventricular dilatation cannot be ruled out but seems at odds with the observed loss of appetite in sick birds.

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