CEVA HANDBOOK
OF POULTRY DISEASES

5 RUNTING & STUNTING SYNDROME

RUNTING & STUNTING SYNDROME

DEFINITION

Runting and Stunting Syndrome (RSS) affects chickens and turkeys at a young age, and is characterised by heterogeneous growth development of the flock, with retarded growth following caused by feed utilisation and diarrhoea

HISTORY AND SYNONYMS

According to Zavala & Sellers (2005) the term "runting-stunting" is not well defined and may yet be altered in the future depending on the etiological agent(s) associated with the state. In poultry terminology, "runt" means a bird which is smaller than its flock mates. "Stunting" is associated with subjects with retarded growth. Therefore, the authors defined "runting-stunting" as a syndrome in which some of the birds within a flock appear considerably smaller due to a lower growth rate. Most birds of unusually smaller size present a lower average live weight.

RSS has been known for many years, and was first described in the 1970s. Since that time, it has been reported in different parts of the world (Olsen, 1977; Kouwenhoven et al., 1978). Due to the lack of a definite etiological agent, the term is often used as a synonym for malabsorption syndrome (Rebel et al., 2006), infectious stunting syndrome (Bracewell et al., 1981), broiler runting syndrome (McNulty et al., 1984), brittle bone disease (Van der Heide et al., 1981) and helicopter syndrome (Kouwenhoven et al., 1978).

Kouwenhoven et al. (1992) have described a problem in broilers appearing at 3 weeks of age with signs similar to those of RSS, called "wet litter syndrome" (WLS).

The clinical signs and the pathology of both states are almost identical. Due to the fact that the authors succeeded in inducing RSS in one-day-old broiler chicks by using WLS homogenate, the wet litter syndrome was believed to be a partial manifestation of RSS.

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

RSS in chickens is a transmissible disease of unknown etiology (Kang et al., 2012). Nevertheless, the RSS-specific clinical signs and microscopic lesions have been reproduced by oral inoculation of filtered and non-filtered intestinal homogenate from infected chickens (Montgomery et al., 1997; Songserm et al., 2000; Songserm et al., 2002).

Although bacterial and environmental factors may contribute to development of the disease (Otto et al., 2006; Rebel et al., 2006), electron microscopy studies and the possibility of recreating the disease state using a bacteria-free filtrate suggest the involvement of small, round non-enveloped viruses as the most probable etiological agents (Sellers et al., 2010). Since RSS has been acknowledged, reovirus (Songserm et al., 2000), rotavirus (Otto et al., 2006), astrovirus (Baxendale & Mebatsion, 2004) and parvovirus (Kisary et al., 1984) have been identified or isolated in affected birds. The latter research team described the presence of parvovirus in the intestines of chickens with RSS. The isolated agent, named ABU, was indeed capable of inducing delayed growth. Other authors, however, have found that the experimental infection of broiler chickens with a reovirus or ABU parvovirus did not result in any signs specific for RSS (Decaesstecker et al., 1986).

Enterovirus-like viruses, whose group is still not well-defined, also play a role in the appearance of enteric diseases.

Until 2000, astroviruses belonged taxonomically to this group (Imada et al., 2000). Astroviruses are associated with gastrointestinal disturbances in different bird and mammalian species (Kang et al., 2012). A reovirus antigen has been detected in epithelial cells in the middle areas and the upper third of intestinal villi of chickens affected by RSS (Songserm et al., 2000).

Through in situ hybridisation (ISH), astrovirus RNA has been detected in villous epithelial cells at those sites (Koci et al., 2003). ISH has also revealed astrovirus-positive cells in the intestinalcrypts of infected turkeys.

The role of these viruses in chickens affected by RSS has not yet been elucidated. They may, in addition, cause RSS in combination with other viruses or unknown factors.

The pathogenicity and target cell predilection of the viruses may differ. It is not clear which of these immune suppression factors would manifest its effects first.

Bearing in mind the early onset of signs, most probably the agents causing clinical RSS induce immuno-suppression by themselves (Sklan, 2001).

The complex etiology of RSS may impede the development of control strategies. New astroviruses have recently been reported in RSS-affected chickens (Sellers et al., 2010). The key to defining the etiology of RSS and elucidating its pathogenesis may lie in the role of the astroviruses and other small round viruses. Microscopic lesions of immune organs and the effect of RSS on live weight have been investigated 12 days after exposure of one-day-old commercial broiler chickens to RSS-contaminated litter.

In the course of this experiment, nucleic acids from three astroviruses and one parvovirus were detected by ISH 1 to 5 days after exposure. The live weight of the RSS-infected group was 70% lower than that seen in the non-infected control subjects. ISH results clearly demonstrated the replication of three astroviruses (chicken astrovirus, avian nephritis virus: ANV-1 and ANV-2) in the duodenum. Nucleic acid of chicken astrovirus was detected during the first two days after the exposure, while nucleic acids of ANV-1 and ANV-2 persisted over several days. Parvovirus-specific nucleic acids were not found in this study. On the basis of simultaneous findings of viral RNA and duodenal lesions, the research team concluded that astroviruses probably play a significant role in RSS (Kang et al., 2012). The authors have emphasized the importance of three members of the Astroviridae family, as well as the possible involvement of immune-compromise in relation to this disease.

It was initially thought that reoviruses were the main etiological agent of RSS after their identification in affected chickens (Kouwenhoven et al., 1988). The typical intestinal lesions of RSS were reported in SPF chickens after reoviral infection (Goodwin et al., 1993). Other evidence, however, suggests that vaccination of breeder flocks against reovirus infection does not reduce the severity of RSS in the affected progeny (Eidson et al., 1985). Viruses from various families Adenoviridae, Parvoviridae and Togaviridae (Kouwenhoven et al., 1978; McNulty et al., 1984; Zsak et al., 2008) were also believed to be

involved in RSS etiology. Ultimately, the exact etiology of the disease has not yet been confirmed and the multi-agent origin of the syndrome is still maintained (Rebel et al., 2006).

The experimental infection of SPF chickens and broiler chickens with reoviruses did not result in any clinical signs or retarded growth despite the replication of viruses, whereas the inoculation with entero-like viruses and field material yielded abnormal consistency of faeces and delayed growth (Decaesstecker et al., 1986). Other researchers have linked reoviral infection of chickens with signs of malabsorption syndrome (MAS) and immunosuppression (Sharma et al., 1994).

The enteropathogenicity of avian reovirus isolates (ARVs) from MAS-affected chickens from the Netherlands and Germany was investigated by experimental inoculation of 7 different virus strains to one-day-old SPF white leghorn chickens. The resulting pathogenicity was compared to that of 2 ARV tenosynovitis isolates: reference S1133 strain and a Dutch strain. Despite the different extent of disease severity, all reoviruses were shown to induce vacuolar degeneration and small intestine epithelium desquamation between post inoculation (PI) days 2 and 7.

The most severe intestinal lesions by PI day 2 were caused by two Dutch and one German ARV isolates, which were subsequently used in a follow-up experiment in SPF broilers. The infection with these reoviruses did not result in weight loss, although the small intestine lesions observed between PI days 1 and 4 were stronger, or more serious than those in white leghorns. As early as the first PI day, one of inoculated groups exhibited apical denuded villi. Seven days after the inoculation of broilers, the appearance of the small intestines was normal. In both white leghorns and broiler chickens, the cytoplasm of enterocytes in the upper and middle parts of affected villi contained reoviral antigens.

The pathogenesis of ARV infection was investigated by comparing a large number of these cells of jejunal villi in one infected and one control group of chicks. The main conclusion was that reoviruses induced intestinal lesions, but did not reduce the live weight of broiler chickens by themselves.

Therefore, the importance of ARVs in the etiology of MAS must be placed in context with the effect of other agents or substances (Songserm et al., 2003).

Recently a new strain of avian orthoreovirus, AVS-B, associated with runting-stunting syndrome in broilers has emerged.

Its discoverers believe that the genetic heterogeneity of the AVS-B genome is a result of mutations (Banyai et al., 2010).

It is still unknown whether the signs of RSS or its synonymous states are pathogenetically due to maldigestion, malabsorption or both. Nor is the role of endocrine glands fully understood. Despite a large number of experiments performed on RSS broiler chickens, the significance of the thyroid function for gastrointestinal events is not yet clear (Rudas et al., 1986).

Other factors which could induce RSS and influence its severity, include the genetic background of broilers, the state of one-day-olds, the feed and the climate (Songserm et al., 2000). In Australia, the role of selenium deficiency in RSS etiology has been studied in broiler chickens. It was demonstrated that dietary selenium deficiency resulted in signs of exudative diathesis and a marked reduction of blood plasma glutathione peroxidase activity, but not in pancreatic atrophy with subsequent fibrosis, nor in increased plasma amylase activities – other RSS-associated findings. Also, in observed RSS outbreaks, there were no substantial differences between infected and healthy birds in terms of the selenium concentrations found in analysed tissue samples. Evidence suggests that pancreatic atrophy and plasma amylase elevation precedes the reduction of blood glutathione peroxidase in cases of RSS (Smart et al., 1985).

EPIDEMIOLOGY

Enteric diseases in poultry are of primary significance due to the substantial economic losses they entail for the industry. Numerous experiments have been conducted in order to detect vertical transmission. The existence of vertical transmission or the possible risk for infection or maternal antibody transmission from parents to the progeny remains unclear. So far, researchers are not able to make any association between breeder flocks and their progeny.

As noted in the previous section, RSS symptoms and/or lesions have been repeatedly reproduced in chickens through filtered or non-filtered homogenates from gastrointestinal content or litter.

CLINICAL SIGNS AND PATHOLOGY

While RSS symptoms can vary greatly, the peak mortality is most commonly observed by 11 days of age (Clark & Jones, 2008). Chickens affected by RSS may be found gathered around feeding or watering troughs or persistently pecking at walls. Feed consumption is usually reduced. Sometimes, the disease may affect a major part of the flock and a significant proportion of those chickens surviving the illness may never recover. Often, there are no changes in the acceptable normal daily mortality rate. The flock uniformity percentage, however, may be as little as half the acceptable level of 70%. Some affected birds display retarded feather growth, with feathers appearing curled. This is particularly obvious for flight feathers on the wings (helicopter disease) (Zavala & Sellers, 2005). The colour of the legs and the beak may be less intense (pale bird syndrome), rickets or more fragile legs may also be detected (brittle bone disease) (Rebel et al., 2006).

Fig.1

Significantly retarded growth of chickens affected by RSS compared to their healthy flock mates.

At necropsy, the most pronounced findings are alterations in the small intestine, which is distended with a thinner, semi-transparent wall. Upon incision, the lumen is found to contain a large amount of fluid and undigested feed. In RSS, the normal intestinal development of the jejunum, where the major part of feed digestion and absorption occurs, is impaired (Rebel et al., 2006). Common features in descriptions of the gross lesions of 2-week-old broiler chickens with RSS in Australia are pancreatic atrophy and fibrosis, which are also reported in different parts of the world (Smart et al., 1985). The result of atrophy, degeneration and repair processes in the pancreas is the reduction of digestive enzymes. The watery faeces moisten the litter and increase the risk from secondary infections (Zavala and Sellers, 2005). Frequently, at a macroscopic level the liver appears smaller and the gallbladder enlarged. The bursa of Fabricius and the thymus are initially of normal size, but in later stages are atrophied. The kidneys are moderately enlarged and filled with urates.

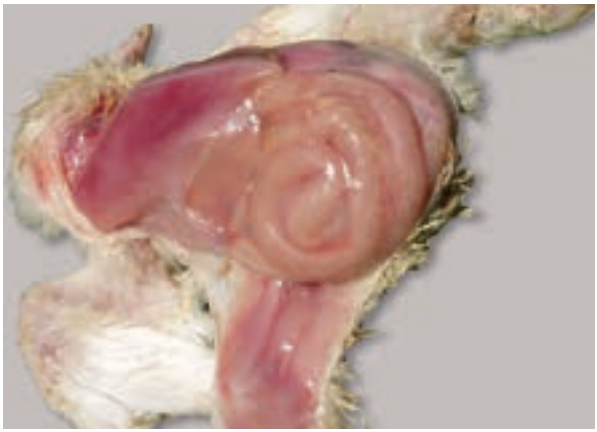

Fig.2

The small intestine is pale, dilated, often curled up into a ball in the caudal part of the pleuroperitoneal cavity and visible through the abdominal wall.

Fig.3

Upon incision, the lumen reveals a copious amount of fluid and undigested feed.

Fig.4

In some instances, the entire intestinal tract is affected, including the caeca. The intestinal walls are pale, semi-transparent, and the lumen if filled with watery content, sometimes mixed with gas.

Histopathological changes in birds affected by RSS are mainly observed in the small intestine. A common lesion in spontaneous and experimental RSS is cystic enteropathy (Otto et al., 2006; Sellers et al., 2010). The epithelial cells lining the crypts degenerate and desquamate into the lumen, forming cystic crypts lined by squamous epithelium. The severity of clinical signs depends on the extent of villous atrophy, which results from the loss of epithelium. Nevertheless, the actual cause and details of the pathogenesis of intestinal lesions remain unclear, although enhanced apoptosis among the epithelial cells lining intestinal villi and crypts has been observed. Approximately 2–3 weeks after experimental reproduction, repair of the covering epithelial was observed (Zekarias et al., 2005).

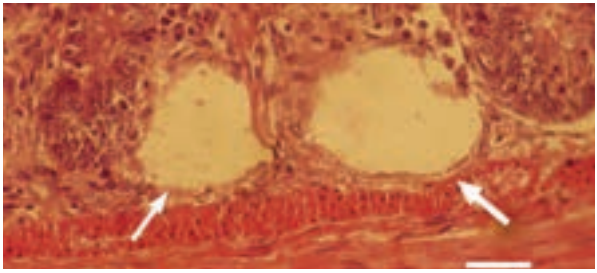

Fig.5

Appearance of cystic crypts (arrows) in duodenal mucosa. The primary cause may be retention resulting from the compression of the inflammatory proliferate in the mucous coating. H/E, Bar = 35 µm.

An interesting fact is that, on average, cystic lesions were detected in 80% of small intestines after 24-hour exposure, with the peak being attained after 4 days followed by decrease by the end of the astrovirus challenge infection period (Kang et al., 2012). A certain dynamic in the presence of lesions is described in existing research reports, with the first cystic lesions appearing 3 days after inoculation, but this was found to be due to the sampling protocol (Smart et al., 1988). The appearance of intestinal cystic lesions in the aforementioned study was followed by lymphocytic depletion of immunocompetent organs.

DIAGNOSIS

The tentative diagnosis of RSS is made on the basis of flock heterogeneity, diarrhoeic faeces and moistened litter during the first two weeks of life of broilers.
The presence of duodenal cystic lesions throughout the histological examination is not always a reliable index of RSS taken on its own, but can corroborate observed clinical signs and retarded growth.
Until recently, only the isolation of reoviruses in cell cultures was possible. Now, the advantages of molecular biology techniques such as PCR and RT-PCR provide valuable tools for the detection of viruses which are difficult to develop in culture (Sellers et al., 2010). Although coronaviruses may play a role in RSS, it is avian reoviruses and avian astroviruses that induce clinical signs, either solely or in combination.

DIFFERENTIAL DIAGNOSIS

Diagnosis should take into consideration the pathology status of chickens at an early age, manifested clinically and morphologically through diarrhoea and enteritis, resulting in retarded growth. This could be a result of enteritis caused by bacterial infections (Salmonella spp., E.coli etc.) or non-infectious illnesses (alimentary disorders, environmental factors etc.).

PREVENTION AND CONTROL

Biosecurity

Experience shows that RSS may suddenly appear and just as suddenly disappear. This complicates the task of establishing effective measures of control. Clark & Jones (2008) recommend focusing efforts for RSS control on three main priorities: biosecurity, proper management of poultry houses and farms, and vaccination. Biosecurity measures aim to

prevent the exposure of susceptible chickens to RSS. Generally speaking, they include the usual hygienic measures limiting the access of unauthorised persons and covering matters such as the collection and destruction of carcasses, maximum reduction of contacts with pests (rodents, wild birds, insects) etc.

Management procedures must ensure optimal handling of environmental threats, compliance with the all-in all-out principle and proper sanitation of poultry houses, including replacement of the litter before each repopulation of premises. The utilisation of acidifiers in drinking water helps to slow the spread of RSS. The use of oral antibiotics may limit the severity of disease and the additional involvement of bacterial pathogens.

Vaccines

So far, there is no effective, commercially-available vaccine for controlling the disease, due to the continued uncertainty regarding the etiological agents. Some reovirus strains, such as 1733 and 2408, have been used in vaccine development. According to some authors, while the vaccination of broilers against RSS may only be efficient in 50% of cases, the regular vaccination of breeders often results in a long-lasting immunity (Van der Heide, 2000).

Recently, a recombinant vaccine was described on the basis of a baculovirus transcribing a new astrovirus capsid protein (Sellers et al., 2010). A new viral sequence, probably belonging to a member of the Astroviridae family was identified in the gastrointestinal content of RSS-affected chickens. The protein was purified and used for production of an experimental vaccine in broiler breeders. The aim was to protect the progeny by providing the chicks with maternal antibodies. The presence of specific antibodies was detected by ELISA. The research team concluded that a partial protection against RSS could be attained in the progeny of a breeder flock, after triple immunisation with a recombinant astroviral capsid protein. The partial protection achieved in this experimental process is attributed to the possible role of other agents in the etiology of the syndrome. So far, this is the first report of a candidate vaccine inducing some kind of protection against RSS in chickens.

REFERENCES

- Banyai K., E. Dandar, K. M. Dorsey, T. Mato & V. Palya, 2011. The genomic constellation of a novel avian orthoreovirus strain associated with runting-stunting syndrome in broilers, Virus Genes, 42, 82–89.
- Baxendale, W. & Mebatsion, T., 2004. The isolation and characterisation of astroviruses from chickens. Avian Pathology, 33, 364-370.
- Bracewell, C.D. & P. Wyeth, 1981. Infectious stunting of chickens. Vet. Rec., 109, 64.
- Clark F.D. & Jones F.T., 2008. Runting-Stunting Syndrome in Broilers. (http://www.thepoultrysite.com/articles/1110/runtingstunting-syndrome-in-broilers).
- Decaesstecker, M., Charlier, G. & Meulemans, G., 1986. Significance of parvoviruses, entero-like viruses and reoviruses in the aetiology of the chicken malabsorption syndrome. Avian Pathology, 15, 769-782.
- Eidson, C.S., Kleven, S.H. & Fletcher, O.J., 1985. Performance of broiler progeny of breeder flocks vaccinated with inactivated oil emulsion malabsorption syndrome virus vaccine. Poultry Science, 64, 2081-2086.
- Goodwin, M.A., Davis, J.F. & Player, E.C. 1993. Reovirus-associated enteritis in Georgia broiler chicks. Avian Diseases, 37, 229-233.
- Imada, T., Yamaguchi, S., Mase, M., Tsukamoto, K., Kubo, M. & Morooka, A., 2000. Avian nephritis virus (ANV) as a new member of the family Astroviridae and construction of infectious ANV c DNA. Journal of Virology, 74, 8487-8493.
- Kang K.-I., M. El-Gazzar, H.S. Sellers, F. Dorea, S. M. Williams, T. Kim, S. Collett & E. Mundt, 2012. Investigation into the aetiology of runting and stunting syndrome in chickens, Avian Pathology, 41, 1, 41-50.
- Kisary, J., Nagy, B. & Bitay, Z., 1984. Presence of parvoviruses in the intestine of chickens showing stunting syndrome. Avian Pathology, 13, 339-343.
- Koci, M.D., Moser, L.A., Kelley, L.A., Larsen, D., Brown, C.C. & Schultz-Cherry, S., 2003. Astrovirus induces diarrhea in the absence of inflammation and cell death. Journal of Virology, 77, 11798-11808.
- Kouwenhoven, B., Davelaar, F.G. & Van Walsum, J., 1978. Infectious proventriculitis causing runting in broilers. Avian Pathology, 7, 183-187.
- Kouwenhoven, B., M. H. Vertommen & J.H.H. van Eck, 1988. Runting and leg weakness in broilers: involvement of infectious factors. Vet. Sci. Commun. 2, 253-259.
- Kouwenhoven, B., R.M. Dwars & J.E.M. Smeets, 1992. Wet litter and high feed conversions, a new problem in broilers. In: New and evolving virus diseases of poultry.
- M S. McNulty and J.B. McFarran, eds. In: Proc. Seminar European Commission, Brussels, Belgium. pp. 243-251.
- McNulty, M.S., G.M. Allan, T.J. Connor, J.B. McFerran & R.M. McCracken, 1984. An entero-virus-like virus associated with the runting syndrome in broiler chickens. Avian Pathol. 13, 429-439.
- Montgomery, R.D., Boyle, C.R., Maslin, W.R. & Magee, D.L., 1997. Attempts to reproduce a runting/stunting-type syndrome using infectious agents isolated from affected Mississippi broilers. Avian Diseases, 41, 80-92.
- Olsen, D.E., 1977. Isolation of a reovirus-like agent from broiler chicks with diarrhea and stunting. Proceedings of the 26th Western Poultry Diseases Conference, pp. 131-139. Davis, California.
- Otto, P., Liebler-Tenorio, E.M., Elschner, M., Reetz, J., Lohren, U. & Diller, R., 2006. Detection of rotaviruses and intestinal lesions in broiler chicks from flocks with runting and stunting syndrome (RSS). Avian Diseases, 50, 411-418.

- Rebel, J.M. J., Balk, F.R. M., Post, J., Van Hemert, S., Zekarias, B. & Stockhofe, N., 2006. Malabsorption syndrome in broilers. World's Poultry Science Journal, 62, 17-30.
- Rudas, P., G. Salyi & J. Szabo, 1986. Decreased thyroxine, triiodothyronine and 5'-deiodination levels in malabsorption syndrome (runting or stunting syndrome) in artificially inoculated broilers. Avian Dis., 30, 293-297.
- Sellers, H., Linneman, E., Icard, A.H. & Mundt, E., 2010. A purified recombinant baculovirus expressed capsid protein of a new astrovirus provides partial protection to runting-stunting syndrome in chickens. Vaccine, 28, 1253-1263.
- Sharma, J.M., Karaca, K. & Pertile, T., 1994. Virus-induced immunosuppression in chickens. Poult.. Sci., 73, 1082-1086.
- Sklan, D., 2001. Development of the digestive tract of poultry. World's Poultry Science Journal, 57, 415-428.
- Smart I.J., D.H. Embury, D.A. Barr, A.J. Sinclair, H. Karunajeewa, I. Ewing, R.L. Reece, W.M. Forsyth & P. T. Hooper, 1985. Absence of a Role for Selenium Deficiency in the Runting Syndrome of Broiler Chickens in Australia, Avian Diseases, 29, 4, 1201-1211.
- Smart, I.J., Barr, D.A., Reece, R.L., Forsyth, W.M. & Ewing, I., 1988. Experimental reproduction of the runting-stunting syndrome of broiler chickens. Avian Pathology, 17, 617-627.
- Songserm, T., Engel, B., van Roozelaar, D.J., Kok, G.L., Pijpers, A., Pol, J.M. & ter Huurne, A.A. 2002. Cellular immune response in the small intestine of two broiler chicken lines orally inoculated with malabsorption syndrome homogenates. Veterinary Immunology and Immunopathology, 85, 51-62.
- Songserm, T., Pol, J.M., van Roozelaar, D., Kok, G.L., Wagenaar, F. & ter Huurne, A.A., 2000. A comparative study of the pathogenesis of malabsorption syndrome in broilers. Avian Diseases, 44, 556-567.
- Songserm, T., van Roozelaar, D., Kant A., Pol, J.M., Pijpers A. Ter Huurne A., 2003. Enteropathogenicity of Dutch and German avian reoviruses in SPF white leghorn chickens and broilers, Vet. Res., 34, 285–295.
- Van der Heide, L., 2000. The history of avian reovirus. Avian Dis., 44, 638-641.
- Van der Heide, L., D. Liitticken & M. Hor-zinek, 1981. Isolation of avian reovirus as a possible etio-logic agent of osteoporosis («brittle bone disease»; «femoral head necrosis») in broiler chickens. Avian Dis., 25, 847-865.
- Zavala G. & Sellers H., 2005. Runting - stunting syndrome. The poultry informed professional, 85, 1-10.
- Zekarias, B., Stockhofe-Zurwieden, N., Post, J., Balk, F., van Reenen, C., Gruys, E. & Rebel, J.M., 2005. The pathogenesis of and susceptibility to malabsorption syndrome in broilers is associated with heterophil influx into the intestinal mucosa and epithelial apoptosis. Avian Pathology, 34, 402-407.
- Zsak, L., Strother, K.O. & Kisary, J. 2008. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathology, 37, 435-441.