

Field experiences with ERS type reovirus infections in diseased broilers reared under Western European field circumstances

Ervaringen uit het veld met ERS-type reovirusinfecties bij zieke vleeskuikens opgefokt onder West-Europese veldomstandigheden

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ABSTRACT

Between August 2001 and October 2006, enteric reovirus strain (ERS) infections were diagnosed in 21 Belgian broiler flocks. ERS isolates were derived either from liver, gastrocnemius tendon, bursa, pancreas, intestinal tract and trachea, or from pools of multiple organs.

The clinical complaints were very similar in all infected flocks and consisted of uneven or retarded growth, wet litter and/or lameness in 71%, 38% and 29% of the cases, respectively. Increased mortality occurred in 52% of the flocks. In 81% of the cases the clinical signs had been showing up in multiple successive rounds, often for over a year. The most consistent lesions found were hepatitis, myocarditis, pancreatitis, proventriculitis, enteritis and tenosynovitis of the gastrocnemius tendon. Concurrent infections with *E. coli*, *O. rhinotracheale*, FAV or IBV were observed in 48% of the flocks, predominantly those that were experiencing increased mortality.

It has been concluded that ERS type reoviruses frequently infect Belgian broilers, causing a disease entity which can be aggravated by concurrent infections with other agents. Since the parents of most of the affected broiler flocks had been well vaccinated against reovirus with vaccines containing classic reovirus strains, the present observations may indicate insufficient protection from these vaccines against ERS strains.

SAMENVATTING

Tussen augustus 2001 en oktober 2006 werden in 21 Belgische vleeskuikentomen enteric reovirus strain (ERS) infecties gediagnosticeerd. ERS-isolaten werden bekomen uit de lever, hakpees, bursa, pancreas, het darmstelsel en trachea, of uit pools van meerdere organen.

De klinische klachten waren erg gelijkend in al de geïnfecteerde tomen en bestonden uit tweewas of groeivertraging, nat strooisel en/of manken in respectievelijk 71%, 38% en 29% van de gevallen. Verhoogde sterfte trad op in 52% van de tomen. In 81% van de gevallen waren de klinische symptomen reeds in meerdere opeenvolgende ronden opgetreden, vaak zelfs over een periode van meer dan een jaar. De meest consistent waargenomen letsels waren hepatitis, myocarditis, pancreatitis, proventriculitis, enteritis en tendovaginitis van de gastrocnemius hakpees. Gelijktijdige infecties met *E. coli*, *O. rhinotracheale*, FAV- of IBV-virus werden waargenomen in 48% van de tomen, vooral in de groepen die af te rekenen kregen met verhoogde sterfte.

Er werd besloten dat ERS-type reovirussen een frequente infectie vormen bij Belgische vleeskuikens en de oorzaak zijn van een ziektebeeld dat verergerd kan worden door gelijktijdige infecties met andere agentia. Aangezien de ouderdieren van de meeste aangetaste vleeskuikentomen goed gevaccineerd werden tegenover reovirus met vaccins die klassieke reovirusstammen bevatten, kunnen de huidige waarnemingen erop duiden dat deze vaccins onvoldoende bescherming induceren tegenover ERS-stammen.

INTRODUCTION

Reoviruses have been isolated worldwide from chickens affected by various disease conditions, predominantly including viral arthritis/tenosynovitis, stunting syndrome, enteric disease, malabsorption syndrome and immunosuppression (Glass *et al.*, 1973; Goodwin *et al.*, 1993a,b; McNulty, 1993; Jones, 2003; Rosenberger, 2003). Economic losses caused by reovirus infections are frequently the result of elevated

mortality, increased slaughterhouse condemnations and poor performance, including diminished weight gains and high feed conversions (Dobson and Glisson, 1992; De Herdt *et al.*, 1999; Rosenberger, 2003). For this reason, vaccination of chickens against reovirus is practiced in most parts of the world and has been proven efficacious. Vaccination of breeders can protect young broilers through the transfer of maternal antibodies.

In 1998, however, reovirus was held responsible for

major disease outbreaks in broiler flocks in Poland, notwithstanding the fact that their parents had been well vaccinated (van Loon *et al.*, 2001). Infected broiler flocks suffered from high mortality and signs of malabsorption. At necropsy, lesions including hydropericardium, enlarged livers with multiple necrotic foci and swollen spleens were found. The signs and lesions seen under field conditions were experimentally reproduced in SPF chicks through intramuscular and oral challenge with a reovirus strain that was isolated from the broilers affected under field circumstances. Recently, central nervous disorders were also ascribed to this type of reovirus infection in chickens (Van De Zande *et al.*, 2007).

Reoviruses can be classified into different serotypes using the plaque reduction assay. In this test, reovirus strains isolated from diseased broilers in Poland could not be neutralized by antisera against known reoviruses (van Loon *et al.*, 2001). Furthermore, characterization of the strains with a panel of monoclonal antibodies revealed a reaction pattern that was different from the reovirus strains described in the literature (Johnson, 1972; van der Heide *et al.*, 1974; Hieronymus, 1983; Rosenberger *et al.*, 1989; van Loon, 2001). Therefore it was concluded that the reovirus strains from Poland belonged to a new serotype. They were subsequently called Enteric Reovirus Strains (ERS). Screening in the field during the following years demonstrated that strains of ERS type reovirus are prevalent in many European countries, the USA, Argentina, the United Arab Emirates, South Africa, the Philippines and Indonesia (Van De Zande and Lin, 2005).

As in other Western European countries, breeders in Belgium are usually vaccinated during the rearing period with commercially available live and inactivated reovirus vaccines containing non-ERS strains. Broilers are not vaccinated against reovirus. Little information is available on the epidemiology and significance of ERS reoviruses in disease outbreaks of broilers under these circumstances. It was therefore the aim of this study to examine the prevalence of ERS reovirus infections in 70 Belgian broiler flocks suffering from clinical disease. The clinical signs and lesions observed in the ERS-infected flocks were analyzed in more detail.

MATERIALS AND METHODS

Flock data and diagnostic procedures

Between August 2001 and October 2006, 70 Belgian broiler farms that experienced either clinical disease with signs of lameness, poor growth and/or wet litter (55 farms) or outbreaks of increased mortality without clinical signs (15 farms), were visited in order to obtain detailed case history data and to collect the samples needed for making a diagnosis. Standard diagnostic procedures included necropsy of 4 - 10 birds per farm and cytological examination of multiple internal organs, most often trachea, lungs, heart, liver,

kidneys, pancreas, jejunum and cecum. For the cecum, impression smears were stained with the Hemacolor (Merck, Darmstadt, Germany) staining reagents and observed microscopically at a magnification of x1000. Routinely in all flocks the presence of reovirus was checked through virological examination of organs that showed gross or microscopic lesions. In order to make a diagnosis of other infectious disease agents, appropriate virological, PCR, bacteriological and histological techniques were used.

In 65 of the 70 examined broiler flocks, the vaccination status of their parents against reovirus was known. Except for two flocks that had been left unvaccinated, these breeders had received a single administration of live S1133 vaccine and inactivated vaccine containing reovirus strains 1733 and 2408 during the rearing period.

Isolation and identification of reovirus

Isolation and identification of reovirus were done as described (van Loon *et al.*, 2001). Briefly, selected organ samples were homogenized either individually or as a pool (Table 1). The supernatant obtained after centrifugation of the homogenized tissues was filtered (0.2 µm filter) and inoculated onto freshly prepared primary chicken embryo liver cells. After 4 to 8 days of incubation, the monolayers were inspected for the presence of a cytopathic effect. Reovirus was identified by the immunofluorescence technique using rabbit polyclonal anti-reovirus serum. Further characterization of reovirus isolates was done using the monoclonal antibodies 154, 14-67-INT, INT-14-11, INT-13-6 and 15-1-INT (van Loon *et al.*, 2001). This allowed distinguishing ERS strains from other serotypes.

RESULTS

Reovirus isolates

The presence of reovirus in diseased chickens was demonstrated in 22 broiler farms. In 21 of these it concerned ERS type of strains. The ERS reovirus isolates were obtained from the liver, the gastrocnemius tendon, the bursa, the pancreas, the intestinal tract and the trachea, or else from pools of multiple organs (Table 1).

In 19 of the 21 ERS infected broiler flocks, the chicks were derived from breeders that had been vaccinated with currently available vaccines against reovirus.

Clinical signs

The nature and onset of clinical signs observed in the ERS infected broilers are summarized in Table 1. Increased mortality rates and symptoms of uneven or retarded growth, wet litter and/or lameness were seen in 52%, 71%, 38% and 29% of the flocks, respectively.

Table 1. Characteristics of ERS reovirus infections observed in 21 flocks of broiler chicks.

Flock	ERS isolated from	First occurrence of signs	Clinical observations					Concurrent infections
			Retarded growth	Wet litter	Lameness	Increased mortality	Recurrent nature*	
1	Pancreas	Week 1		x**			x	
2	Pool heart - liver - pancreas	Week 1	x	x		x	x	<i>O. rhinotracheale</i>
3	Pool liver - pancreas and Pool small intestines - cecum	Week 1	x				x	
4	Pool heart - liver - pancreas - bursa	Week 1	x			x	x	FAV
5	Liver	Week 2				x	x	<i>E. coli</i>
6	Pool liver - bursa - pancreas - heart	Week 2	x		x	x	x	<i>E. coli</i>
7	Bursa	Week 2				x	x	<i>E. coli</i>
8	Liver	Week 2	x				x	
9	Pool liver - heart - bursa	Week 2	x	x			x	
10	Pool liver - heart - pancreas	Week 2	x	x			x	QX-IBV
11	Liver	Week 3	x			x	x	
12	Pool liver - pancreas	Week 3	x	x		x		
13	Liver	Week 3	x	x		x	x	
14	Gastrocnemius tendon	Week 3			x			
15	Gastrocnemius tendon	Week 4	x		x	x	x	<i>E. coli</i>
16	Gastrocnemius tendon & liver	Week 4	x		x	x	x	<i>E. coli</i>
17	Gastrocnemius tendon	Week 4			x			
18	Bursa	Week 4	x	x			x	FAV
19	Pool liver - pancreas	Week 4	x		x		x	
20	Gastrocnemius tendon	Week 5	x	x			x	
21	Bursa and trachea	Week 5				x	x	FAV and <i>E. coli</i>

*Recurrent nature = similar signs were observed in successive rounds on the same farm

**x = present

The first occurrence of these problems varied from week 1 to week 5 of age, and the symptoms always lasted until the end of the growing period (week 6).

In 17 of the 21 infected farms, the clinical signs had already posed a recurrent problem for several rounds, and even for over one year in 9 of them.

Concurrent infections

Concurrent infections were found in 10 of the 21 ERS infected flocks. Bacterial co-infections with *E. coli* and/or *O. rhinotracheale* were diagnosed in 7 flocks, in all of which increased mortality was prominent. Fowl adenoviruses (FAV) were concurrently isolated with ERS from 3 flocks in which the clinical problems consisted, respectively, of poor growth, poor growth together with wet litter, and increased mortality without signs. One flock appeared to be positive for the presence of the QX genotype of infectious bronchitis virus (IBV) (Liu and Kong, 2004) along with ERS. Poor growth and wet litter were the main symptoms in this flock.

Gross lesions

Chicks that were derived from ERS infected flocks suffering from concurrent bacterial infections showed lesions of polyserositis. In chicks from ERS positive flocks without bacterial co-infections, gross lesions were most frequently observed in the liver (10 farms). This organ usually had a pale aspect and/or was swollen and in a few cases contained petechiae. In

chicks from 6 farms, paleness and the small size of the pancreas were apparent. Watery contents of the proventriculus, the small intestines and/or the ceca were typical also in chicks from 6 farms. Furthermore, paleness of the heart muscle and/or hydropericardium (Figure 1) were seen in 5 cases. Small bursas were observed in chicks from 4 farms, including the three farms that had experienced increased mortality without clinical signs. In rather sporadic cases, paleness or swelling of the kidneys was seen. Femoral head necrosis was present in chicks from 7 farms. In 3 farms the predominant lesion was a swelling of the gastrocnemius tendon on one or both legs (Figure 2). In some of the birds this swelling had an edematous nature, while in others it was very hard due to fusion of tendon sheaths with the surrounding tissues.

Microscopic lesions

For the results of the cytological examinations, birds suffering from concurrent bacterial polyserositis were not taken into consideration. Cytology revealed consistent inflammatory lesions in the livers and heart muscles of almost all birds from all farms infected with the ERS virus. Inflammatory infiltrations predominantly consisted of lymphocytes in the liver and both lymphocytes and heterophilic granulocytes in the heart muscle. Focal hepatic necrosis was seen in two cases. Infiltration of lymphocytes was also a common finding on cytology of the pancreas. Although hepatic necrosis lesions were seen in chicks that derived from almost all the infected farms, their

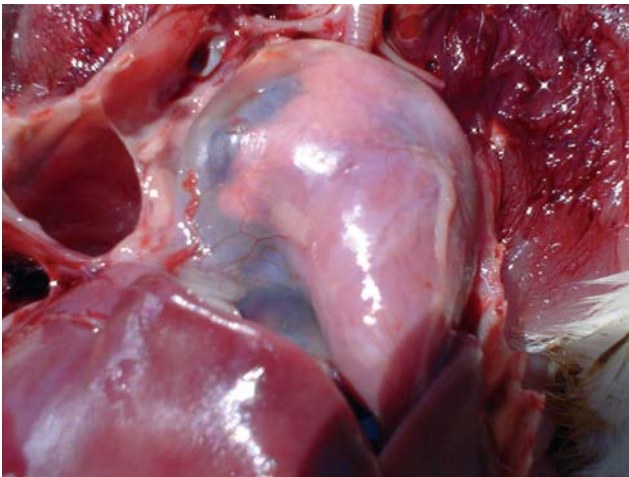


Figure 1. Five-week-old broiler chick infected with ERS type reovirus under field circumstances, showing excessive volumes of clear fluid in the pericardial sac, as well as a pale aspect of the heart muscle and liver. Concurrent infections were not demonstrated.



Figure 2. Five-week-old broiler chick infected with ERS type reovirus under field circumstances, showing a marked swelling above the right hock due to tenosynovitis of the gastrocnemius tendon. Concurrent infections were not demonstrated.

prevalence within the flocks appeared rather low. Lymphoid infiltrations of the jejunum were observed in 2/3 of the submissions. Cytological lesions of the kidneys and the proventriculus were seen in one or more birds in approximately 35% of the groups examined. No inflammation was found in the bursas. In chicks from two farms from which ERS isolates were obtained from the bursas, however, the nucleus of some bursal lymphocytes had a more intense basophilic staining. In birds that suffered from lameness, tenosynovitis of the gastrocnemius tendon with high numbers of lymphocytes, heterophilic granulocytes and in some cases bacteria were observed.

DISCUSSION

In the present paper, ERS reovirus was found in 21 of the 70 Belgian farms that submitted broiler chicks for necropsy because of clinical disease problems. This may indicate that ERS reoviruses are frequently involved in outbreaks of clinical disease in broilers kept under Western European field circumstances.

Since 1957 (Olson *et al.*, 1957), lesions of tenosynovitis and arthritis have traditionally been associated with reovirus infections. Most reports on outbreaks of this type of disease have come from the USA. Reovirus-associated lameness has also been observed in Europe (poultry practitioners, personal communications), especially between 1984 and 1987, when the vaccination of broiler breeders was not yet common practice, but papers on the subject are rare. In 29% of the ERS infections described in the present article, lameness due to tenosynovitis of the gastrocnemius tendon was observed. This may demonstrate that lameness resulting from reovirus infections forms a problem in Europe at the current time.

The predominant clinical signs observed in the ERS

infected flocks of the present study were retarded growth and, to a lesser extent, the production of wet litter. In the affected birds, lesions were found in the liver, the pancreas and/or the intestinal tract. ERS isolates were consistently obtained from one or more of these organs. Since these are all organs involved in the digestion process, the findings may indicate that reovirus infections can lead to malabsorption and/or maldigestion. Many authors have associated malabsorption and retarded growth with infections with reovirus strains (Lenz *et al.*, 1998; Songserm *et al.*, 2000; McNulty and Jones, 2001; Songserm *et al.*, 2002; Songserm *et al.*, 2003). However, the causal relationship between the reoviruses and the clinical signs observed could often not be proven because of the inconclusive results of the experimental studies (Jones, 2003). For ERS strains, on the contrary, growth retardation was clearly reproduced through oral or subcutaneous inoculation of day-old commercial broiler and SPF chicks (van Loon *et al.*, 2001; personal observations).

The administration of ERS virus to SPF birds resulted in high mortality rates (van Loon *et al.*, 2001). Under the field circumstances of this study, increased mortality was observed in 11 of the 21 ERS infected flocks, 8 of which suffered from concurrent infections, predominantly consisting of bacterial polyserositis and septicemia. This may indicate that the increased mortality in ERS infections under field conditions is especially important in flocks that at the same time are suffering from concurrent infections. Avian reoviruses have indeed been shown to enhance the pathogenicity of other infectious agents of chickens such as *E. coli* (Rosenberger *et al.*, 1985) and IBDV (Moradian *et al.*, 1991). The possible types of interactions between the ERS virus and the concurrent infecting agents are unknown. Facilitation of invasion through primary colonization of the intestinal or respiratory tract and/or immunosuppression arising from multiplication in the

bursa may be involved. Indeed in the present study ERS virus was isolated from all of these organs. Further research on this aspect of the pathogenesis is required.

Nineteen of the 21 broiler flocks that appeared to be infected with ERS were derived from breeders that had been vaccinated with currently available vaccines against reovirus. This may indicate that these vaccines provide insufficient protection against viral multiplication and the occurrence of clinical disease in the progeny. Vaccines that provide stronger protection against ERS reovirus therefore seem necessary. Van De Zande and Lin (2005) demonstrated that broiler chicks can be protected against challenge with ERS through the vaccination of breeders with a mineral oil adjuvated vaccine containing an ERS strain.

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