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.

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 Arabic 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.
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 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

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

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

*Recurrent nature = similar signs were observed in successive rounds on the same farm
**x** = present
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 adjuvanted vaccine containing an ERS strain.

REFERENCES


