

EPIDEMIOLOGICAL, CLINICAL AND MORPHOPATOLOGICAL RESEARCHES IN AN OUTBREAK OF REOVIRUS IN A BROILER FLOCK

CERCETĂRI EPIDEMIOLOGICE, CLINICE ȘI MORFOPATOLOGICE ÎNTR-UN FOCAR DE REOVIROZĂ LA UN EFECTIV DE BROILERI

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ABSTRACT | REZUMAT

Avian reovirus is an infectious and contagious disease spread in intensive poultry, especially broilers, evolving with anatomoclinical multiple forms. In this paper are presented the results of epidemiological, clinical and morphopathological exams conducted in a broiler flock, in which the two syndromes of avian reovirus have occurred. The epidemiological investigation has shown that reovirus was present only at the respective generation of broilers, and that the disease was transmitted vertically. Specific symptoms of malabsorption syndrome have occurred after 7 days, being well expressed up to 3 weeks. It was observed an unevenness in broilers, suggesting the existence of broilers of different ages. Specific signs of the tenosynovitis-arthritis syndrome appeared after 3 weeks of age. Histological lesions in proventriculus, small intestine, pancreas and liver, show an alteration of digestion, followed by a poor conversion of food, and infiltrative and hyperplastic lesions, show a local and general infectious process. A confirmation of reovirus diagnosis was done through RT-PCR.

Key words: broilers, malabsorption syndrome, tenosynovitis-arthritis syndrome

Reoviroza aviară este o boală infectocontagioasă virotică, întâlnită la puii de găină și curcă, manifestată clinic prin mai multe forme clinico-lezionale. În acest articol sunt prezentate rezultatele examenelor epidemiologice, clinice și morfopatologice realizate într-un efectiv de broileri în care boala a evoluat sub forma a două sindroame. Ancheta epidemiologică, efectuată în fermă, a evidențiat transmiterea verticală a reovirozei. Simptomele specifice sindromului de malabsorbție au apărut după vârsta de 7 zile fiind bine exprimate la vârsta de 3 săptămâni. A fost remarcată neuniformitatea accentuată a puilor, sugerând existența unor pui de vârste diferite. Semnele specifice sindromului de artrită-tenosinovită au apărut după vârsta de 3 săptămâni. Leziunile histologice din proventricul, intestinul subțire, pancreas și ficat pledează pentru alterarea digestiei, urmată de conversia redusă a furajelor, iar leziunile de tip infiltrativ și hiperplazic, pledează pentru un proces infecțios local și general. Confirmarea diagnosticului de reoviroză a fost făcută prin RT-PCR.

Cuvinte cheie: broileri, sindrom de malabsorbție, sindrom de artrită-tenosinovită

Reovirus infections in broilers and turkeys are frequently found in countries with intensive poultry and intensive commerce with poultry material (1,2,8).

Avian reovirus is clinically manifested through more pathological entities, represented by: tenosynovitis-arthritis syndrome, malabsorption syndrome, respiratory infections, intestinal infections, and also immunosuppression conditions (3,4,6,7,10,11).

The disease causes significant economic damages, represented by mortality, a poor conversion of food and unequal growth, a decrease in egg and meat productions, slaughterhouse forfeitures and immunosuppression induced by reoviruses (8,9).

In 1954, Fahey and Crawley isolated a virus in sick

birds, Petek et al. (1957) confirming isolation of this virus. Then, Olson et al. (1957), in the same year, describe synovitis, named by Dalton and Henry (1967) viral tenosynovitis-arthritis (8,13,14). In broilers, Kouwenhoven et al. (1978) describe a disease named runting syndrome, also seen in the next years, under several names, being accepted as malabsorption syndrome, proposed by Veltmann et al. (1985) (8,13,14).

In Romania, the occurrence and spread of reovirus infections in broilers and turkeys are poorly documented, without to offer a clear clinical picture for clinicians in order to facilitate the diagnosis of the disease, either at farm, or laboratory level. However, the importance of the monitoring of different understudied pathogens in different animal species, including poultry, in order to increase the biological safety of the resulted meat for the consumer (12) has been recently underlined in a recent review (5). Thus, further studies are recommended (5, 12).

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The present study aimed to investigate the epidemiological, clinical and anatomopathological features occurred in a primary reovirus outbreaks in a western Romanian broiler farm.

MATERIALS AND METHODS

In a poultry farm, from west county, in a series of broiler flock, from Cobb hybrid, imported, where seen, after 10 days of age, losses in mortality, reduced weight intake and characteristic symptoms of avian reovirus.

In order to do researches, there was monitored a broiler flock of 10,500, raised on soil, through epidemiological, clinical and anatomopathological study. Based on examinations it was thought to be avian reovirus, and lab tests confirmed the disease.

Necropsy examination was done twice a week, starting from the first week, and then after 7-8 days of age, when the symptoms of malabsorption syndrome appeared and after 21 days of age when appeared symptoms of tenosynovitis-arthritis syndrome.

From broilers with reovirus characteristic macroscopic lesions were taken organs samples: proventriculus, small intestine, spleen and liver needed for microscopic examination and PCR. Histopathological examination was carried out by haematoxylin-eosin methylene blue staining and Pappenheim (Pph) staining methods. A confirmation of reovirus diagnosis was made by RT-PCR.

RESULTS AND DISCUSSIONS

Research conducted on broilers series taken in this study have provided important results on the development of avian reovirus, with two syndromes, the malabsorption syndrome and the tenosynovitis - arthritis syndrome. The epidemiological investigation carried out, in the form of a detailed investigation, aimed to identify the sources of infection, to find favourable factors and ways of transmitting the disease on the farm.

On the farm, the biosecurity and general prophylaxis measures have been partially applied, with a frequent movement of people and means of transport on the premises, and at the gate, broilers, of different ages, are frequently sold to population.

The feeds administered to the chicks corresponded qualitatively and quantitatively to each age, and the main microclimate parameters, monitored by the farmer respectively humidity, air currents, ammonia, NTGMA and coliforms, were within the limits allowed until the age of 4 weeks after which their values they grew progressively. Weight gain, determined by weekly weighing, showed a lower feed conversion. Thus, the average weight was 30% lower than the standard weight of that hybrid, and the growth time was about two weeks longer. The evolution of weight gain is the consequence of malabsorption syndrome, in the literature it is mentioned

that weight gain can be reduced by 25% -66% (1, 7, 8).

The epidemiological investigation showed that reovirus was present only in the respective series of broiler chickens, the transmission being vertical, through eggs infected with reoviruses from the breeding flocks (parents). No other sources of infection were found on the farm because in the previous series, broiler chickens, reovirus was absent.

Losses expressed by mortality were registered daily, with absolute value, being quantified as relative value by cumulative mortality (Table 1).

Table 1
Evolution of cumulative mortality weekly

Week	Cumulative mortality	%
I	165	1.65
II	134	1.34
III	98	0.98
IV	72	0.72
V	69	0.69
VI	34	0.34
VII	44	0.44
Total	616	6.16

Analysing the evolution of cumulative mortality weekly, it appears that the limits were between 0.34% and 1.65%, the maximum being in the first week. From the third week cumulative mortality decreased progressively until the sixth week, then increased again, in the last week of growth. This evolution is correlated with the results of pathological examination, which revealed the presence of specific lesions of infectious diseases. This indicator was preferred because it reveals mortality losses, reported to the total number of chickens, which are at risk towards this disease within a clearly specified time. The decreasing values of cumulative mortality suggest the infection with avian reoviruses by vertical transmission, in the first weeks of life being reported a higher number of diseases in chickens, which is correlated with losses through mortality. With the increase of the chicks' age, the cumulative mortality values were lower, which pleads for the lack of horizontal transmission of the disease on the farm. Weight gain determined by weekly weightings showed a lower feed conversion, average weight being 35% lower than standard weight and the growing period was higher by about two weeks. Evolution of weight gain is the result of malabsorption syndrome, in the scientific literature stating that weight gain can be reduced by 25 % - 66% (1,2,3).

Specific symptoms of malabsorption syndrome have occurred after 7 days, being well expressed up to 3 weeks. First was noted a marked unevenness of chickens, suggesting the existence of chickens of different ages. Other symptoms reported were diarrhoea, whimsical appetite, increased water intake, adynamism and

fluff. After the age of 3 weeks, the changes in the feathers on the wings were very obvious. They grew and oriented sideways or upwards, being characteristic of "helicopter chicks" (Fig. 1).



Fig. 1. Malabsorption syndrome – helicopter chickens

Symptoms specific to tenosynovitis - arthritis syndrome appeared in chickens after 3 weeks of age. Sick chickens showed difficult gait, limping, uni- or bilateral arthritis in the tibiotarsus metatarsal joint. The joints were enlarged, fluctuating and painful. For these reasons, the chicks refused to move, to drink water and to feed, which is why the progressive weight loss occurred.

Histopathological examination revealed microscopic lesions in several organs. Thus, the delimitation between the proventriculus, oesophagus, and stomach was absent and highlight the necrosis of proventricular papilla, necrosis of the lenticular glands, peeling of serous membrane with accumulation of oedema (Fig. 2).

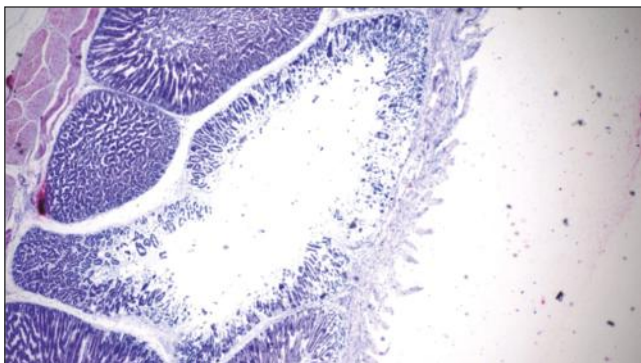


Fig. 2. Proventriculus - necrosis of proventricular papilla (col. HEA, ob x20)

In the small intestine was revealed catarrhal enteritis. At the level of intestinal villi, in sections, was observed scaling at the top of the villi due to enterocyte necrosis, decapitation of the villi and unequal villi with rare lymphocytes in their axis; rare caliciform cells irregularly disseminated on the flanks of villi, alternating with enterocytes; infiltration in the submucosa with small lymphocytes, histiocytes, lymphoblasts and rare

plasma cells (Fig. 3).

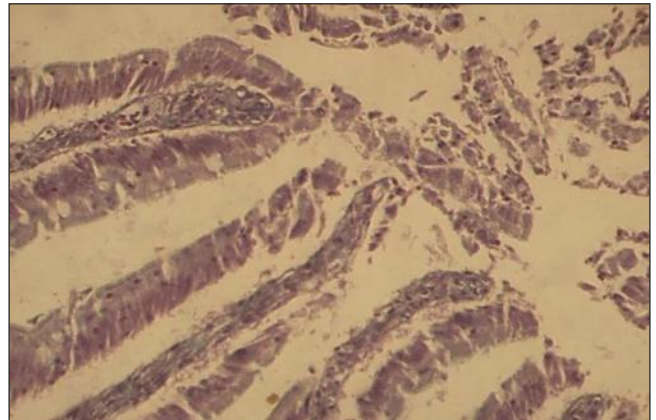


Fig. 3. The small intestine - infiltration in the submucosa with small lymphocytes, histiocytes, lymphoblasts and rare plasma cells (col. HEA, ob x20)

In the liver was highlighted: Disse space oedema, hepatocyte tumefaction, dystrophy vacuolated, lymphocytic infiltration and hypertrophy, and hyperplasia of Kupffer cells. In bursa were identified the following lesions: interstitial oedema, a large number of burso-cytes, proliferations with lymphoblasts with nuclear mitosis and a small number of macrophages in the structure of bursal follicles, respectively in the cortical and medullary area. In the pancreas, by the HEA staining method, congestive, inflammatory and necrotic histopathological lesions were highlighted, represented by: congestion and oedema of the interstitial connective tissue, degeneration of the glandular acini, obliteration of the glandular acini ducts, necrosis of the glandular acini (optic infiltrated areas) (Fig. 4).

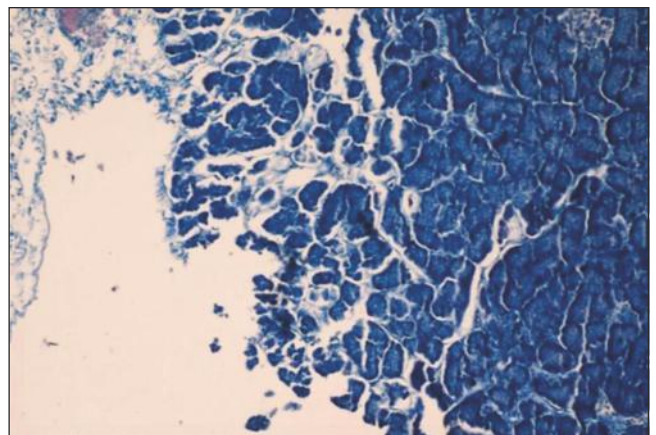


Fig. 4. Pancreas - congestion and oedema of the interstitial connective tissue, degeneration of the glandular acini (col. HEA x20)

In the spleen, Pappenheim staining revealed histopathological lesions that advocate a post-infectious immune response: numerous lymph nodes with a medium

-developed lymphocyte crown, reticulohistiocytic proliferation, a pronounced dilation of the splenic vessels with numerous splenocytes in the lumen and primary (active) and secondary (in the process of activation) splenic follicles, with hypochromic appearance (Fig. 5).

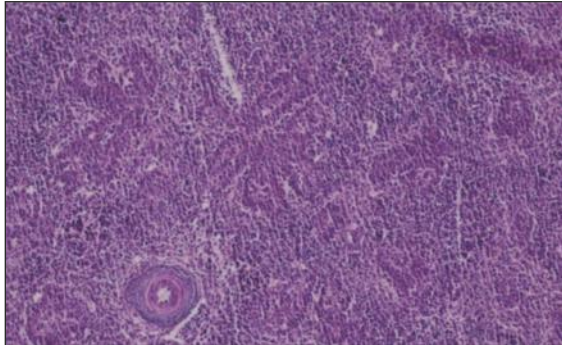


Fig. 5. Spleen – primary (active) and secondary (in the process of activation) splenic follicles, with hypochromic appearance (col. PPH x40)

Histological changes of the spleen and bursa highlights the installation of a moderate response, reported by other authors. In the tenosynovitis - arthritis syndrome is marked the following lesions: oedema of the tarsal and metatarsal tendon sheaths, of digital flexors and gastrocnemius tendon rupture and serofibrinous arthritis. Using the RT-PCR test, avian reovirus was detected in several organs taken from chickens of different ages in the monitored flock, thus confirming the evolution of avian reovirus. The fact that in two samples of proventriculus, taken from corpses aged 6 days and 28 days, viral RNA was not detected, is not an argument for the exclusion of this disease, because the reovirus was present in other organs. The results provided by the RT-PCR technique can be correlated with the results of the histological examination that revealed characteristic lesions, in several organs, produced by the avian reovirus following the multiplication (Fig. 6).

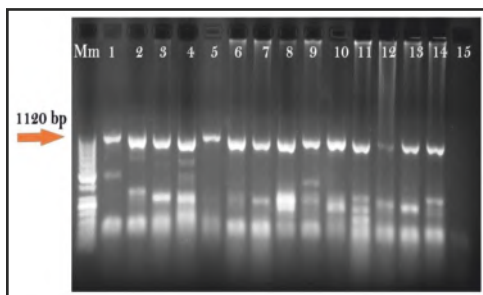


Fig. 6. Agarose gel image with amplification samples for ARV identification. Mm: molecular marker 100bp; 1: positive control; 2-14: exanimated samples; 15: negative control

CONCLUSIONS

In the monitored flock, the symptoms with which the

reovirus evolved were characteristic of the malabsorption syndrome and the tenosynovitis – arthritis syndrome, whose evolution was concomitant. Histological lesions in the proventriculus, small intestine, pancreas and liver, advocate altered digestion, followed by reduced feed conversion, and infiltrative and hyperplastic lesions, advocate a local and general infectious process. RT-PCR technique has proven to be an extremely useful method for confirming reovirus infection but it can also be recommended in monitoring infection in farms with a history of disease.

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