RUNTING IN BROILERS

B. Kouwenhoven, M.H. Vertommen and E. Goren.

Poultry Health Institute
P.O. Box 43, 3940 AA Doorn
The Netherlands

ABSTRACT

The main symptoms and pathological changes of the runting and stunting (malabsorption) syndrome as they are observed in many countries are described. Criteria used to define successful experimental infections are formulated.

Results of experimental work are presented from which it appeared that runting is an infectious condition in which the intestine is the target organ. Birds developed all clinical and pathological aspects of the syndrome only if they were infected during the first days of life. The main spread of the infectious agent(s) is horizontally and it occurs very rapidly. Although vertical transmission is suggested by circumstantial evidence, this has not been proved.

While a viral aetiology was suspected, attempts to reproduce the disease with reoviruses and coronaviruses isolated from field cases were unsuccessful. The original theory of a viral aetiology was questioned when it appeared that the pathogenicity of a diluted homogenate could be sedimented by low speed centrifugation. Moreover in a series of 3 bird passages of a bacteria-free filtrate of the homogenate, the pathogenicity decreased rather than increased.

The syndrome could be reproduced by inoculation of a combination of isolated aerobic and anaerobic bacteria and a bacteria-free filtrate, but not with each of these inocula separately. These results indicate involvement of both virus(es) and bacteria(e) in the aetiology.

General features of the disease

Runting and stunting, a disease of mainly broilers but sometimes also of broiler breeders, was described for the first time approximately 8 years ago in The Netherlands. Since then identical or similar syndromes have been observed in broilers in other European and non European countries (Belgium, England, N. Ireland, Italy, Denmark, German Federal Republic, Spain, The Near East, Australia, Mexico, South America, USA). There are also reports of the syndrome in turkeys. These observations indicate a worldwide distribution of the disease. While it seems to come and go "in waves", in many areas runting is the most important cause of economic loss in broiler production.

Clinically the disease combines the non-specific characteristics of several different diseases such as impaired growth and bad feathering. However the presence of many, sometimes up to 20 per cent, birds that seem

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not to grow at all, is characteristic of the runting syndrome. This can be observed from as early as 5 days of age. At 6 weeks birds can still have the appearance of a chick just a few days old and they weigh less than 100 g. A varying fraction of such birds shows feathering abnormalities. The replacement of down, especially at the head, is retarded, leading to "yellow heads". Feather growth is poor and wing feathers may point to different directions, causing an appearance called "helicopter chicks".

The excretion of yellow-orange, wet to mucoid droppings in which much maldigested feed is present, has also not been observed in other diseases. The yellow droppings are observed only when birds are fed a diet containing yellow corn or carotenoid pigments in another form. Then also another criterion, ie the presence of birds with pale shanks, can be observed.

At necropsy, pale swollen small intestines with thin liquid maldigested contents and sometimes gas accumulation are found up to an age of 2 weeks. Microscopically by day 3 and 7 necrotic and cystic changes are observed in the Lieberkühn glands of the small intestine. Thereafter up to about 3 weeks of age, the main changes are multilayering of the intestinal epithelium and the presence of great numbers of goblet cells, indicating a metaplasia of the epithelial cells to mucus producing cells. Proventriculitis (Kouwenhoven et al., 1978) is definitely not a part of the syndrome; it should be regarded as a completely separate cause of growth retardation.

From 2 - 3 weeks of age a large proportion of birds develop changes of rachitis and/or osteoporosis of the rapidly growing bones such as tibia and femur. Widened epiphyseal growth lines can be seen macroscopically on longitudinal sections of the proximal parts of these bones. More rarely, there is a hyaline enlargement of the tuberculae and capitulae costarum.

While pancreatic fibrosis is thought to be a part of the disease in England and Australia, this has not been observed in other countries.

Both experimentally and in field cases the disease goes together with long lasting serum concentrations of carotenes and fat soluble vitamins A, D and E. (Encephalomalacia, due to low vitamin E concentration, can also be an aspect of the syndrome). The alkaline phosphatase (ALP) activity in the serum is increased up to about 4 weeks pi and it could be demonstrated that this was most likely of intestinal origin.

In experimental studies described below a successful infection was determined by a combination of growth impairment up to 3 weeks after

infection of one-day-old chicks, excretion of yellow-orange mucoid to wet droppings from approximately day 4 up to 3 weeks pi, an increased plasma ALP activity and decreased carotenoid concentration up to 3-4 weeks pi and macroscopically widened epiphyseal growth plates of the proximal tibia 3-4 weeks pi.

The infectious nature of the syndrome was demonstrated by experimental reproduction of all its characteristics by oral administration of intestinal homogenates to one-day-old broiler chicks kept in isolators. The syndrome could again be reproduced with intestinal homogenates made from these birds 2 weeks after infection and so on. These observations, together with the clinical symptoms (diarrhoea, decreased carotene plasma concentrations) make it most likely that the digestive tract is the primary target for the infectious agent.

An extensive series of experiments has been carried out in order to study the infectiosity, the epidemiology and spread of the infection and the etiology.

To some of the questions we can give quite a detailed answer. However, with respect to the etiology the definite answer cannot be given (at least not by us today), but we can give some indications.

Epidemiology and spread of the disease

So far the disease has been observed mainly in broilers. There have been some cases in broiler breeders. This means that it is a disease of the heavy fast growing breeds. The disease is even connected with the growth rate. Symptoms after experimental infection were less serious in broilers kept on layer feed than in birds that were forced to grow fast on broiler feed. This shows that the syndrome is a typical luxury disease of the fast growing chick.

Although there is circumstantial evidence pointing to vertical transmission of the infection, there is no experimental proof of it.

Age related resistance

An important observation concerning the epidemiology was an age related resistance; the younger the birds, the more sensitive they are to the infection. So when significant pathological changes are present then the infection has taken place at a very young age. This is illustrated in Table 1. Although birds inoculated at 7 days of age developed general

TABLE 1 Influence of age at infection on disease parameters

Infected at	Body weight	Day 29 ALP	Bone changes
Day 1	680	1100	Yes
Day 7	790	438	No
Day 14	978	n.d.	No
Uninfected	977	n.d.	No
Day 1	472		Yes
Day 3	688		No
Uninfected	716		No

disease symptoms, especially diarrhoea (from day 21 (= day 14 pi) until day 29 even more than those infected at one-day old), they contracted no bone abnormalities. It is remarkable that in these birds the ALP activity was low at day 29 in contrast to that in the birds infected at one-day old. So obviously bone disorders develop only when pathological changes take place (in the intestine) during the first days of life. In this connection it is remarkable that mineralization in the normal broilers used in these experiments, does not take place before the 7th or 8th day of life. Inoculation at 14 days of life did not result in any clinical symptom nor in bone changes. In another experiment depicted in Table 1 there was a great difference even between inoculation at an age of one day or at three days. The latter birds obviously are already much less sensitive to the disease than the day-olds (just hatched).

Spread of the disease

The disease spreads easily and apparently very rapidly. This is shown in Table 2. When birds were placed in contact and 50% or 25% were infected, the contact birds acquired the disease 100%. They suffered at least as much from the syndrome as the infected birds. They also developed bone changes at least as much and to the same extent as the infected birds. Since we have seen that after infection at 3 days of age no bone abnormalities developed and that also weight losses were much smaller than after infection at one day of age, it can be concluded only that infection spreads very rapidly from one bird to another, most likely within one day.

TABLE 2 Spread of the infection

Isolator	Body weight	Day 22 ALP	Bone changes
A. 60 birds inoculated	379	1747	+
B. 30 birds inoculated	377		+
30 birds not inoculated	349	2747	+
C. 60 birds not inoculated	627	499	-
D. 10 birds inoculated			
30 birds not inoculated	210	2100	+
E. 40 birds not inoculated	425	500	-

Etiology

Reoviruses

The disease was reproduced to a certain degree with bacterium-free 450 and 220 nm filtrates as shown in Table 3.

TABLE 3 Effect of filtration and treatment with reovirus hyperimmune serum

	Body weight	Day 19 Carotene	ALP	Osteoporosis
Homogenate	366	0.43	1641	+
450 nm	436	0.57	1826	+
220 nm	436	0.46	1153	+
Homogenate + broth	424	0.46	1340	+
Homogenate + anti REO	444	0.14	958	+

Since then the attention was directed to a viral etiology.

From all field cases we investigated, a reovirus was isolated and from experimentally infected birds reovirus was isolated from the intestines (or faeces) even 5 weeks after oral inoculation with the crude homogenate.

However, with none out of 8 different reoviruses that were isolated from 8 different infectious homogenates, was it possible to reproduce the syndrome. These isolates were from 7 Dutch field cases and one Italian.

On the other hand reoviruses were more or less pathogenic after oral inoculation. They caused a more or less serious diarrhoea during 1-2

weeks. However, this diarrhoea was not of the yellowish mucoid type. Birds excreted watery sometimes mucoid faeces which had a grey black colour like normal faeces; sometimes it was a little yellow.

Table 4 gives the parameters of a typical reovirus infection as compared with infection with a crude homogenate. It is clear that reovirus caused a temporary growth retardation. However, even after 2 weeks the difference with the uninfected birds was over. Equally the increased ALP activity and the decreased carotenoid concentration was over by 3 weeks. This is in contrast to the birds inoculated with the infected homogenate. Here ALP and carotenes were still high/low at 4 weeks of age. So these parameters were changed for 2 weeks longer than in the reovirus infected birds.

We also infected with intestinal homogenate from reovirus infected birds, without success (bird passage).

TABLE 4 Comparison of reovirus and crude homogenate

Age (days)	Reovirus			Infected homogenate			Uninfected		
	Body wt.	ALP	Car.	Body wt.	ALP	Car.	Body wt.	ALP	Car.
3 days	58	1357	2.1	51	1232	1.5	61	864	2.1
1 week	97	3473	0.35	59	1486	0.37	122	1238	0.71
2 weeks	247	1731	0.43	129	2534	0.19	318	1122	0.57
3 weeks	499	684	0.65	214	1988	0.16	501	650	0.50
4 weeks	761	615	0.45	501	1009	0.14	785	397	0.35
	No Osteoporosis			Osteoporosis			No Osteoporosis		

In another important experiment (Table 3) the crude inoculum was treated for some hours with an anti reovirus hyperimmune serum and an equal volume as a control with broth. From the antiserum-treated inoculum reovirus could not be isolated (not even after 23 CKC passages); from the broth treated inoculum it was readily isolated. Equally from the intestines from inoculated birds reovirus was isolated/not isolated at 7 and 14 days after infection. However, with both inocula the disease was reproduced to the same extent, although not completely. So obviously the absence of reovirus in the inoculum did not mean the absence of infectiosity. However, there are also results of an experiment that revealed, in contrast with the results just mentioned, some role for reovirus (Table 5).

TABLE 5 Influence of reovirus infection at day-old

Inoculation	Body weight	Bone changes
Homogenate day 1	472	Yes
Homogenate day 3	688	No
Reo day 1 + homogenate day 3	540	Yes
Reo day 1	592	No
Not inoculated	716	No

Birds were inoculated at 3 days of age and they developed practically no symptoms (weight loss, bone abnormalities) as compared with control birds inoculated at day 1. But when birds had been infected at day-old with reovirus, they developed the complete syndrome after inoculation at day 3, although the growth retardation in the day-old infected group was still significantly greater than in the reo 1 + crude 3 inoculated group. The weight loss in this group was mainly due to reovirus which alone produced a significant weight loss but no bone abnormalities. This role of reovirus acting as a trigger or a preinfection could not clearly be established in another experiment. So we concluded that reovirus as such cannot cause the disease but that an early infection with reovirus under some unknown conditions may facilitate infection with the true agent.

Viruses other than reoviruses

We isolated some adenoviruses. They were of no pathological significance.

After treatment of our standard crude inoculum with anti reovirus serum we isolated both from the inoculum and from the intestine of infected birds a virus which proved to be a coronavirus. This isolate, designated B29C5, on experimental infection caused diarrhoea and weight loss comparable with that caused by the crude inoculum. But this diarrhoea was, although mucoid and wet, not yellow enough. ALP activity was increased until day 21 but 4 days later it was normal and so was the carotene concentration. However in 4 out of 29 infected birds we observed ribknob swelling. In a further experiment (Table 6), the virus used had been adapted to growth in the allantoic sac. It had undergone 15 embryo passages (5 yolk sac and 10 all. sac) and had a titre of $10^{6.2}$ EID50. This amount was inoculated in

experimental birds. These birds developed a long lasting diarrhoea and weight loss, longer than in the control infected birds. But in these birds the diarrhoea was mucoid and yellow which was not the case in the birds inoculated with the isolate.

Birds did not develop bone abnormalities which were present in the controls. It can be seen also that neither ALP values nor carotene concentrations showed significant changes in this group, again in contrast with the infected controls. Also birds developed antibody against B29C5 after infection with this isolate but not with the crude inoculum from which it was isolated.

TABLE 6 Comparison of B29C5 coronavirus and crude homogenate

	Day 22				Day 50
	Body w.	ALP	Carot.	Bone abnorm.	Antibodies to B29C5
Uninfected	743	low	high	-	No
Crude homogenate	590	high	low	+	No
B29C5 coronavirus	585	low	high	-	Yes

In the next series of experiments it was attempted to remove the coronavirus from the intestinal homogenate by incubation with an antiserum. In these experiments an incubation step for 30 minutes followed by centrifugation at 3000 g was also involved and the supernatants were used as inoculum. Experimental infections showed that the pathogenicity of such supernatants had decreased considerably. However the pathogenicity of homogenates after similar treatment with saline (inoculated control groups) had decreased to the same extent. A possible explanation for these results was sought in the effect of the centrifugation, keeping in mind that the centrifugal force and time applied was too low to sediment viruses.

A most important finding was obtained in further experiments, as presented in Table 7. Centrifugation was again for 30 minutes at 3000 g and 4°C. Birds in group 3 were slightly ill only at day 4 and 5 pi and they excreted some yellow faeces. In contrast birds in the inoculated groups 4, 5 and 6 were seriously ill from day 2 through 12 or 14 pi during which time they excreted typically yellow mucoid dropping. The clinical picture in these groups was the same and much more serious than that in group 3,

TABLE 7 Effect of centrifugation of crude homogenate

Group	1	2	3	4	5	6
Treatment	Infected control	Uninf. control	Supernatant of homogenate 1 vol. + saline 4 vols. after centrif.	Sediment of homogenate 1 vol. + saline 4 vols. after centrif.	Supernatant as in 3 + sediment as in 4, re-combined	Homogenate 1 vol. + saline 4 vols. Not centrif.
Body weight	345[+]	708	523	360	415	386
ALP	1763	706	2404	3482	2106	2692
Carotene	0.063	0.345	0.274	0.160	0.161	0.105
Bone changes	13/13*	0/16	10/16 (slight)	14/15	14/14	14/14

[+]All parameters measured at 22 days pi

*Number of birds with changes in the proximal tibia/number examined

but slightly milder than that in group 1.

From this experiment it was concluded that the pathogenicity of an inoculum consisting of a crude intestinal homogenate diluted 1:5 in saline could be sedimented easily for the greatest part by centrifugation for only 30 minutes at a relatively low centrifugal force of 3000 g and that after recombination of the more pathogenic sediment and the less pathogenic supernatant, pathogenicity was recovered.

These results pointed strongly to involvement of bacteria in the etiology or of a virus that could be sedimented as easily as bacteria, eg attached to easily sedimented particles. Regarding the latter, one thinks of eg rotaviruses. Sera taken from broilers 7 weeks after experimental inoculation with a pathogenic homogenate, had no antibodies against rotavirus.

In order to clearly define the significance of any virus present in the supernatant in the etiology, the experiments presented in Tables 8, 9 and 10 were carried out. The principle was simple: if any virus were the causative agent, it would be present in a 450 nm filtrate, hence this filtrate would be pathogenic on its own, or at least it would be pathogenic in a subsequent second or third bird passage. This pathogenicity would increase rather than decrease during these bird passages. The filtrate

TABLE 8 Effect of filtration of crude homogenate

Group Treatment	1 None	2 450 nm filtrate (1st bird passage)	3 Crude homogenate
Body weight	319[+]	255	143
ALP	760	3933	3176
Carotene	0.618	0.302	0.123
Bone changes*	1	2	4

[+]All parameters measured at day 14 pi.

*Maximum score can be 4.

used (Table 8) was made by centrifuging the homogenate for 30 minutes at 3000 g, passing the supernatant through a 450 nm filter, centrifuging again for 10 minutes at 3000 g (the small amount of sediment produced proved bacteriologically sterile) and passing this supernatant again through a 450 nm filter. From this filtrate a reovirus was easily isolated.

It appears from Table 8 that the control birds inoculated with crude homogenate displayed all parameters of full pathogenicity. The filtrate, although pathogenic, caused retardation, lowered carotene concentrations and bone deformations that were intermediate between those of the inoculated and uninoculated control birds. Five birds showed a great growth inhibition, 24 were moderately inhibited. The ALP activities in this group were higher than in the inoculated control birds.

Intestines were collected from 8 birds of group 1 and 3 and inoculated in groups 1 and 3 of the next experiment (Table 9). From 4 birds of group 2 that showed the greatest growth inhibition, intestines were collected separately from those of 8 other birds that had shown the intermediate grade of growth inhibition. From these intestines also homogenates were made and inoculated in groups 2.a and 2.b (Table 9).

It appears clearly that the filtrate had lost pathogenicity by the second bird passage. This observation reduced the possibility of a virus as the only causative agent considerably. If it were a virus, pathogenicity should have increased during the first bird passage.

In the experiments presented in Table 10 the intestinal homogenates made from birds of group 2.b (Table 9) were inoculated in birds of group 2, representing a third bird passage. The birds in group 1 were inoculated with intestinal homogenates made from the birds of group 1, Table 9, representing a second bird passage of intestinal homogenate from not inoculated birds. Carotene concentrations are not presented. It appears that in a third bird passage of filtrate made from the original homogenate, pathogenicity again has not increased. From this series of experiments it was concluded that a virus could be excluded as the primary and only causative agent of the runting and stunting (mabsorption) syndrome.

The involvement of bacteria or of bacteria and viruses was then investigated. Both aerobic and anaerobic bacteria were isolated from intestines 3 days after infection with the crude homogenate. It was attempted to reproduce the disease with these bacteria or with a combination of a bacteria-free filtrate as before, plus a mixture of these bacteria. The filtrate was supposed to contain any virus (reovirus and coronavirus were present, possibly others also). The bacterial part of the inoculum was composed of hundreds of different colonies. The bacteria were not identified. No virus could be isolated from this bacterial inoculum.

The main results are presented in Table 11 from which appears that the filtrate caused a limited growth inhibition as before and also a limited

TABLE 9 Effect of passage in birds on pathogenicity of filtered crude homogenate

Group / Treatment		Homogenate uninf. control birds group 1(Table 8) 1st bird pass.	Homogenate smallest bird group 2(Table 8) 2nd bird pass.	Homogenate intermed. birds group 2(Table 8) 2nd bird pass.	Homogenate control birds group 3(Table 8) 2nd bird pass.	Inoculated control	Not inoculated control
Body weight	Inoculated(15)*	397+	332	300	212	124	361 (25)
	Contact (10)	411	347	313	217	154	
ALP	Inoculated	983	3036	2547	3768	1187	542
Carotene	Inoculated	0.409	0.314	0.355	0.140	0.134	0.573
ALP	Contact	846	2121	2412	4179	1558	
Carotene	Contact	0.384	0.334	0.345	0.119	0.116	
Bone changes	Inoculated	0	0.4	1.1	2.4	1.9	0
	Contact	0	0.3	1.25	2.3	2.7	

*( ) number of birds

+All parameters measured at day 17 pi

TABLE 10 Effect of passage in birds on pathogenicity of filtered crude homogenate

Group / Treatment		1 Homogenate group 1, Table 9 2nd bird pass. not inoculated	2 Homogenate group 2.b Table 9 3rd bird pass. filtr.	3 None	4 Inoculated Controls
Body weight	Inoculated (15)*	334+	306	407	166
	Contact (10)	334	267		142
ALP	Inoculated	1345	1408	794	1674
	Contact	1243	986		1224
Bone changes	Inoculated	0.3	0.1	0.27	1.2
	Contact	0.6	0.9		1.7

*( ) number of birds

+At day 16 pi

TABLE 11 Investigation of involvement of bacteria in etiology of runting.

Group		1	2	3	4	5
Treatment		Filtrate	Filtrate + bacteria	Bacteria	Crude homogenate	None
Body weight	Inoculated	398[+] (19)[*]	299(19)	411(20)	294(19)	478(21)
	Contact	408 (5)	297(5)	355(5)	314 (5)	544(5)
ALP	Inoculated	1469	1411	740	1085	722
	Contact	1688	1207	1161	1873	617
Carotene	Inoculated	0.563	0.352	0.660	0.234	0.993
	Contact	0.709	0.346	0.630	0.254	0.908
Bone changes		0.42	0.84	0.76	1.44	0.46

* ( ) number of birds.
[+] Measured at day 20 pi.

decreased carotene concentration. The ALP activity was like that in the inoculated control group. Equally the birds inoculated with the bacteria showed about the same slight changes.

In contrast the bacteria and filtrate together caused a growth inhibition and ALP values that were the same as in the inoculated control group. The carotene concentrations were also considerably lower than in the birds inoculated with bacteria or filtrate only and not much higher than in the inoculated controls. Birds in group 2 excreted yellow coloured mucoid faeces like the birds in group 4 until about 14 days pi. Bone changes in group 2 were less than in group 4.

It can be concluded that in this experiment the syndrome was almost completely reproduced by inoculation of a bacteria-free filtrate of an infectious homogenate plus various bacteria isolated from intestines of birds at day 3 pi. These results confirmed our theory based on experiments described above that the etiological agent could not be a 450 nm filtrate agent only, but that a bacterial component was also involved.

REFERENCE

Kouwenhoven, B., Davelaar, F.G. and van Walsum, J. 1978. Infectious proventriculitis causing runting in broilers. Avian Pathol. 7, 183-187.