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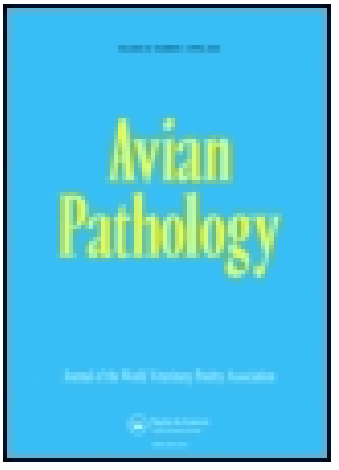

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Experimental infection of chicken embryos and day-old chickens with parvovirus of chicken origin

J. Kisary [a]

[a] Veterinary Medical Research Institute , Hungarian Academy of Sciences , P.O. Box 18, H-1581, Budapest, Hungary

[b] Université Libre de Bruxelles, Laboratoire de Biophysique et Radiobiologie , Rue des Chevaux 67, 1640 , Rhode-Saint-Genèse, Belgium

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EXPERIMENTAL INFECTION OF CHICKEN EMBRYOS AND DAY-OLD CHICKENS WITH PARVOVIRUS OF CHICKEN ORIGIN

J. KISARY[1]

Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, P.O. Box 18, Hungary

SUMMARY

Specific pathogen free (SPF) White Leghorn chicken embryos and day-old chickens and day-old commercial broiler chickens were infected with a parvovirus (strain ABU) isolated from chickens with stunted growth. As a result, egg hatchability was significantly decreased and hatched chickens had enteritis and low vitality. Serious growth retardation, bad feathering and bone disorders were observed in infected broiler chickens. Similar signs were not seen in SPF White Leghorn chickens.

INTRODUCTION

It has been reported recently (Kisary *et al.*, 1984) that virus particles tentatively classified as parvovirus were demonstrable in the intestinal tract of 10-day-old chicks showing markedly retarded growth, poor feathering, and very pale intestinal tracts. Electron microscopy revealed the presence of viral particles 19-24 nm in diameter in their intestinal contents, and after ultracentrifugation in CsCl, the buoyant density of the viral particles was 1.43 g/ml.

Since parvovirus play an important role in diseases of young animals of other species (Bachmann *et al.*, 1975), a possible involvement of the parvoviruses in the aetiology of the stunting or malabsorption syndrome was considered. This disorder is one of the economically most important diseases of broiler chicks. In an attempt to examine this possibility, chicken embryos, day-old specific pathogen-free (SPF) White Leghorn chicks and commercial broiler chicks were infected with the parvovirus derived from the stunted chickens. The results of these experiments are presented here.

MATERIALS AND METHODS

Virus

A parvovirus strain designated ABU, isolated from chicks under conditions described previously (Kisary *et al.*, 1984), was used to infect both chicken embryos

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[1] *Present address: Université Libre de Bruxelles, Laboratoire de Biophysique et Radiobiologie, Rue des Chevaux 67, 1640 Rhode-Saint-Genèse, Belgium.*

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and day-old chickens. The virus was propagated in chicks as follows. Commercial day-old broiler chicks were given 0.1 ml of the parvovirus-containing suspension *per os.* On the 8th day post-infection (p.i.) the chicks were killed and homogenates were prepared from the entire intestinal portion between the gizzard and the rectum. First the coarse debris was removed from the homogenate by low-speed centrifugation, then the supernate was layered onto a 45% (w/w) sucrose cushion. After centrifugation in an SW 27 rotor at 150,000 *g* for 2 hours the pellet was re-suspended in PBS. The suspension obtained was layered onto CsCl of 1.41 g/ml average density and centrifuged in an SW 41 rotor at 150,000 *g* for 24 hours. The virus band formed during centrifugation in the 1.42 to 1.43 g/ml density range was harvested through a hole pierced in the side of the tube, and dialysed against PBS to remove CsCl. The dialysed virus suspension was made up to 100 ml with PBS and stored at -20°C until use.

Chicken embryos and day-old chicks

To determine the embryo-pathogenicity of the virus, 60 SPF White Leghorn chicken embryos were infected with 0.1 ml of the virus suspension into the allantoic cavity after incubation for 9 to 16 days, respectively.

SPF White Leghorn and commercial broiler chicks were used in experiments aimed at determining the pathogenicity of the virus to day-old chicks.

In each of two consecutive experiments, 30 day-old SPF White Leghorn chicks were infected *per os* with 0.1 ml suspension of the ABU parvovirus strain. After infection the chicks were weighed weekly and their weight was compared with that of chicks kept uninfected in a separate room but under identical conditions and given the same feed.

In each of two consecutive experiments 30 day-old commercial broiler chicks were infected orally with 0.1 ml of the ABU parvovirus suspension. Uninfected chicks of the same origin and number were kept in a separate room and served as controls. The chicks were kept under observation during a 4-week period and their weight was recorded weekly.

RESULTS

Experimental infection of chicken embryos

In each of three consecutive experiments, 20 9- and 16-day-old chicken embryos were infected, incubated further and hatched. On average, 15% of the infected 9-day-old embryos died between the 12th and 20th day of incubation. The dead embryos were bacteriologically sterile, showed oedematous swelling and haemorrhages dispersed over the body. The internal organs also contained haemorrhages; however, none of the organs showed striking lesions. The surviving embryos began to break through the egg-shell 24 to 48 hours later than the uninfected controls, and, on average, only 30 to 40% of the embryos hatched. The chicks were poorly developed, of low vitality, and 80 to 90% of them died within 1 week after hatching. Prior to their death, the chicks were reluctant to move, stayed close to the heat source and sat on their hock joints (Fig. 1). A high proportion of them had diarrhoea and excreted light mustard-yellow faeces. The intestines of the dead chicks were filled with mucoid mustard-yellow contents saturated with ill-smelling gas and the intestinal wall was pale. No other characteristic pathological lesions were seen.

Eighty to 90% of the embryos infected at 16 days of age hatched out. The remaining

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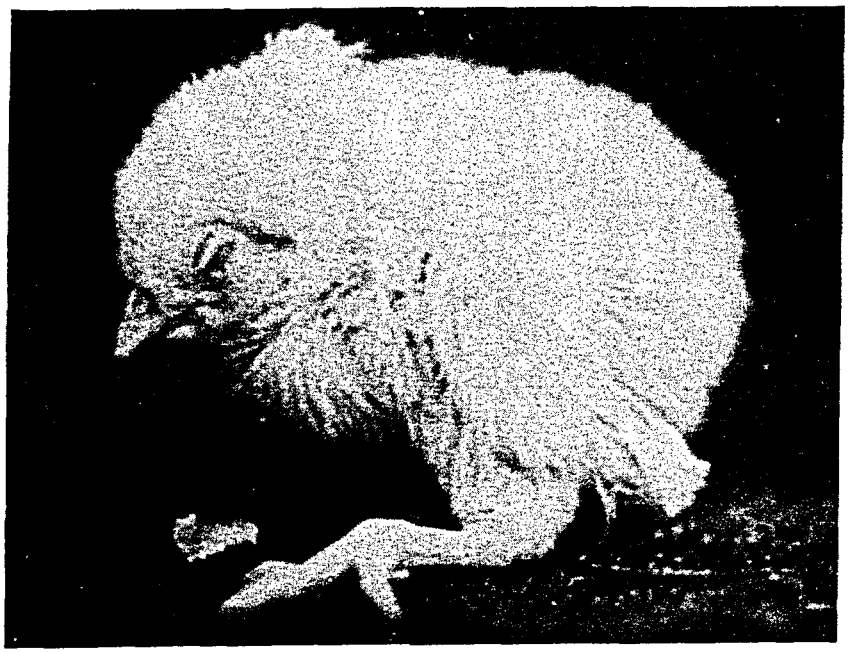

Fig. 1. Two-day-old SPF chicken infected with parvovirus strain ABU as embryo on the 9th day of incubation.

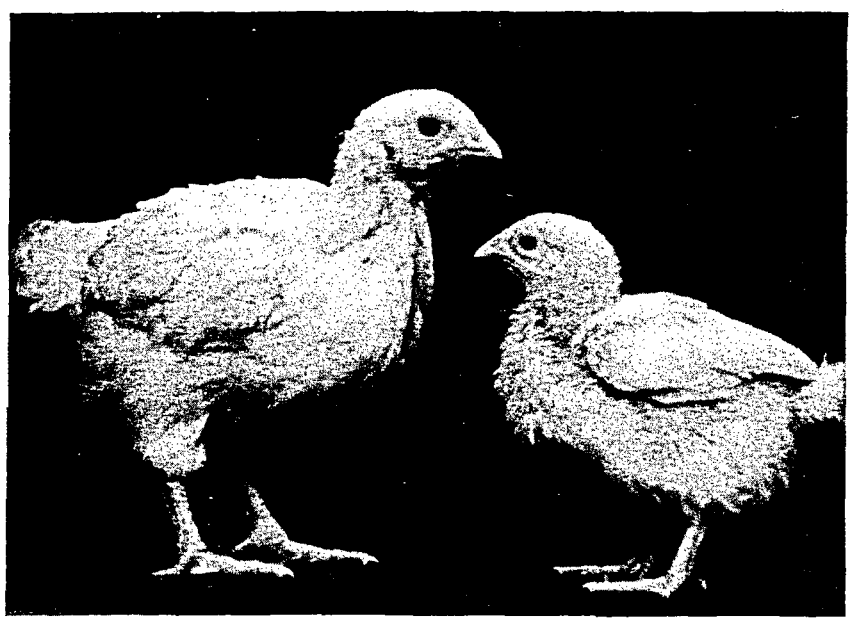

Fig. 2. Four-week-old broiler chicken infected with parvovirus strain ABU at day-old (right). The left one is an uninfected control of the same age.

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embryos broke the egg shell but were unable to hatch and died. About 30 to 40% of the hatched chicks died in the 1st week of life showing the clinical signs and pathological lesions described above.

Experimental infection of day-old chicks

During the 4-week observation period the SPF White Leghorn chicks showed no clinical signs. Little retardation in weight gain was recorded, except for a 5 to 10% weight difference in favour of the controls observed between the 10th and 20th days of life which became negligible by the 4th week.

To determine whether the parvovirus had multiplied in the cells of the intestine in the absence of clinical signs, five SPF White Leghorn chicks were killed on the 8th day p.i. and homogenates were prepared from the portion of their intestines between the gizzard and the rectum. Large amounts of parvovirus were detected in the homogenates by the method described,. It should be noted that the intestinal tracts of the chicks killed were, as before, paler than those of the uninfected controls.

As can be seen from Table 1, by the 4th week of life the body weight gain of broiler chicks infected with the ABU parvovirus suspension was nearly 40% lower than that of the controls. The difference was measurable by the 7th day of life, and rapidly increased at subsequent weekly weighings. Retardation in growth was especially striking in the 3rd and 4th weeks when there were considerable differences in body weight among the infected birds. The difference in growth between uninfected and infected chicks can be observed in Fig. 2.

Table 1. Mean body weight (g) of broilers infected orally with chicken parvovirus strain ABU at 1 day of age.

Age in days	Uninfected		Infected		Retardation in growth (%)
	Weight	Growth rate	Weight	Growth rate	
1	34.1	–	34.1	–	0
7	84.1	2.46	80.1	2.34	4.77
14	204.5	2.43	185.1	2.31	9.50
21	398.0	1.94	298.7	1.61	24.96
28	586.0	1.47	369.1	1.23	37.02

In addition to their retarded growth, the infected birds showed mild watery diarrhoea and excreted light-yellow or mustard-yellow faeces for 7 to 10 days after infection. Apart from this, the chicks were lively and their feed and water consumption was normal. It can be seen in Fig. 2 that the infected chicks showed disturbed feathering, their feathers being crumbled or absent from large areas of the body surface.

The chicks were killed and autopsied at 30 days of age at the end of the experiment. No characteristic lesions were found. However, it should be noted that the metatarsal bones of poorly-developed chicks were much more flexible than those of the uninfected controls.

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On the 8th day p.i. five broiler chicks infected with the ABU parvovirus suspension at day-old were killed. These chicks also had very pale intestines and the parvovirus could be detected in their intestinal homogenates by ultracentrifugation.

DISCUSSION

Infection of chicks with the ABU parvovirus suspension (Kisary *et al.*, 1984) consistently produced the most important sign of the infectious stunting syndrome, i.e. severe retardation of growth, but only when rapidly growing broiler chicks were infected (Kouwenhoven *et al.*, 1983). The nearly 40% retardation of growth which was found in comparison with the uninfected controls in the 4th week of life is considered to be clearly significant. In addition, the infected birds had deficient, low-quality feathering and their metatarsal bones occasionally showed rubber-like flexibility. The parvovirus used could be detected on the 8th day p.i., which proved its replication in the intestinal tract. As opposed to the broilers and in agreement with published information on the stunting disease syndrome, from experiments in which crude intestinal homogenates were used (Kouwenhoven *et al.*, 1983), inoculation of more slowly growing SPF White Leghorn chicks with the ABU parvovirus suspension did not produce the disease entity described, although the virus had replicated in the birds.

Experimental infection of SPF White Leghorn chicken embryos has shown that infection with the chicken parvovirus resulted in a considerably decreased hatchability of eggs, especially if infection occurred in the early stage of incubation. Chicks that had been infected as embryos showed markedly lower vitality, and most of them died of enteropathy in the 1st week of life.

The aetiology of the infectious stunting syndrome (also known as malabsorption disease or helicopter disease) has not yet been elucidated (Kouwenhoven *et al.*, 1983). However, it has been established that the putative pathogenic agent responsible for the main signs of the disease (i.e. severe retardation in growth, disturbed osteogenesis and feather development) exerts its effect in the intestine, resulting in poor absorption and consequent metabolic disorders (Kouwenhoven *et al.*, 1983). Chicks affected with this syndrome had significantly lower levels of essential minerals and vitamins.

The results of the present experiments do not allow us to attribute this economically very important disease exclusively to parvovirus, not least because in poultry kept under commercial conditions numerous other infective agents are present and should also be considered. However, our experiments do indicate that parvovirus infection of broiler chicks may play an important role in decreasing their growth rate below the expected level.

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RESUME

Infection expérimentale d'embryons de poulets et de poussins d'un jour par un parvovirus isolé chez le poulet

Des embryons de poulets et poussins d'un jour provenant de poules Leghorn blanches exemptes d'organisms pathogènes spécifiés (SPF) ainsi que de poulets de chair conventionnels âgés d'un jour ont été infectés avec un parvovirus (souche ABU) isolé de poulet présentant un retard de croissance avec rabougrissement. Sur le plan des résultats, l'éclosabilité a été significativement diminuée et les poussins éclos ont présenté de l'entérite et une baisse de la vitalité. Un important retard de croissance, un mauvais emplumement et des troubles osseux ont été observés chez les poulets de chair infectés. Les mêmes signes n'ont pas été observés chez les poulets Leghorn blancs SPF.

ZUSAMMENFASSUNG

Experimentelle Infektion von Hühnerembryonen und Eintagsküken mit Parvovirus aus Hühnern

Embryonen und Eintagsküken von spezifisch pathogenfreien (SPF) Weißen Leghornhühnern und Eintagsküken von kommerziellen Broilerzuchten wurden mit einem Parvovirus (Stamm ABU) infiziert, der aus Hühnerküken mit verzögertem Wachstum isoliert worden war. Als Folge trat eine Abnahme der Schlupffähigkeit auf. Die geschlüpften Küken zeigten Enteritis und eine herabgesetzte Vitalität. Bei den infizierten Broilerküken wurden eine schwere Wachstumsverzögerung, schlechte Befiederung und Knochenveränderungen beobachtet. Bei den SPF Weißen Leghornküken wurden diese Symptome nicht festgestellt.

RESUMEN

Infeccion experimental en embriones de pollo y en pollitos de un dia de edad con parvovirus originarios de pollo

Embriones de pollo Leghorn blancos, así como pollitos de un día de edad y pollitos de engorda comerciales de un día de vida, todos ellos libres de patógenos específicos (SPF), fueron infectados con un parvovirus (cepa ABU) aislado de pollos que presentaban un retraso en el crecimiento. Como resultado, se observó un descenso significativo en la incubabilidad, además de que los pollos nacidos presentaron una enteritis y una baja viabilidad. Se observó un retraso severo del crecimiento, mal emplumado y desórdenes óseos en los pollos de engorda inoculados. Signos similares no fueron vistos en pollos Leghorn blancos libres de patógenos específicos.

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