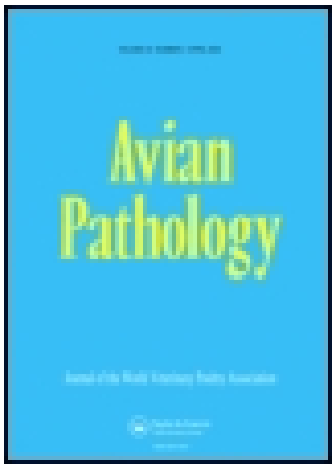

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Indirect immunofluorescence as a diagnostic tool for parvovirus infection of broiler chickens

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SHORT COMMUNICATION

INDIRECT IMMUNOFLUORESCENCE AS A DIAGNOSTIC TOOL FOR PARVOVIRUS INFECTION OF BROILER CHICKENS

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SUMMARY

Nuclear fluorescence was seen in the epithelial cells of the duodenum of broiler chicks infected experimentally at 1-day-old with a parvovirus of chicken origin. No antigenic relationship was detected by this method between chicken and goose parvoviruses.

Small viral particles of 19-24 nm in diameter with a buoyancy in CsCl of 1.43 g/ml were detected recently in the intestine of broiler chicks with stunting disease (Kisary *et al.*, 1984). The virus, tentatively classified as a new member of the Parvoviridae, caused decreased hatchability, stunted growth, poor feathering and bone disorders when chicken embryos and 1-day-old broiler chicks were experimentally infected (Kisary, 1984). These findings strongly suggest that the parvovirus of chicken origin may play a fundamental role in the aetiology of infectious stunting or malabsorption syndrome of broilers.

Since attempts at cultivating the parvovirus in chicken embryos and cultured chicken embryo fibroblasts have failed we have elaborated an indirect immunofluorescence (IF) technique to monitor the parvovirus infection of broiler flocks. This technique has also been used to determine the primary target cells in the gut for virus infection and to gain further information about the site of virus replication in an infected cell.

One-day-old commercial broiler chicks were purchased from a farm where infectious stunting has never been observed. They were experimentally infected *per os* with the purified chicken parvovirus strain designated ABU (Kisary *et al.*, 1984) as previously described (Kisary, 1984). On post-infection (p.i.) days 1, 3, 5, 10, 14 and 18 two infected and two uninfected control birds were killed and smear preparations were taken from the mucous membranes of the part of the duodenum surrounding the pancreas. The smear was air-dried and fixed with acetone at 4°C for 15 min. The fixed preparations were covered with 1:10 dilution of serum against parvovirus strain ABU prepared as follows. Guinea-pigs were inoculated intraperitoneally with parvovirus purified on a CsCl gradient on the 1st and 21st

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days and then bled 10 days after the second injection. The serum was inactivated at 56°C for 30 min and stored in aliquots at -20°C. Anti-guinea-pig-IgG sheep serum conjugated with fluoroscein isothiocyanate (FITC) was purchased from the Human Institute for Sero-bacteriological Production and Research (Budapest, Hungary). The smears covered with anti-parvoviral serum were placed in a wet chamber at 37°C for 1 hour. After incubation they were washed three times with phosphate buffered saline (PBS) each for 10 min. A drop of 1:20 dilution of the anti-guinea-pig-IgG sheep serum conjugated with FITC was then placed on the smears followed by incubation in wet chamber at 37°C for 1 hour. Subsequently, the slides were washed in PBS as above. For the control preparations anti-goose parvovirus serum produced in guinea-pigs was used instead of anti-ABU serum. All preparations were mounted with glycerin-PBS (9:1) and examined with a fluorescent microscope.

Immunofluorescent positive cells were visible in all specimens taken from the infected birds between days 1 and 18 p.i. Brilliant nuclear staining was seen in the epithelial cells of the gut, the most characteristic representatives of which are shown in Fig. 1. The antigen filled up the whole nucleus, however, a more brilliant nuclear margin or granules scattered throughout the nucleus were often seen. In preparations made from uninfected birds or when anti-chicken parvovirus serum was replaced by anti-goose parvovirus serum neither nuclear nor cytoplasmic fluorescence was observed.

These findings indicate that the virus studied replicates in the nucleus of infected cells. This result together with the properties of the viral particles described before (Kisary *et al.*, 1984) suggests that this virus belongs to the Parvoviridae. It appears that there is no antigenic relationship with the goose parvovirus since no specific fluorescence could be detected when anti-goose parvovirus serum was used. This agrees with our observation that the goose parvovirus is not neutralised serum against chicken parvovirus (Kisary, unpublished data).

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RESUME

Immunofluorescence indirecte appliquée au diagnostic de l'infection par les parvovirus chez les poulets de chair

La fluorescence du noyau a été observée dans les cellules épithéliales du duodénum de poulets de chair infectés expérimentalement à l'âge d'un jour avec un parvovirus isolé chez le poulet. Aucune relation antigénique n'a été décelée par cette méthode entre les parvovirus du poulet et ceux de l'oie.

ZUSAMMENFASSUNG

Die indirekte Immunofluoreszenz, eine Möglichkeit für die Diagnose von Parvovirusinfektionen bei Broilern

In den Epithelzellen des Duodenums wurde bei den experimentell als Eintagsküken

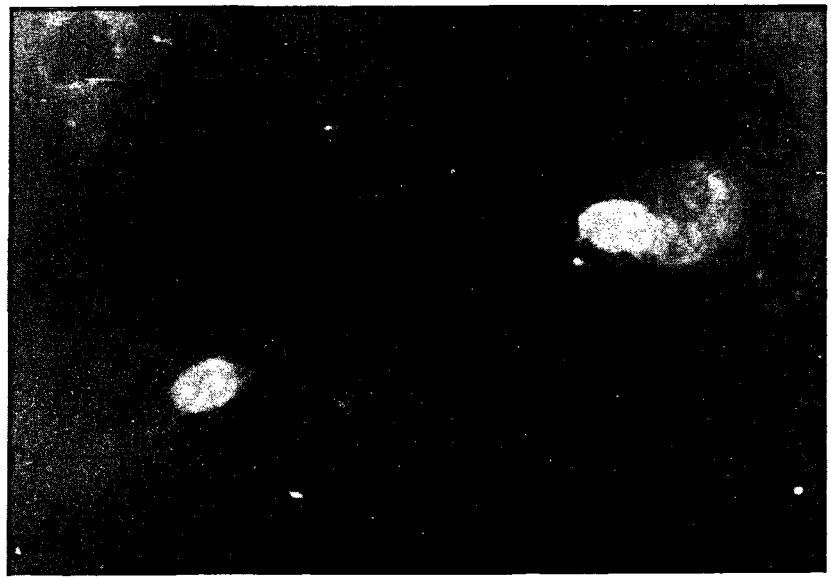

Fig. 1. Nuclear fluorescence in the epithelial cells of the duodenum of chickens infected experimentally at 1-day-old with the chicken parvovirus strain ABU.

mit Hühnerparvovirus infizierten Broilern Kernfluoreszenz beobachtet. Mit dieser Methode konnte keine Antigenverwandtschaft zwischen Hühner- und Gänseparvovirus nachgewiesen werden.

RESUMEN

La inmunofluorescencia como un instrumento de diagnostico de la infección por parvovirus en pollo de engorda

Se ha observado fluorescencia en los núcleos de células epiteliales del duodeno de pollitos infectados experimentalmente a un día de edad con un parvovirus aislado a partir de pollos. No se detectó una relación antigénica por medio de este mêtodo entre los patvovirus de pollo y de ganso.

Se han observado recientemente pequeñas partículas virales de 19-24 nm de diámetro con una flotación en CsCL de 1.43 g/ml, en el intestino de pollitos con síntomas de enanismo (J. Kisary *et al.*, 1984). Dicho virus ha sido clasificado tentativamente como un nuevo miembro de la familia Parvoviridae. Estos virus causaron baja de la incubabilidad, reducción del crecimiento, mal emplumado y desórdenes óseos cuando se infectaron experimentalmente a embriones de pollo y a pollitos de un día de edad (Kisary, 1984). Estos hallazgos sugieren fuertemente que los parvovirus originarios de pollo pueden jugar un papel fundamental en la etiología del enanismo infeccioso o síndrome de la mala absorción en pollos.

Debido a que han fallado los intentos de cultivo del parvovirus en embrión de pollo y en fibroblastos de embrión de pollo, hemos elaborado una técnica de inmunofluorescencia (IF) indirecta para monitorear las infecciones por parvovirus en parvadas de pollo.

Esta técnica ha sido también empleada para determinar las células blanco primarias en el intestino para la infección viral y de esta manera obtener más información acerca del sitio de replicación del virus en una célula infectada.