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17) USE OF REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR) FOR THE DIAGNOSIS OFAVIAN VIRAL ARTHRITIS

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Summary
Viral arthritis or infective tenosynovitis is a contagious disease of chickens and more rarely, turkeys. It causes articular symptoms and lesions of the synovia tendon structure. There are various diagnostic tests used today including RT-PCR (reverse transcription-polymerase chain reaction) which can be used both as support diagnosis, as a confirmation of infected cell cultures as well as directly in the homogenised materials obtained from pathological tissue taken during necropsy
Key words: avian reovirus, RT-PCR, viral arthritis tenosynovitis.

Applicazione della reverse transcriptase-polymerase chain reaction (RT-PCR) nella diagnosi dell'artrite-tenosinovite virale

Riassunto
L'artrite virale o tenosinovite infettiva è una malattia contagiosa del pollo e, più raramente, del tacchino. Si manifesta con sintomi articolari e lesioni a carico delle strutture sinoviali tendinee. Diversi sono gli strumenti diagnostici attualmente in uso, tra questi, la tecnica di RT-PCR (reverse transcriptase-polymerase chain reaction), applicabile sia come diagnosi di supporto e conferma alle colture cellulari infettate, sia direttamente all'omogenato ottenuto da tessuti patologici prelevati in sede autoptica.
Parole chiave: reovirus aviare, RT-PCR, artrite-tenosinovite virale

Introduction

Avian reovirus is associated in broilers to a series of pathologies including contagious arthritis tenosynovitis and malabsorption syndrome. Viral arthritis mainly affects heavy meat producing breeds from an age of between one and three months. The disease is transmitted by both vertical and horizontal pathaways. Contaminated faeces, food and drink are the most important contagious sources. Morbidity varies according to age and mortality is usually low except when there are bacterial complications. The symptoms occur in the chicks at three to four weeks of age and include mono- and bilateral lameness. In breeding birds there is delayed growth and lameness, sometimes with complete immobility of the tibiotarsic articulation sometimes leading to the breaking of the gastrocnemium tendons with subsequent haemorrhage of the peri-articular tissue. This causes economic loss, which results not only from mortality but also from the reduced performances and the inability to consume birds with obvious articular lesions when slaughtered. Direct (bio-security) and indirect (vaccination of the breeders) preventive techniques have partially contained the spread of this disease even if this is not complete. It is ever more necessary therefore, to have an early diagnostic technique that can allow us to organise intervention plans adapted to this pathology. Thus, PCR that can be applied directly to pathological samples has been considered particularly useful.

Material and methods

Six clinical cases were taken which had an anatomo-pathological profile which could be attributed to avian viral arthritis. These were broilers of between three and five weeks of life, with articular symptoms including lameness and ankylosis. From an anatomo-pathological point of view there was mainly bilateral arthritis and tenosynovitis, shortening of the tibia, flattening and lateral slipping of the gastrocnemium tendons as well as peritendonal and intra-articular yellow serous exudates

The samples for the virological exam (cell cultures and RT-PCR) were prepared starting with the tendons and exudates. At the same time other samples of the same materials were taken for the microbiology laboratory and the PCR for the *Mycoplasma synoviae* as differential diagnoses.

Viral Isolation

The initial sample was homogenised with antibiotated PBS, centrifuged at 3000 x g and filtered at 450nm. The primary liver cultures of chicken SPF (specific pathogen free)(Spafas®, USA) embryos (13 days) were used for the avian reovirus (ARV) () research. The primary liver cultures were then infected once they had fully confluenced They were observed using an optical microscope once every 24 hours to check for any possible syncytium cytopathic effects, which are typical of ARV. An electronic microscope test was used as confirmation of the virus (Lavazza et al. 1990).

ARV identification by RT-PCR

The extraction and purification of viral RNA was carried out both directly on the pathological material taken from the autoptical site and on the cell suspension usingTrizol-Reagent (Invitrogen®, USA): this is a monobasic phenol and isothiocyanate gaunidinium solution used according to the modified technique described by Chomezynski and Sacchi (1987). Once we had obtained our RNA sample we proceeded to the retro-transcription using the ProstarTM Kit (Stratagene®, USA).

The product of the retro-transcription was then used directly in the PCR with the addition of the relevant reagent and the tampon (Accuprime TaqPCRx DNA Polymerase) (Invitrogen®, USA). The MK87 (5' GGT GCG ACT GCT GTA TTT GGT AAC 3') forward primer and the reverse primer MK88 (5' AAT GGA ACG ATA GCG TGT GGG 3') acted as the trigger for the amplification reaction. These primers were selected on the basis of published data on the S1 gene of the avian reovirus (Zhixun et al. 1997) , that is different from the mammal reovirus (Shapouri et al, 1995)., and they produce a fragment of 532 bp DNA contained in the S1 gene sequence.

The reaction occurred according to the following amplification profile: 94°C for 5 minutes, (hot start) followed by 35 cycles of three steps: 94°C for one minute (denaturation), 55°C for one minute (annealing), 72°C for one minute (extension), at the end of cycles there was an additional 10 minutes at 72°C for any possible extensions. The amplified result then underwent an electrophoresis course in agarose gel at 1% with ethidium bromide and was visualised using a UV trans-illuminator.

Results and Discussion

Four of the six samples analysed from different clinical cases were positive on the cell cultures and confirmed by electronic microscopy. Their positivity was further confirmed by the RT-PCR technique, both directly on the pathological samples after approximately 48 hours and on the cell suspension after 15 days.

Conclusions

These first results show the validity of RT-PCR in identifying avian reovirus even from pathological material taken from the necroscopic site. Moreover, this method represents a rapid and sensitive alternative to traditional culture methods which require specialised media and reagents and are time-consuming. Results of PCR test can be obtained in two days, as opposed to the usual one to three weeks for isolation and identification of reovirus.

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