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Real-time PCR for diagnosis of three common infectious diseases in caged birds: chlamydophilosis, psittacine beak and feather disease and avian polyomavirosis

INTRODUCTION

Although clinical signs in affected birds can lead to a presumptive diagnosis, definitive diagnosis of infectious diseases often involves demonstration of virus/bacteria presence. Pathogen genome detection by PCR provides helpful results either for a clinical case investigation or for the detection of asymptomatic carriers. A quantitative tool can make interpretation easier. Three real-time PCR assays for caged-birds infections and their interest are described below.

SUMMARY

***Chlamydophila psittaci* (CP), Beak and Feather Disease Virus (BFDV) and Avian Polyomavirus (APV) are common pathogens in psittacine birds. Diagnosis testing can be used to confirm or invalidate a clinical suspicion. Furthermore, as these diseases are very contagious, asymptomatic carriers have to be identified to prevent dissemination and economic losses in facilities and to reduce the zoonotic risk with Chlamydophila.**
The Polymerase Chain Reaction (PCR) is an effective method for the detection of these three different pathogens. Whereas these assays can be sensitive and specific, the provided qualitative results are often inconclusive: what is the meaning of the presence of the detected pathogen? For example, is it an acute or a subclinical infection?
Real-Time PCR is an advanced PCR-based technology which permits to assess the quantity of one specific agent in various biological samples. Here, this technique is described and the interest to perform Real-Time PCR analyses in birds medicine is shown through some clinical examples. Direct diagnosis with a quantitative approach produces a conclusive result when others assays may sometimes not provide any solution for avian practitioners.

MATERIALS AND METHODS

Biological samples and DNA isolation

EDTA blood and feather calamus samples have been screened for BFDV and APV and cloacal swabs for *C. psittaci*. DNA was extracted from each field sample with High Pure PCR Template Purification Kit (Roche).

PCR Internal Control

A synthetic DNA was added in each sample at the first step of the DNA extraction. The extraction yield and the absence of PCR inhibitors were checked with a specific real-time PCR.

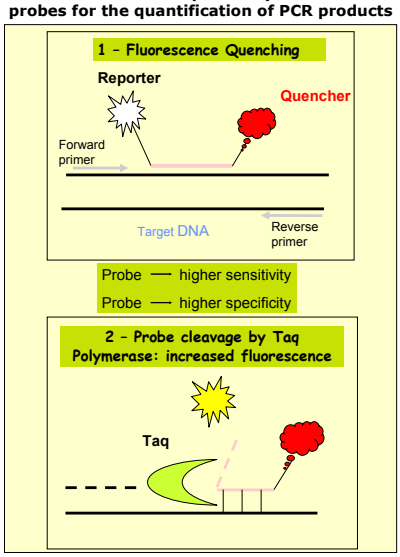
BFDV conventional PCR

A specific assay was used to amplify a fragment of BFDV genome of 690 bp. 40 PCR cycles were performed and PCR products run in an 1.5 % agarose gel.

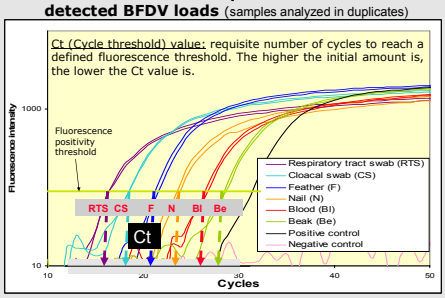
Real-Time PCR

The TaqMan® system, an improved PCR-based technology, was used on an 7900HT SDS (Applied Biosystems). Conditions: 2X UDG Platinum® Quantitative PCR (Invitrogen), a specific probe (100 nM) and primers (600 nM) for 50 cycles. The limit of detection of PCR was assessed on quantified plasmids.

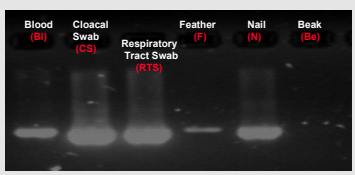
Real-time PCR in TaqMan® system: a PCR with internal probes for the quantification of PCR products



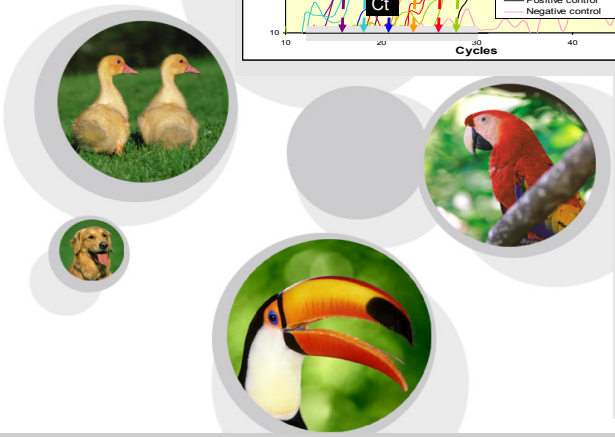
A: Relative real-time quantification of the detected BFDV loads (samples analyzed in duplicates)



B: BFDV conventional PCR



Samples from a 9-years old *Cacatua alba*, clinically infected with BFDV for 5 years, with severe feathers and beak disorders.



C: Real-time *vs* conventional PCR: different samples from this clinical case of PBFD

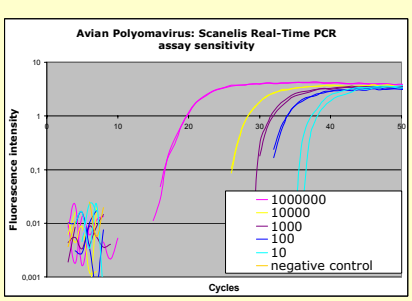
Quantification	
Conventional PCR	Real-Time PCR
CS ≈ RTS	RTS ≈ 10 x CS
Bl > F	F ≈ 100 x Bl

• **End-point analysis (conventional PCR):**
no possible quantification

• **Real-time PCR:**
quantitative or semi-quantitative results

RESULTS: examples of the interest of Real-Time PCR in birds medecine

1. A higher sensitivity for a better detection of healthy carriers



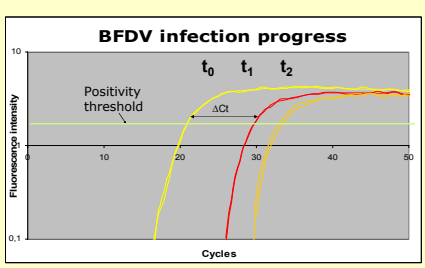
As the detection limit for our APV, BFDV and CP assays is less than 10 copies, the detection of asymptomatic but infected birds is therefore improved. Then, any trace of virus/bacteria can be detected, allowing prevention of pathogen dissemination in the breeding.

2. A tool to interpret chlamydophilosis testing results

❑ The bacterial load can be helpful to assess the role of the detected *Chlamydophila psittaci* in the clinical signs observed:
- *Chlamydia* can be shed in droppings by asymptomatic birds: generally, low bacterial load is then detected in cloacal swab.
- However, very significant load can be detected in air sac swab in an animal with respiratory disorders or in conjunctival cells in a case with ocular signs.
Taking account of the bird clinical signs, the quantitative result makes the interpretation easier to distinguish healthy carrier from ill animals.

❑ Shedding measurement could also be used to assess the zoonotic potential (**Psittacosis**) of an infected bird.

3. Viral/Bacterial load monitoring



❑ For one bird, the relative quantification of the viral load at two different times provides very interesting information about the infection progress.

❑ In this example, the ΔCt between t_0 and t_1 (≈ 8) indicates that the virus load was 200-fold decreased.

❑ Other application: to assess the efficiency of a vaccine

CONCLUSIONS

There are many applications of the real-time PCR in the veterinary field. Few examples were presented in this poster. Compared to conventional PCR, the higher sensitivity and the possible quantification improve the reliability of the result.
So Real-Time PCR presents essential assets for routine diagnostic: quantitative results, high specificity and sensitivity, no carry-over contaminations and possibility of high-throughput analyses.

Suspicion of chlamydophilosis: think of PBFD also!

In four recent clinical suspicions of chlamydophilosis, no chlamydia but high load of BFDV was detected in parrot organs.
A sudden-death but also various signs were observed as nervous signs, hepatomegaly or splenomegaly, …

PERSPECTIVES

To increase the diagnostic possibilities for the vet, development of other molecular tests are in progress

- in the infectious diseases field: a Pacheco's disease test, a quantitative assay for Aspergillosis diagnosis,
- in the genetic field: a new bird sexing test.