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Research in Veterinary Science 79 (2005) 259–264
RVS
www.elsevier.com/locate/rvsc

A PCR technique for the detection of Jaagsiekte sheep retrovirus in the blood suitable for the screening of ovine pulmonary adenocarcinoma in field conditions

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Accepted 15 February 2005

Abstract

Ovine pulmonary adenocarcinoma (OPA) is a naturally occurring contagious lung neoplasia caused by jaagsiekte sheep retrovirus (JSRV). Although no specific circulating antibodies against the virus can be detected in infected sheep, JSRV proviral DNA sequences can be found in peripheral blood leukocytes (PBLs) in clinically affected and in a proportion of in contact animals. In this study, existing hemi-nested PCR procedure is compared with a new one-step PCR technique that was developed to minimise potential DNA contamination and reduce sample and reagent handling. Different blood preparations were assessed and the best results were achieved on DNA prepared from buffy coat. The sensitivity of this PCR was lower in JSRV infected sheep without lesions of OPA than in clinically affected sheep, which indicate that this PCR may not be not fully appropriate for screening of individual sheep, but rather to provide results at flock level. This PCR is the only currently available blood test for detection of JSRV infected sheep and may be useful in epidemiological studies and in control programmes of OPA.

Keywords: Jaagsiekte; Retroviruses; Sheep; Blood test; PCR

1. Introduction

Ovine pulmonary adenocarcinoma (OPA, sheep pulmonary adenomatosis, Jaagsiekte) is a contagious lung cancer that occurs naturally in sheep and rarely in goats. It is caused by an exogenous betaretrovirus, known as jaagsiekte sheep retrovirus (JSRV) (Palmarini et al., 1999). The tumours are derived from Type II pneumocytes and Clara cells and as these cells are secretory, a considerable amount of fluid can be generated and accumulated in the respiratory tract of the affected animal. Diagnosis of OPA is possible when clinical signs or tumours are observed (De las Heras et al., 2003) and the presence of JSRV can be confirmed in lung fluid or tumours by immunoblotting (Sharp and Herring, 1983), ELISA (Palmarini et al., 1995) or PCR (Bai et al., 1996; Palmarini et al., 1996a) However, it is more difficult to identify infected animals during the pre- clinical period due to the lack of detectable JSRV proteins outside the tumour (Palmarini et al., 1995) and of circulating JSRV-spe-

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0034-5288/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2005.02.003

cific antibodies (Sharp and Herring, 1983; Ortín et al., 1998). In spite of this, using a specific PCR for JSRV-LTR, proviral DNA and JSRV RNA transcripts were found in tissues of the lymphoreticular system (LRS) and peripheral blood mononuclear cells (PBMC) from sheep affected by OPA and not in unaffected sheep or control animals (Palmarini et al., 1996b). Improved PCR techniques have demonstrated JSRV proviral DNA in LRS and PBMC prior to the onset of neoplasia in experimentally inoculated lambs (Holland et al., 1999) and during the pre-clinical period of the natural disease in commercial sheep flocks (Gonzalez et al., 2001). These previous reports employed a hemi-nested PCR procedure (U3hn PCR) which is very sensitive and specific, but suffers from the drawback of a high risk of contamination, particularly when large numbers of samples are processed, giving an increased chance of false positives. We present in this paper a modified single-step PCR protocol (U3 PCR) and its application to blood samples from commercial sheep flocks. The comparison between U3hnPCR and U3PCR performed on different blood preparations are presented, as well as the assessment of the performance of the U3PCR on DNA extracted from the buffy coat of animals with different JSRV/OPA status.

2. Materials and methods

2.1. Animals

2.1.1. Group A: JSRV infected sheep with no evidence of disease

Six sheep aged 8–10 years belonging to a Rasa Aragonesa breed flock with a long history of OPA were bled at 20 day intervals on three occasions and humanely killed according to local euthanasia regulations. Both PCR procedures described for JSRV detection were performed on DNA extracted from whole blood (WB), peripheral lymphocytes (LP) and white blood cells (WBC, buffy coat). None of these animals showed gross or histopathological OPA lesions, but the mediastinal lymph nodes were positive for JSRV proviral DNA by U3PCR, by U3hn PCR or by both (Table 1).

2.1.2. Group B: sheep with OPA lesions but no clinical evidence of the disease

Five sheep from two flocks with a long history of OPA showed small lesions of OPA at post-mortem examination, which were confirmed histologically and immunohistochemically using previously described procedures (Palmarini et al., 1995). WBC from one bled of these sheep was tested by U3PCR.

Table 1
PCR results for sheep from group A comparing U3 and U3hn PCR and different methods for preparation of DNA from blood

Sheep no.	Sample	Lymphoprep		Buffy coat		Whole blood		Mediastinal lymph node	
		U3	U3hn	U3	U3hn	U3	U3hn	U3	U3hn
1	1	2	1	1	0	0	0		
	2	1	0	1	3	0	1		
	3	1	2	0	0	0	0	1	2
2	1	2	2	2	2	0	1		
	2	1	0	0	1	1	1		
	3	0	0	0	0	0	1	1	1
3	1	2	0	1	1	1	0		
	2	0	1	0	0	0	1		
	3	0	2	0	0	0	1	1	1
4	1	0	2	0	0	1	0		
	2	0	0	1	0	0	2		
	3	0	2	0	0	1	0	1	1
5	1	0	1	1	0	2	1		
	2	0	0	0	0	0	2		
	3	0	3	1	0	0	0	1	2
6	1	0	0	2	2	0	3		
	2	1	0	2	0	0	0		
	3	1	1	0	0	0	0	1	1
Animals pos. ($n = 6$)		4	6	6	4	4	6	6	6
Samples pos. ($n = 18$)		8	10	9	5	5	10		
Concordance		44%		67%		39%			
Replicates pos. ($n = 54$)		11	17	12	9	6	14		

Three bleeds taken at 20 day intervals were analysed for each of six sheep. Results are shown as number of positive PCR results from triplicate reactions. Results for mediastinal lymph nodes are also noted.