BIOCHEMICAL CHANGES ASSOCIATED WITH EXPERIMENTAL ORF INFECTION IN SHEEP AND GOATS

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ABSTRACT

The inoculation of sheep and goats with contagious pustular dermatitis (orf) virus manifested typical clinical signs indicative of orf viral infection. The inoculated animals sero converted and showed significant decrease in total proteins. A significant increase was seen in the rectal temperature, haematocrit values, concentration of cortisol, glucose, creatinine, aspartate aminotransferase and creatinine kinase but not in lactic dehydrogenase.

Key words: Biochemical changes, orf infection, sheep, goats.

INTRODUCTION

Contagious pustular dermatitis (CPD) or orf is a specific disease of sheep and goats, caused by aparapox virus and occurs almost in all countries where sheep and goats are raised (Darbyshire, 1961; Mazur and Machado, 1989). The disease causes huge losses among animals by reducing the growth rate of affected lambs and kids and inducing serious pain and distress to ewes and sucking lambs.

In an earlier study (Housawi *et al.*, 1993), a clinico-pathological and serological response of experimentally orf-infected sheep and goats was investigated. The primary purpose of the present study was to determine biochemical changes associated with orf infection in sheep and goats.

MATERIALS AND METHODS

Reference orf scabs (Makkah strain) was prepared and used as described by Housawi *et al.* (1991) and Housawi *et al.* (1993).

Experimental animals:

Twenty mature (6 months old) each of Najdi sheep and Awassi goats of either sex were used in this study. Animals of each species were divided randomly into two equal groups A and B. Group A was kept as uninfected control and Group B was used as infected group. All animals were allowed free access to hay and water.

Experimental infection:

Dermal skin around the mouth of experimental sheep and goats was scarified and injected with inoculum using a previously described procedure (Housawi *et al.*, 1993). The animals were examined daily for development and progression of orf lesions. Blood samples were collected from Jugular vein, serum was separated and kept at –20 °C until used. Scabs from infected animals were collected in sterile container awaiting identification, as reported elsewhere (Housawi and Abu-Elzain, 2000).

Clinical observations:

All animals were closely observed for clinical signs. Their rectal tempertures and weights were recorded for 5 weeks.

Assays of samples:

Haematocrit and total proteins were determined by a microhaematocrit technique and a refractometer (Bellman and Stanly Ltd., UK), respectively. Blood chemistry analyses were conducted on each sample using 32µl of serum per assay and examined using a dry chemistry analysis system (RefLotron, Boehringer-Mannhein, Germany). Serum cortisol concentration was measured by radioimmunoassay kit (Cambridge Medical Technology, Billerica, MA, USA). The intra and inter-assay coefficients of variation were 7 and 9% respectively. Extraction efficiency was 86% and results were corrected for procedural losses.

The virus was reisolated and identified. The virus was isolated in vero cells and re-identified by Agar precipitation test and serum neutralization test, as reported earlier (Housawi and Abu-Elzain, 2000).

Data analysis:

The data were examined for statistical differences by analysis of variance and least significance difference tests.

RESULTS

Typical orf lesions were observed on the skin of the infected sheep and goats. The lesions started by ecthyma 3 day post infection (Pi). This was followed by the appearance of small papules and pustules. A week later, the pustules increased in size and developed into irregular crusty scabs which remained for 5 weeks before eventually falling off.

Rectal temperature of control animals remained relatively constant, while that of infected animals was significantly ($P<0.05$) elevated by 1-2 °C. Excessive salivation and anorexia were observed concommittently with skin lesions.

Haematocrit values became elevated ($P<0.01$) by day 2 Pi (Table 1). The effects of orf infection on plasma concentrations of aspartate aminotransferase (ASAT), creatinine kinase (CK), lactic dehydrogenase (LD) and cortisol are shown in Table 2. The inoculated animals showed a significant ($P<0.01$) increase in the activity of ASAT (45-55%), CK (33-55%) and cortisol (47%) but not in LD concentration by day 2 Pi compared to control animals. Serum creatinine (50%)

Table 1: Mean ± (SD) rectal temperature (T,°C), PCV (%) and body weight (BW,Kg) of sheep and goats experimentally infected with orf virus.

Experimental Period (weeks)	Sheep						Goats					
	Control			Infected			Control			Infected		
	T	PCV	BW	T	PCV	BW	T	PCV	BW	T	PCV	BW
1	39.3 ± 0.2	28.1 ± 0.1	29.2 ± 0.3	41.1* ±0.2	34.2* 0.1	27.3 ±0.3	39.3 ±0.2	28.1 ±0.1	27.0 ±0.3	40.9* ±0.2	34.1* ±0.1	29.1 ±0.3
2	39.5 ± 0.2	26.0 ± 0.1	29.6 ± 0.3	39.3 ±0.2	32.6 ±0.1	26.6 ±0.2	39.2 ±0.2	29.1 ±0.1	27.3 ±0.3	39.2 ±0.2	29.4 ±0.7	29.2 ±0.2
3	39.6 ± 0.2	27.9 ± 0.1	31 ± 0.2	39.5 ±0.2	28.1 ±0.1	27.1 ±0.2	39.1 ±0.2	28.8 ±0.1	27.8 ±0.3	39.3 ±0.2	26.3 ±0.1	28.8 ±0.2
4	39.2 ± 0.2	28.1 ± 0.1	31.2 ± 0.3	39.4 ±0.2	29.1 ±0.1	27.2 ±0.3	39.3 ±0.2	29.1 ±0.1	28.3 ±0.3	39.4 ±0.2	28.0 ±0.1	28.9 ±0.3
5	39.4 ± 0.2	29.0 ± 0.1	31.6 ± 0.3	39.5 ±0.2	26.9 ±0.1	27.4 ±0.3	39.4 ±0.2	30.0 ±0.1	28.5 ±0.2	39.2 ±0.2	29.1 ±0.1	29.3 ±0.2

* Significantly different from controls ($P<0.05$)

Table 2: Total protein (TP, g/dl), glucose (G, mmol/L) and creatinine (C, umol/L) concentrations of sheep and goats experimentally infected with orf virus.

Experimental Period (weeks)	Sheep						Goats					
	Control			Infected			Control			Infected		
	TP	G	C	TP	G	C	TP	G	C	TP	G	C
1	7.4 ±0.2	2.7 ±0.1	61 ±3	7.4 ± 0.2	4.5* ± 0.1	91 ± 3	7.3 ± 0.2	3.4 ± 0.1	60 ± 3	7.2 ± 0.2	4.2 ± 0.1	89 ± 3
2	7.2 ±0.2	3.2 ±0.1	60 ±2	5.6* ± 0.2	3.4* ± 0.1	85 ± 3	7.4 ± 0.2	3.3 ± 0.1	62 ± 3	5.5* ± 0.2	3.6* ± 0.1	79 ± 3
3	7.3 ±0.2	3.4 ±0.1	62 ±3	5.5* ± 0.2	3.7 ± 0.1	65 ± 3	7.2 ± 0.2	3.6 ± 0.1	60 ± 2	5.6* ± 0.2	3.9 ± 0.1	62 ± 3
4	7.2 ±0.2	3.8 ±0.1	59 ± 3	5.4* ± 0.2	3.4 ± 0.1	60 ± 3	7.3 ± 0.2	3.3 ± 0.1	64 ± 3	5.7* ± 0.2	3.3 ± 0.1	60 ± 3
5	7.3 ±0.2	3.5 ±0.1	58 ± 3	5.6* ± 0.2	3.5 ± 0.1	62 ± 3	7.4 ± 0.2	3.5 ± 0.1	57 ± 3	5.7* ± 0.2	3.4 ± 0.1	61 ± 3

* Significantly different from controls ($P<0.01$)

Table 3: Serum aspartate aminotransferase (AST, U/L), creatinine kinase (CK, U/L), lactic dehydrogenase (LD,U/L) and cortisol (C, mmol/L) levels in sheep and goats experimentally infected with orf virus.

Experimental Period (weeks)	Sheep								Goats							
	Control				Infected				Control				Infected			
	AST	CK	LD	C	AST	CK	LD	C	AST	CK	LD	C	AST	CK	LD	C
1	90 ± 3	60 ± 2	820 ± 15	86 ± 2	130* ± 8	80* ± 2	810 ± 10	120 ± 7	86 ± 3	62 ± 2	790 ± 10	89 ± 3	142* ±10	96* ± 3	810 ±10	130* ± 8
2	92 ± 3	65 ± 3	750 ± 15	90 ± 2	110 ± 8	75 ± 2	760 ± 15	95 ± 3	91 ± 4	59 ± 2	795 ± 10	92 ± 2	115 ± 6	85 ± 3	710 ± 10	95 ± 3
3	89 ± 3	62 ± 3	780 ± 15	92 ± 2	96 ± 3	66 ± 2	770 ± 15	80 ± 2	90 ± 3	62 ± 3	802 ± 15	94 ± 3	94 ± 3	60 ± 3	720 ± 12	80 ± 2
4	90 ± 2	64 ± 3	805 ± 15	88 ± 2	92 ± 3	65 ± 3	800 ± 15	85 ± 2	92 ± 4	64 ± 2	800 ± 10	85 ± 3	93 ± 3	65 ± 2	800 ± 12	85 ± 2
5	93 ± 3	65 ± 3	810 ± 15	95 ± 2	92 ± 3	64 ± 2	815 ± 15	80 ± 2	91 ± 3	63 ± 3	780 ± 10	83 ± 3	90 ± 3	60 ± 2	810 ± 12	83 ± 3

* = Significantly different from controls ($P<0.01$)

concentration and glucose (20%) were significantly ($P<0.01$) elevated (Table 3) but the concentration of total protein decreased in infected animals compared to controls.

DISCUSSION

All inoculated animals showed typical orf lesions and course of disease similar to that reported earlier (Housawi *et al*., 1993). The virus was reisolated.

The transient haematocrit increase during the febrile period may reflect a shift in body fluid distribution which was expected to be short-lived (Van Miert *et al*., 19830. as fever subsided by next day. The increase in ASAT and CK activity suggests that the virus induced liver and skeletal muscle damage. The lack of increase in the activity of LD indicates that damage to cardiac muscles was minimal and that is not surprising, since it is known that at Moredun Research Institute MRI, UK orf virus strain is adapted to grow in fetal lamb muscle tissue culture (Peter Nettleton, personal communication).

Infection in animals is characterized by a negative nitrogen balance (Southern and Thompson 1986) due to an increased rate of protein breakdown of skeletal muscles (Baracos *et al*., 1983). Indeed, the low protein concentration and elevated plasma creatinine observed in this study reflected an increased rate of skeletal muscle breakdown.

The inoculation of animals with orf virus caused a consistent increase in the concentration of cortisol compared to controls. This effect could be a direct one on the adrenals, as virus can induce a release of corticosterone (Smith *et al*., 1982. Al-Afaleq 1998). The increase in glucose may represent a direct effect of cortisol by virtue of its glucogenolytic and gluconeogenetic properties (Kataria *et al*., 2000).

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