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# Characterisation of putative immunomodulatory gene knockouts of lumpy skin disease virus in cattle towards an improved vaccine

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ABSTRACT

Lumpy skin disease virus (LSDV) is responsible for causing severe economic losses to cattle farmers throughout Africa, the Middle East, and more recently, South-Eastern Europe and Russia. It belongs to the *Capripoxvirus* genus of the *Poxviridae* family, with closely related sheeppox and goatpox viruses. Like other poxviruses, the viral genome codes for a number of genes with putative immunomodulatory capabilities. Current vaccines for protecting cattle against lumpy skin disease (LSD) based on live-attenuated strains of field isolates passaged by cell culture, resulting in random mutations. Although generally effective, these vaccines can have drawbacks, including injection site reactions and/or limited immunogenicity. A pilot study was conducted using a more targeted approach where two putative immunomodulatory genes were deleted separately from the genome of a virulent LSDV field isolate. These were open reading frame (ORF) 005 and ORF008, coding for homologues of an interleukin 10-like and interferon-gamma receptor-like gene, respectively. The resulting knockout constructs were evaluated in cattle for safety, immunogenicity and protection. Severe post-vaccinal reactions and febrile responses were observed for both constructs. Two calves inoculated with the ORF008 knockout construct developed multiple lesions and were euthanised. Following challenge, none of the animals inoculated with the knockout constructs showed any external clinical signs of LSD, compared to the negative controls. Improved cellular and humoral immune responses were recorded in both of these groups compared to the positive control. The results indicate that at the high inoculation doses used, the degree of attenuation achieved was insufficient for further use in cattle due to the adverse reactions observed.



## 1. Introduction

Lumpy skin disease (LSD), caused by lumpy skin disease virus (LSDV), is an acute, subacute or in-apparent infectious disease of cattle [1]. LSDV belongs to the *Capripoxvirus* (CaPV) genus of the *Poxviridae* family, with closely related sheeppox (SPP) and goatpox (GTP) viruses [2].

LSD impacts negatively concerning trade in livestock and livestock products, especially in regions where it is endemic and vaccination is not routinely practiced [3]. It is of major economic significance due to production losses incurred; decrease in hide value, and amongst severely infected cattle, prolonged recovery periods resulting in reduced growth rates [4,5]. In LSD-free countries, the disease can have a considerable financial impact due to treatment, control and trade restrictions [3].

LSD, considered an African disease, is now endemic in most Middle East countries having spread recently via Turkey and Cyprus into the European Union for the first time in 2015, with outbreaks occurring in Greece [6], and now most parts of South-Eastern Europe, with incursions into southern Russia [7] (Supplementary Fig. 1).

In endemic countries like South Africa, the disease is controlled by vaccination with live-attenuated LSD vaccines [8]. Locally, LSD vaccines available are ones produced by Onderstepoort Biological Products® (herein referred to as OBP vaccine), MSD (Lumpyvax®) and Deltamune (HerbivacLS®).

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The OBP vaccine, developed by multiple passages in cell culture does not cause generalised infection or severe signs of illness in cattle [1]. It produces a granulomatous local reaction, 1–2 cm in diameter, at the inoculation site in 50% of vaccinated animals, coupled to a temporary drop in milk yield in dairy cattle negatively affecting use of this vaccine [1]. Local reaction disappears within four to six weeks without evidence of necrosis [1]. Ben-Gera et al. [9] reported, in Israel following vaccination with the OPB vaccine, 0.38% cattle developed mild clinical disease, 0.04% severe disease, however overall reactions were minimal and the vaccine deemed safe. In contrast, during outbreaks in Greece, some OBP-vaccinated animals developed painful injection site swellings of up to 20 cm, accompanied by decline in milk production, reduction of appetite and food intake [6,10].

Since live-attenuated LSD vaccines were developed by inducing random mutations in genomes of field strains through serial passages in cell culture, the precise genetic modifications required for attenuation are unknown [11]. Homologous recombination can be used to generate live-attenuated recombinant vaccine constructs by knocking out genes specifically involved in immune modulation and/or virulence in order to attenuate the virus [12].

Our aim is to use a targeted approach to knock out (KO) putative immunomodulatory genes from the LSDV genome to develop an improved LSD vaccine. In this pilot study, we generated two LSDV constructs from which the putative interleukin-10-like (IL-10) and interferon gamma receptor-like (IFN-γR) genes were removed separately from a virulent field isolate. The two KO constructs were evaluated in Friesen-Holstein calves for safety, efficacy and ability to induce protective immune responses against virulent LSDV challenge.

## 2. Materials and Methods

### 2.1. Cells

Primary and secondary bovine cell cultures used for generation, selection and growth of the LSDV KO constructs, growth of the OBP vaccine, including determination of virus titres. Details: Supplementary Methods, 1.1.

### 2.2. Viruses

The virulent LSDV Warmbaths field isolate (LSDV_WB) used as parental virus in the development of the recombinant LSDV KO constructs, including challenge virus. A high titre of the LSD OBP vaccine (ht_LSD_OBP) used as the positive control virus. Details: Supplementary Methods, 1.2.

### 2.3. Generation and selection of recombinant KO virus

The recombinant LSDV_WB005KO construct with removal of its IL-10-like gene described by Boshra et al. [13]. Construction, generation and selection of LSDV_WB008KO with removal of its IFN-γR-like gene described in Supplementary Methods, 1.3.

### 2.4. Animals

Details: Supplementary Methods, 1.4.

### 2.5. Inoculation and challenge of cattle with LSDV

Calves injected subcutaneously (SC) with titres of $1.4 \times 10^7$ $TCID_{50}$ (tissue culture infection dose) with either LSDV_WB005KO, LSDV_WB008KO or ht_LSD_OBP vaccine (Table 1). Twenty-eight days post-inoculation (dpi) calves challenged via the intradermal (ID) route with $1.4 \times 10^7$ $TCID_{50}$/ml of the LSDV_WB isolate. Details: Supplementary Methods, 1.5.

**Table 1**
Calf identity numbers per group, and the inoculum and titre used.

| Group | Animal identity numbers | Inoculum | Titre |
|---|---|---|---|
| 1 | 33<br>41<br>44<br>48<br>50 | Cell culture medium | |
| 2 | 34<br>39<br>45<br>47<br>52 | ht_LSD_OBP vaccine[a] | $1.4 \times 10^7$ $TCID_{50}$ |
| 3 | 35<br>40<br>42<br>43<br>49 | LSDV_WB005KO | $1.4 \times 10^7$ $TCID_{50}$ |
| 4 | 36<br>37<br>38<br>46<br>51 | LSDV_WB008KO | $1.4 \times 10^7$ $TCID_{50}$ |

[a] High titre of the LSD OBP vaccine.

### 2.6. Virus isolation and detection

Details: Supplementary Methods 1.6 and 1.7.

### 2.7. Measurement of humoral and cell-mediated immune responses.

Details: Supplementary Methods 1.8–1.14.

## 3. Results

### 3.1. Generation and selection of the recombinant LSDV_WB008KO virus

A recombinant LSDV KO construct deficient of its IFN-γR gene homologue (ORF 008) generated as described in Supplementary Results, 1.

### 3.2. Safety of the LSDV_WB005KO and LSDV_WB008KO constructs

Following inoculation, all five calves in the negative control group (Group 1) had no inoculation site reactions, while one (52) of the five calves in the positive control (Group 2), developed a lesion (2 × 3 cm in size) at 5 dpi, eventually resolving by 15 dpi. Febrile responses were not observed in calves in groups 1 and 2.

Four of five LSDV_WB005KO-inoculated calves 35, 42, 43 and 49 (Group 3) developed inoculation site reactions early as 4 dpi, while calf 40 developed no reaction. Swellings around these sites were between 2 and 5 cm in size. Eight dpi, calves 35 and 43 had swellings of 15 and 30 cm respectively, while 9 dpi calves 42 and 49 had swellings of 5 and 10 cm respectively (Supplementary Fig. 5A). Fourteen dpi, 6 nodules observed in calf 49, while calf 43 developed multiple diffuse nodules around the right cranial area ranging from 2 to 3 mm in diameter. Twenty-five dpi, calf 35 died of rumen acidosis, unrelated to the LSDV inoculation.

Four of the LSDV_WB005KO-inoculated calves 35, 40, 43 and 49 displayed increased rectal temperatures lasting 6, 7 and 8 days in calves 43, 49 and 35 respectively. Calf 40 had a rise in temperature only on 16 dpi (Fig. 1A).

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