

Evaluation of homologous and heterologous protection induced by a virulent field strain of orf virus and an orf vaccine in goats

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Objective—To evaluate cross protection provided by administration of contagious ecthyma vaccines against strains of orf virus in goats.

Animals—126 Boer-Spanish crossbred goats (3 to 20 days old).

Procedures—85 goats were vaccinated with a goat-derived contagious ecthyma vaccine. Of these, 41 were challenge exposed with the virus strain for the contagious ecthyma vaccine, 40 were challenge exposed with a more virulent field strain of orf virus, and 4 were lost to predation or died. Another 41 goats were vaccinated with a vaccine produced from a more virulent field strain of orf virus; of these, 18 were challenge exposed with the virus strain of the goat-derived contagious ecthyma vaccine, 18 were challenge exposed with the more virulent field strain of orf virus, and 5 were lost to predation or died.

Results—Vaccination with the goat-derived contagious ecthyma vaccine did not significantly reduce the number of goats with lesions or lesion severity caused by challenge exposure with the more virulent field strain of orf virus. Vaccination with the vaccine produced from the more virulent field strain of orf virus significantly reduced the number of goats with lesions attributable to challenge exposure with the virus strain of the goat-derived contagious ecthyma vaccine, but it failed to significantly reduce lesion severity.

Conclusions and Clinical Relevance—Vaccination did not result in cross protection for the 2 strains of orf virus. This may have been attributable to antigenic differences and may be a factor in outbreaks of contagious ecthyma in vaccinated goats. (*Am J Vet Res* 2012;73:86–90)

The first vaccine for CE (commonly referred to as orf, scabby mouth, sore mouth, and contagious pustular dermatitis) was developed at the Texas Agricultural Experiment Station (currently the Texas AgriLife Research Center) at Sonora, Tex, in the 1930s.^{1,2} The vaccine was labeled for use in sheep, but it was efficacious when used in goats. The etiologic agent that causes the disease of CE in sheep and goats is referred to as orf virus or CE virus. In the late 1990s, there were anecdotal reports of epizootics of a persistent, generalized form of CE in goats vaccinated with the vaccine labeled for use in sheep. A study³ conducted to evaluate the effectiveness of the vaccine revealed that it was ineffective for protecting goats against the strain of orf virus that caused the persistent, generalized form of CE in goats. Results of that study³ prompted development

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ABBREVIATION

CE Contagious ecthyma

of a CE vaccine from viruses obtained during outbreaks of CE in goats.⁴

In Texas, the goat-derived CE vaccine was used successfully in goats for a few seasons. However, in the early and mid-2000s, outbreaks of more virulent CE, mainly in Boer goats and Boer-Spanish crossbred goats, were consistently being reported in goats vaccinated with the goat-derived CE vaccine^{5,a} (Figure 1). The specific reasons for the vaccination failures are unknown, but variability among animals and breeds,⁵ nutritional differences,⁶ incorrect vaccine administration,⁷ antigenic differences in strains of the orf virus,^{5,8} and environmental conditions⁹ have been postulated.

Therefore, the objective of the study reported here was to determine whether young goats properly vaccinated with the goat-derived CE vaccine would be protected when challenge exposed with a field strain of the orf virus obtained from more virulent CE infections (virulent strain of orf virus). Furthermore, we evaluated whether vaccination with a vaccine developed from the virulent strain of orf virus would induce protection against challenge exposure with the strain of virus contained in the goat-derived CE vaccine.

Materials and Methods

Animals—Boer-Spanish crossbred goats (3 to 20 days old; $n = 126$) were used in the study. Boer-Spanish crossbred goats are a prevalent goat breed found in the goat-raising area of Texas and are the animals predominantly reported to have the more virulent form of CE. Goat kids were from dams that had been vaccinated with the CE vaccine labeled for sheep when the dams were kids. Dams and kids were pastured together on approximately 145 hectares. None of the goats had clinical signs of CE at the time of the study. The study was approved by the Institutional Animal Care and Use Committee of Texas A&M University.

Preparation of vaccine and material for challenge exposure—Two vaccine preparations were evaluated. The goat-derived CE vaccine was prepared as described elsewhere.⁴ The vaccine for a virulent field strain of orf virus was prepared from scabs collected from active-disease lesions located around the mouth and nares of goats that had been vaccinated with the goat-derived CE vaccine. The vaccine for the virulent field strain of orf virus was prepared by modification of a procedure described elsewhere.⁴ Briefly, scabs were combined and placed in a desiccator for 4 to 7 weeks. Scabs were then ground slowly in a grinding mill. The ground scabs were passed through a coarse sieve to remove hair and then were further ground manually by use of a mortar and pestle. The material was desiccated for an additional 2 to 3 weeks. Finally, the material was ground in a ball grinder. The powdered scab material was divided into 65-mg aliquots and stored in glass vials at 4°C until used. At the time of vaccination or challenge exposure, each aliquot of powdered scab material was mixed with 6 mL of 30% glycerin-saline (0.9% NaCl) solution.

Vaccination and challenge exposure—The study involved parallel groups. Goats were randomly allotted into 2 groups. Group 1 ($n = 85$) was vaccinated with the goat-derived CE vaccine, and group 2 (41) was vaccinated with the vaccine created from the virulent strain of orf virus. Predation loss ($n = 7$) and death (2) resulted in 81 goats in group 1 and 36 goats in group 2 for use in challenge exposure.

Ten weeks after vaccination, goats in each of the 2 groups were randomly allocated for challenge exposure with the strain of the goat-derived CE vaccine or the virulent strain of orf virus (every other kid in each vaccination group was assigned to the same challenge exposure). Consequently, there were 4 subgroups in the study. Group 1A ($n = 41$) was vaccinated with the goat-derived CE vaccine formulated for goats and subsequently challenge exposed with the strain of the goat-derived CE vaccine, group 1B (40) was vaccinated with the goat-derived CE vaccine and subsequently challenge exposed with the virulent strain of orf virus, group 2A (18) was vaccinated with the vaccine created from the virulent strain of orf virus and subsequently challenge exposed with the strain of the goat-derived CE vaccine, and group 2B (18) was vaccinated with the vaccine created from the virulent strain of orf virus and subsequently challenge exposed with the virulent strain of orf virus. Vaccination and challenge inoculation were performed on the hairless skin at the medial aspect of

the right and left thigh, respectively. The skin was scarifed, and approximately 0.05 mL of the reconstituted vaccine or challenge-exposure solution was applied to the scarified region.

Assessment of lesions—Lesion formation (ie, scabs) was assessed 12 days after vaccination and 10 days after challenge exposure. Scabs were graded as present or not present. If scabs were present, a lesion



Figure 1—Goats vaccinated with a goat-derived CE vaccine that developed mild (more typical) lesions of orf (A) and excessively proliferative lesions potentially attributable to the more virulent strain of the orf virus (B).

severity score was assigned by use of an ordinal scale from 1 to 4 (1 = formation of a few small scabs, 2 = mild scab formation, 3 = moderate scab formation, and 4 = excessive and severe scab formation).⁴ Assessment of goats and scoring of lesions were performed by the same investigator (JMBM).

Data analysis—Scab formation due to vaccination or challenge exposure was analyzed by use of χ^2 analysis of contingency and the Fisher exact test. Lesion severity scores were analyzed by use of the Mann-Whitney rank sum test. A value of $P \leq 0.05$ was considered significant for all analyses.

Results

Scab formation and lesion severity score—The number of goats that developed lesions as a result of challenge exposure with the virulent strain of orf virus did not differ significantly ($P = 0.482$) between the goats vaccinated with the vaccine produced from the virulent strain of orf virus (group 2) and the goats vaccinated with the goat-derived CE vaccine (group 1B). Similarly, there was no significant ($P = 0.460$) difference in lesion severity score between group 2 and group 1B.

The proportion of goats that developed lesions as a result of challenge exposure with the strain of virus in

Table 1—Lesion formation (ie, scabs) and median lesion severity score in young goats vaccinated with a goat-derived CE vaccine or a vaccine produced from a more virulent field strain of orf virus and subsequent challenge exposure (10 weeks after vaccination) with the virus strain of the goat-derived CE vaccine or the virulent field strain of orf virus.

Group*	No. of goats†	Lesion formation‡	Lesion severity score§
1	85	83/85	2.0 (2.0–2.0)
1A	41	18/41	0.0 (0.0–1.0)
1B	40	35/40 ¶	2.0 (1.0–3.0) ¶
2	41	38/41	2.0 (2.0–3.0)
2A	18	14/18#***	2.5 (1.0–3.0) ¶
2B	18	8/18††‡‡	0.0 (0.0–1.0)††§§

*Group 1 was vaccinated with the goat-derived CE vaccine, and group 2 was vaccinated with the vaccine created from the virulent strain of orf virus. Group 1A was vaccinated with the goat-derived CE vaccine and subsequently challenged exposed with the strain used for the goat-derived CE vaccine, group 1B was vaccinated with the goat-derived CE vaccine and subsequently challenge exposed with the virulent strain of orf virus, group 2A was vaccinated with the vaccine created from the virulent strain of orf virus and subsequently challenge exposed with the strain used for the goat-derived CE vaccine, and group 2B was vaccinated with the vaccine created from the virulent strain of orf virus and subsequently challenge exposed with the virulent strain of orf virus. †Nine goats (4 in group 1 and 5 in group 2) were found dead ($n = 2$ goats) or lost as a result of predation (7) sometime after vaccination but before viral challenge exposure. ‡Value reported is the number of goats with lesions/number of goats in the group. §Value reported is median (interquartile range). Lesion severity score was assigned by use of an ordinal scale from 1 to 4 (1 = formation of a few small scabs, 2 = mild scab formation, 3 = moderate scab formation, and 4 = excessive and severe scab formation). || Value differs significantly ($P < 0.001$) from the value for group 1. ¶ Value differs significantly ($P < 0.001$) from the value for group 1A. # Value differs significantly ($P = 0.007$) from the value for group 1. *** Value differs significantly ($P = 0.034$) from the value for group 1A. †† Value differs significantly ($P < 0.001$) from the value for group 2. ‡‡ Value differs significantly ($P = 0.001$) from the value for group 1B. §§ Value differs significantly ($P < 0.001$) from the value for group 1B. ||| Value differs significantly ($P < 0.001$) from the value for group 2A.

the goat-derived CE vaccine following vaccination with the vaccine produced from the virulent strain of orf virus (group 2A; 14/18) was significantly ($P = 0.007$) smaller than the proportion of goats vaccinated with the goat-derived CE vaccine (group 1; 83/85). However, there was no significant ($P = 0.498$) difference in lesion severity score between group 2A and group 1 (Table 1).

Discussion

Vaccination with the goat-derived CE vaccine did not provide heterologous protection from challenge exposure with the virulent strain of orf virus, but it did provide homologous protection from infection with the virus strain of the goat-derived CE vaccine. Clinical signs in naïve sheep and goats generally begin as localized erythema and development of pustules and vesicles at the site of infection. The pustules and vesicles rupture and form scabs. Lesions heal by 4 to 8 weeks after infection.^{10–16} In goats with protective immunity, infection results in lesions in fewer animals, less severe lesions, and a shorter resolution time of approximately 1 to 3 weeks.^{3,11,12,14–18} In goats exposed to the virulent strain of orf virus, there were no significant differences in the number of goats that developed lesions or in lesion severity between goats vaccinated with the vaccine developed from the virulent strain of orf virus (group 2) and goats vaccinated with the goat-derived CE vaccine (group 1B). These results support findings of other studies,^{3,5,8,19–21} which suggest that strains of orf virus antigenically distinct from those in the goat-derived CE vaccine are a cause of disease outbreaks in sheep and goats.

It did not appear that vaccination with the vaccine produced by use of the virulent strain of orf virus provided protective immunity against infection with the virus strain of the goat-derived CE vaccine. If vaccination had stimulated protective immunity, the number of goats with lesions and severity of the lesions should have been reduced.^{3,11,12,14,15,18} In the present study, significantly fewer goats vaccinated with the vaccine produced by use of the virulent strain of orf virus (group 2A) formed scabs following challenge exposure with the virus strain of the goat-derived CE vaccine than did goats vaccinated with the goat-derived CE vaccine (group 1). However, there were significantly fewer goats with scabs when goats were vaccinated with the goat-derived CE vaccine prior to challenge exposure (group 1A). Lesion severity scores did not differ significantly between groups 1 and 2A. These findings suggest that protection derived from vaccination with the vaccine produced by use of the virulent strain of orf virus may not have biological importance.

Reduced protection from vaccination has been attributed to physiologic variability among individual animals and breeds,⁵ genetic factors,²² nutritional differences,⁶ environmental conditions,¹¹ the method of inoculation,²⁰ viral titers at the time of inoculation and challenge exposure, incorrect vaccine administration,⁷ and antigenically distinct strains of the orf virus.^{5,8,20} The methods used in the present study accounted for most of these variables, except for potential antigenic differences between strains of the orf virus; thus, antigenic differences in epitope and virulence proteins

likely were factors in the differences in protection and apparent vaccine failures.

Research has been conducted on neutralization epitopes and virulence proteins of the orf virus.^{16,23,24} The orf virus encodes a range of immunomodulatory and virulence genes for substances that interfere with host antiviral immune responses.²⁵ These include vascular endothelial growth factor,²⁶ interferon resistance factor,²⁷ inhibitor of ovine granulocyte-macrophage colony-stimulation factor,²⁸ inhibitor of interleukin-2,²⁸ and a cytokine interleukin-10 orthologue.²⁹ Immune responses of sheep to the orf virus involve neutrophils, dendritic cells, T cells (of which CD4+ predominate), B cells, and antibodies.^{16,18,28,30,31} Increases in CD4+ and CD8+ T cells, B cells, inflammatory cytokine interleukin-1 β and chemokine interleukin-8, and the humoral response have been used to evaluate protection provided by a vaccine.^{14,16,20} Relative correlates, which are immune responses with a relationship to protection,³² are useful measures, but they do not always evaluate actual protection from disease. For example, even in animals with sufficient antibody titers, neutralizing antibodies against orf virus are rare and passive transfer with hyperimmune serum or colostrum is not protective against infection and disease.^{30,33,34} In the present study, the variables of interest were the clinical signs of lesion formation and lesion severity. Duration of clinical signs, which is reduced with protective vaccination,^{11,12,14,15} would have been useful to measure in the present study; however, pasture location and logistics precluded us from determining the duration.

In the present study, goats vaccinated with the vaccine produced by use of the virulent strain of orf virus did not develop the excessively proliferative reactions that potentially can be seen with infections attributable to the virulent strain of orf virus in field settings. However, lesion severity scores of these goats (group 2) were higher than those of goats vaccinated with the goat-derived CE vaccine (group 1). Individual susceptibility factors within animals,⁵ anatomic location for and method of virus introduction,³⁵ and lack of secondary compounding infections³⁶ may have factored into the absence of excessively proliferative lesions in the present study. Additionally, processing the virus in the scabs for use in the vaccine and for challenge exposure may have attenuated the virulence of the virus.³⁷

In the present study, there was homologous protection after vaccination, protection against challenge exposure with the same virus strain that was used in the vaccine, which significantly reduced the number of goats with scabs and lesion severity. Nonetheless, the proportion of vaccinated goats that formed scabs after challenge exposure with the virus strain of the goat-derived CE vaccine (18/41) or the virulent strain of orf virus (8/18) was higher than that in other studies.^{4,11,14,34} This may reflect differences between a study conducted in a controlled laboratory environment and a study conducted in field conditions. The goats were maintained on a pasture that in previous years contained sheep and goats vaccinated with a commercial orf vaccine labeled for use in sheep. Goats in the present study could have been exposed to a virus strain unrelated to the virus strain used in the study reported here. However, this

should not have biased the results or conclusions because all goats had similar potential exposure to any field strain of the orf virus. Although there is variation in protection among studies, results of the study reported here clearly indicate that the 2 strains of orf virus are not cross protective, which may be attributable to antigenic differences. This lack of protection may be a factor in CE outbreaks in vaccinated goats.

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