

Development of a contagious ecthyma vaccine for goats

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Objective—To identify a strain of contagious ecthyma virus from goats that possesses the appropriate characteristics for an effective vaccine for goats.

Animals—25 goat kids used for vaccine development and 100 goat kids used for evaluation of vaccine efficacy.

Procedures—5 strains of contagious ecthyma virus were tested in a vaccination-challenge study to identify the best strain to be the seed strain for a contagious ecthyma vaccine. The vaccine derived from the chosen viral strain was tested at 2 concentrations for efficacy in a vaccination-challenge study.

Results—2 of 5 viral strains induced moderate to severe scabs following infection, and 3 viral strains protected the goats from wild-type virus challenge following vaccination. Viral strain 47CE was selected as the seed source for the production of a contagious ecthyma vaccine because of the larger vaccine-to-challenge scab formation ratio. Vaccine 47CE protected all goat kids (48/48) following challenge with the wild-type contagious ecthyma virus; all goat kids (32/32) in the control group had scab formation following challenge with the wild-type contagious ecthyma virus, which indicated no protection following administration of vaccine diluent.

Conclusions and Clinical Relevance—A vaccine containing a caprine strain of contagious ecthyma virus used in goats appeared to provide the characteristics needed for an effective vaccine, including good scab production and protection from wild-type infection. This vaccine may potentially provide better protection for goats from contagious ecthyma than currently available vaccines labeled for sheep. (*Am J Vet Res* 2008;69:1366–1370)

Contagious ecthyma, also referred to as soremouth in domestic animals and orf in humans, is a common viral disease of sheep, goats, wild ruminants, and humans with worldwide distribution. Contagious ecthyma virus, a double-stranded DNA virus, is part of the *Parapoxvirus* genera.¹ Damaged skin and mucosa allow contagious ecthyma virus to infect and replicate in regenerating epidermal cells.^{2–4} In sheep and goats, contagious ecthyma is clinically recognized by the appearance of macules and papules that progress to vesicles, pustules, and proliferative lesions mainly at the mucocutaneous junctions of the mouth and nose but can extend to the udder and teats, coronary band, and anus.^{5,6} These lesions usually crust over, rapidly become growing scabs, and heal spontaneously within 4 weeks.⁴ Scabs contain large numbers of the virus and protect the virus from environmental inactivation for up to months and years; scabs are a source of further

infections and contribute to the contamination of pastures and sheds.^{2,3,5,7} Economic losses associated with contagious ecthyma result from reduced growth, poor feed conversion, and increased susceptibility to secondary bacterial infections or maggot infestations of affected animals. Presently, the disease is rarely fatal, but prior to elimination of *Cochliomyia hominivorax* from the United States, screwworm myiasis of contagious ecthyma lesions was a major cause of losses and death in lambs and kids.^{8,9} Concerns about the virus' zoonotic potential and the effects on show animals are additional negative effects of this disease.

The first vaccine for contagious ecthyma was developed at the Texas Agricultural Experiment Station (presently known as Texas AgriLife Research) at Sonora, Tex, in the 1930s.^{8,9} Since that time, more than 100 million doses have been produced at the Experiment Station. Though labeled only for sheep, the contagious ecthyma vaccine seemed efficacious when administered to goats. However in the late 1990s, some goats that had been vaccinated against contagious ecthyma developed a persistent, generalized form of contagious ecthyma. A study evaluating the effectiveness of the Texas AgriLife Research contagious ecthyma vaccine and the only other commercially available contagious ecthyma vaccine^a revealed that neither was effective in protecting goats from the wild-type contagious ecthyma virus found in goats.¹⁰ The apparent failure of the present contagious

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ecthyma vaccines to adequately protect goats coincides with increased numbers of goats in Texas and a dramatic change from Angora goats to Boer cross goats, which may be a factor in this situation. For these reasons, there is a great need for a contagious ecthyma vaccine to protect this increasingly important livestock enterprise.

More than 40 strains of contagious ecthyma virus have been characterized.¹¹ Phylogenetic analyses indicate that sheep and goat contagious ecthyma virus strains cluster on different branches of the genetic tree, which is probably the reason that sheep contagious ecthyma vaccines often fail to protect goats.¹⁰ These studies also reveal that goat contagious ecthyma virus strains are more heterogeneous than sheep strains, indicating that to be effective in goats, contagious ecthyma vaccines need to be prepared with virus strains isolated from goats. Therefore, the objective of the study reported here was to test the efficacy of a contagious ecthyma vaccine developed from caprine viral isolates.

Materials and Methods

Animals—Three- to 30-day-old goat kids ($n = 125$; Boer-Spanish goat crosses) were used in the study. Twenty-five goat kids were used in the vaccine development stage, and 100 goat kids were used to evaluate the vaccines.

Isolation of viral strains—During 2003 and 2004, scab material was collected from 40 goats naturally infected with contagious ecthyma; the goats were from herds in western Texas. Scab material from each goat was combined with Hank balanced salt solution at 1:1 wt/vol, and the suspension was homogenized in tissue mortars. The suspension was centrifuged at $2,000 \times g$ for 15 minutes, and the supernatant was filtered with a $0.45\text{-}\mu\text{m}$ syringe membrane filter and inoculated into Madin-Darby ovine kidney cells. Cell cultures were examined daily for the presence of cytopathic effect and passed to new Madin-Darby ovine kidney cell cultures weekly. When 50% cytopathic effect was observed, the virus-containing media was harvested and frozen at -70°C as the seed viruses.

The seed viruses were used at a 1,000-fold dilution to infect Madin-Darby ovine kidney cell cultures and allowed to replicate until $> 90\%$ of the cells detached; then, the supernatant was centrifuged at $2,000 \times g$ for 15 minutes. An aliquot of the clarified supernatant was used to perform a plaque assay, and if the plaque count ranged between 1,000,000 to 10,000,000/mL, 20 mL of the supernatant, with the addition of 3% sucrose, was lyophilized and stored at 4°C . The procedure was repeated for each individual goat sample of scab material.

Vaccine development and production—Clinical case records of the 40 infected goats from which scab material was collected were examined to determine the relative severity of the contagious ecthyma infection in each goat; 5 goats with the least severe infections were identified. The contagious ecthyma strains chosen were identified as 11CE, 47CE, 131CE, 140CE, and 460CE (Table 1). The 5 lyophilized contagious ecthyma virus strains, corresponding to the goats identified as having the least severe lesions, were reconstituted in 7 mL of a

30% glycerin-saline (0.9% NaCl) solution. For each of the viral strains, 5 goat kids were infected by scarifying the skin of the medial region of the thigh and applying the reconstituted virus solution to this area. Scab formation at the infection site was monitored over a 35-day period; scabs were collected from each goat and combined within strain.

For each strain, the combined scabs were placed in a desiccator for 4 to 7 weeks, then ground slowly in a grinding mill, run through a coarse sieve to remove any hairs, hand ground in a mortar, further desiccated for 2 to 3 weeks, and ground in a ball grinder. Finally, the powdered scab material was divided into 5- and 10-mg aliquots and stored in glass vials at 4°C until used for vaccination. At time of vaccination, the powdered scab aliquot was mixed with a 1-mL 30% glycerin-saline solution.

Vaccination and challenge experimental protocol—In study 1, 4 goat kids for each of the 5 vaccines were vaccinated (Table 1); the skin of the medial region of the thigh was scarified, and approximately 0.05 mL of the reconstituted virus solution was rubbed on the scarified region. Following vaccination, scab formation was monitored over a 35-day period. Sixty-five days following vaccination, the goats were challenged in the contralateral thigh region with a virulent (wild-type) contagious ecthyma virus. Scab formation at the challenge site was assessed and scored 10 days following challenge.

Scoring scab formation was performed by visually classifying scab formation on a scale of 1 to 4 as follows: 1 = no scab, 2 = mild scab, 3 = moderate scab, and 4 = severe scab. A vaccine-to-challenge scab formation ratio was calculated for each viral strain by use of the following equation:

$$\frac{\sum(X_v / X_c)_{n+1}}{(n+1)}$$

where X_v is the scab formation score after vaccination, X_c is the scab formation score after challenge, and $n + 1$ is the number of goats within the viral strain group.

In study 2, 80 goat kids were randomly assigned to 1 of 8 experimental groups. Goat kids in experimental group 1 ($n = 10$) were administered vaccine containing 10 mg of scab in 1 mL of diluent. Goat kids in experimental group 2 ($n = 12$) served as controls and were administered only the diluent. Goat kids in experimental groups 1 and 2 were evaluated for scab formation 7 days after administration, challenged 50 days after administration, and evaluated for scab formation 7 days after challenge.

Goat kids in experimental group 3 ($n = 10$) were administered vaccine containing 5 mg of scab in 1 mL of diluent. Goat kids in experimental group 4 ($n = 10$) were administered vaccine containing 10 mg of scab in 1 mL of diluent. Goat kids in experimental group 5 ($n = 8$) served as controls and were administered only the diluent. Goat kids in experimental groups 3, 4, and 5 were evaluated for scab formation 7 days after administration, challenged 23 days after administration, and evaluated for scab formation 8 days after challenge.

Goat kids in experimental group 6 ($n = 10$) were administered vaccine containing 5 mg of scab in 1 mL

of diluent. Goat kids in experimental group 7 (n = 10) were vaccinated with vaccine containing 10 mg of scab in 1 mL of diluent. Goat kids in experimental group 8 (n = 10) served as controls and received only the diluent. Goat kids in groups 6, 7, and 8 were evaluated for scab formation 7 days after vaccination, challenged 18 days after vaccination, and evaluated for scab formation attributable to challenge 17 days after challenge.

For all groups, vaccine or placebo was administered by scarifying the skin in the medial aspect of the thigh and applying the solution to the scarified area. In study 1 and 2, the wild-type contagious ecthyma virus was obtained from a goat herd neighboring the Texas AgriLife Research facility at Sonora, Tex, that was in the midst of a virulent outbreak of contagious ecthyma. Scabs from the goats were collected, combined, and ground into a powder. Sixty-five milligrams of processed scabs containing the wild-type contagious ecthyma virus was added to 6 mL of a 30% glycerin-saline solution. The process of challenging goats was as follows: the skin of the medial region of the thigh contralateral to the vaccination site was scarified, and the solution of wild-type contagious ecthyma virus was placed topically and rubbed into the scarified area.

For all groups in study 2, scab formation at the site of vaccination or challenge was visually examined and scored as either no or yes; no scab formation after challenge indicated protection attributable to the vaccine, whereas scab formation after challenge indicated no protection conferred from the vaccination.

Results

Contagious ecthyma virus was isolated from the scab material collected from all 40 naturally infected goats. Cytopathic effect and plaque assay results varied among the viral strains (data not shown). The viral strains from the goats with the least severe clinical signs were designated 11CE, 47CE, 131CE, 140CE, and 460CE; these 5 strains were used as seed virus for subsequent vaccine development and testing. Regardless of which of the 5 strains was used, all 25 goat kids infected with the contagious ecthyma virus developed scabs with sufficient quantity of scab material to manufacture separate vaccines for each of the 5 contagious ecthyma strains.

In study 1, viral strains 47CE and 131CE induced the greatest scab formation response to vaccination,

Table 1—Scab formation in goats in response to vaccination with different strains of contagious ecthyma virus and following cutaneous exposure to a wild-type contagious ecthyma virus 65 days after vaccination (study 1). Five vaccines, each produced from a different contagious ecthyma viral strain, were each tested on 4 goat kids.

Variable	Viral strain of vaccinate	Scab formation score* (No. of goats)				Total scab formation score	Mean vaccine-to-challenge scab formation ratio
		1	2	3	4		
Response to vaccination	11CE	0	4	0	0	8	1.8
	47CE	0	0	1	3	15	3.8
	131CE	0	0	2	2	14	3.5
	140CE	1	3	0	0	7	1.3
	460CE	0	3	1	0	9	2.3
Response to challenge	11CE	3	1	0	0	5	
	47CE	4	0	0	0	4	
	131CE	4	0	0	0	4	
	140CE	2	2	0	0	6	
	460CE	4	0	0	0	4	

*Scab formation score: 1 = no scab; 2 = mild scab; 3 = moderate scab; and 4 = severe scab.

Table 2—Response of goat kids (number of goat kids that did or did not form a scab) to vaccination with a contagious ecthyma (strain 47) vaccine and to cutaneous exposure with a wild-type contagious ecthyma virus after vaccination.

Group	Vaccine scab weight (mg)*	No. of goats	Vaccination		Wild virus challenge	
			Scab formed	No scab formed	Scab formed	No scab formed
1	10 mg	10	10	0	0	10
2	Control	12	0	12	12	0
3	5 mg	10	10	0	0	10
4	10 mg	8	8	0	0	8
5	Control	10	0	10	10	0
6	5 mg	10	10	0	0	10
7	10 mg	10	10	0	0	10
8	Control	10	0	10	10	0

*Scab in 1 mL of diluent.
Control = Vaccine diluent only. Groups 1 and 2 = Response evaluated 7 days after vaccination with strain 47 and 7 days after challenge with wild-type virus 50 days after vaccination. Groups 3, 4, and 5 = Response evaluated 7 days after vaccination with strain 47 and 8 days after challenge with wild-type virus 23 days after vaccination. Groups 6, 7, and 8 = Response evaluated 7 days after vaccination with strain 47 and 17 days after challenge with wild-type virus 18 days after vaccination.

whereas vaccination with 47CE, 131CE, and 460CE did not result in any scabs following challenge with the wild-type contagious ecthyma virus (Table 1). Viral strain 47CE had the largest vaccine-to-challenge scab formation ratio and was substantially different from strains 11CE, 140CE, and 460CE, so 47CE was selected as the seed source for the production of the contagious ecthyma vaccine to be examined for efficacy.

In study 2, none of the goat kids (0/32) in the control groups, which received only the diluent, developed scabs at the vaccination site, although all had scab formation following challenge with the wild-type contagious ecthyma virus. In goat kids inoculated with vaccine containing 5 mg of scab or 10 mg of scab in 1 mL of diluent, all (48/48) developed scabs in response to the initial vaccination and none developed scabs following challenge with the wild-type contagious ecthyma virus, regardless of the vaccine's scab concentration or the time frame of challenge or evaluation (Table 2).

Discussion

Throughout many years, the authors have observed that contagious ecthyma vaccinations are not always successful; several outbreaks of contagious ecthyma in vaccinated goats were reported in west Texas during the past 10 years. Reasons for these vaccination failures are not clear, but they could be the result of poor vaccination procedures, improper handling and storage of the vaccine, a field strain with increased virulence, or a viral strain that differed antigenically from the vaccinal virus.¹²

In testing of the isolated contagious ecthyma viral strains to determine which to use as the vaccine seed strain, scab formation following vaccination and challenge with wild-type contagious ecthyma virus differed between the 5 viral strains tested. This was expected because previous reports^{10,11,13-18} indicate that viral strains of contagious ecthyma have differing pathogenicity in cell culture and abilities to induce immune protection against wild-type contagious ecthyma viruses that infect goats. These differences can be attributed to antigenic and virulence differences of contagious ecthyma strains.^{13,15,18,19} Scab formation following vaccination is desirable because it is an indirect indication of successful vaccination, whereas no scab formation at the site of challenge is an indirect assessment of the protective immunity derived from the vaccination. Because 47CE had the largest vaccine-to-challenge scab formation ratio and was substantially different from the strains with lower ratios, it was chosen for development into the vaccine to be examined for efficacy; however, use of viral strain 131CE may have resulted in similar findings.

Results indicated that viral strain 47CE has the potential to be an efficacious vaccine for goats. All goats vaccinated with the vaccine, regardless of the amount of scab used in the vaccine, formed scabs at the vaccination site, indicative of a local infection with the live virus. Following challenge with wild-type contagious ecthyma virus, all goats that had been vaccinated developed no evidence of infection, indicating a protective immunologic response. In contrast, all goats that

received diluent only formed scabs after challenge with wild-type contagious ecthyma virus.

Lesions usually develop between 3 and 14 days after inoculation at the site of inoculation, progressing from maculae to ulcers, over which scabs form in a few days.^{1,20} Because reinfection following vaccination may affect time for lesion development and can result in milder lesions,^{7,10} the authors of the present study used time periods that encompassed occurrence of the lesions; thus, challenge lesions were evaluated at 7, 8, and 17 days following challenge. No differences were detected for the different days; vaccinated goats produced no scabs, whereas the unvaccinated goats produced scabs.

This study evaluated the short-term immune protection induced by the vaccine and did not examine duration of immunity. Immunity following infection with contagious ecthyma, either natural or by vaccination, is only partial and not long lasting. Sheep and goats may repeatedly be infected, but subsequent infections usually are both milder and of shorter duration, which is most likely attributable to an accelerated immune response.^{7,10} Young animals are prone to develop more severe clinical signs, with resistance to the disease increasing with age and exposure.⁸ Presently, vaccine management of contagious ecthyma in Texas consists of initial vaccination of all animals in the herd, with further annual vaccination of only new additions (by birth or purchase) to the herd, and is strategically timed if needed to coincide with particular events (eg, vaccination of kid goats before shows). Vaccination should not be done in the absence of disease in a herd because vaccination will introduce the live virus into the environment.

It was recognized throughout the study that factors such as concomitant disease, age, nutritional status, and environmental conditions could influence the immune response of the animal to wild-type or vaccinate contagious ecthyma virus and thus could have influenced the data.²¹⁻²⁵ These factors were mitigated in our study because the animals were from the same herd, kept in similar conditions, and randomly assigned to groups. Passive transfer of immune factors to contagious ecthyma virus most likely occurred in the goat kids because the dams had been vaccinated as kids; however, passive transfer of immune factors has not been shown to be protective against infection with contagious ecthyma virus.²⁶ Differences clearly exist between neonates and adults in humoral and cell-mediated immune responses, although most studies^{27,28} have examined immune functions in mice and humans. However, T cells are developmentally mature in neonates and able to mount protective responses,²⁸ and DNA vaccines and replicating live vaccines induce similar responses in young and adult mice.²⁷ The authors could find no references to immune response maturation in goats; however, in sheep, cell-mediated immune responses attributable to immunization were not found to be different between newborn lambs and lambs 5 to 6 weeks of age.²⁹ These findings and the random assignment of animals to test groups suggest that the effect of the age range in this study, 3 to 30 days of

age, should have been minimal; however, age as a factor could not be examined because of the lack of records of exact date of birth. Genetic factors may contribute to the immune response with exposure to wild-type contagious ecthyma virus³⁰; genetic factors in Boer goats may be a factor aspect in their observed susceptibility to disease and more severe clinical manifestations of infection with contagious ecthyma virus.¹³ Goats used in our study were all Boer-Spanish goat crosses. These crosses have been reported by veterinarians and producers to be susceptible to contagious ecthyma, so the goats used in our study should have been susceptible to infection with the wild-type contagious ecthyma virus, which was evident by all control goats developing signs of infection upon challenge.

Testing the goat contagious ecthyma virus strains identified a specific strain that appears to provide the characteristics needed in an effective vaccine, including good scab production and protection from wild-type contagious ecthyma infection. Results of the present study indicated that a new vaccine derived from goats may have the potential to provide protection for goats from contagious ecthyma.

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