Evaluation of antibody response and nonspecific lymphocyte blastogenesis following inoculation of a live attenuated bluetongue virus vaccine in goats

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Objectives—To evaluate vaccine safety, antibody response, and nonspecific lymphocyte blastogenesis following inoculation of a commercial monovalent live attenuated bluetongue virus (BTV) serotype 2 vaccine in goats.

Animals—12 nonpregnant and nonlactating Saanen goats.

Procedure—Six goats were inoculated with the monovalent live attenuated BTV serotype 2 vaccine, which has been widely used in Italy during the preceding 2 years. The other 6 goats were unvaccinated and represented negative controls. Nonspecific lymphocyte blastogenesis was evaluated 14 and 7 days before and 7, 21, and 49 days after vaccination by measuring DNA synthesis in peripheral blood mononuclear cells (PBMCs) stimulated with phytohemagglutinin, concanavalin-A, and pokeweed mitogen. On the same days as lymphocyte blastogenesis, blood samples were taken to determine serum concentrations of anti-BTV antibodies.

Results—During the 7 weeks following vaccination, PBMCs obtained from vaccinated goats had a significantly decreased response to mitogens in terms of DNA synthesis, compared with PBMCs from the same goats before vaccination. Conversely during the experiment, no significant change was found in the response of the PBMCs obtained from unvaccinated goats. Starting from 21 days after vaccination, serum from vaccinated goats had anti-BTV antibodies. No anti-BTV antibodies were detected in the serum from unvaccinated goats.

Conclusions and Clinical Relevance—Inoculation of goats with the monovalent live attenuated BTV serotype 2 vaccine described herein resulted in a profound depression of nonspecific lymphocyte blastogenesis, which might compromise the resistance of vaccinated goats to pathogens. (Am J Vet Res 2004;65:1331–1334)

Various authors have described risks or adverse effects resulting from the use of live attenuated vaccines against bluetongue virus (BTV). Hunter and Modumo and Hammoumi et al. presented data on the safety of a monovalent live attenuated vaccine against BTV serotype 2 in sheep, which has been used during the previous 2 years in southern Europe. They concluded that the vaccine was safe because neither viremia nor clinical signs of BTV infection were detected after inoculation.

To our knowledge no published data exist on the safety of the monovalent live attenuated BTV serotype 2 vaccine in species other than sheep. Furthermore, no data have been published on other variables that may contribute to the overall safety profile of the vaccine against infectious diseases.

Results of a previous study in calves indicate that BTV serotype 11 inhibits lymphocyte proliferation induced by mitogens. Other viruses of interest for domestic ruminants also have immunosuppressive effects. The purpose of the study reported here was to evaluate vaccine safety, antibody response, and nonspecific lymphocyte blastogenesis following inoculation of a commercial monovalent live attenuated BTV serotype 2 vaccine in goats.

Materials and Methods

Animals, vaccination, and clinical examination—Twelve nonpregnant and nonlactating Saanen goats of approximately 12 months of age were used for the study. Six goats were inoculated with the monovalent live attenuated BTV serotype 2 vaccine, which has been used during the previous 2 years in Italy and other Mediterranean countries. The vaccine used in the study belonged to lot 15. Inoculation was performed according to the recommendations of the manufacturer by injecting a single 1-mL dose of the vaccine SC. The remaining 6 goats were unvaccinated and represented negative controls. On the day of vaccination, the unvaccinated goats were injected SC with sterile PBS solution. The injection sites were examined daily to detect possible reactions. Rectal temperatures of the 12 goats were measured twice a week during the experiment.

Evaluation of DNA synthesis—Nonspecific lymphocyte blastogenesis was evaluated by measuring DNA synthesis in peripheral blood mononuclear cells (PBMCs) stimulated with mitogens. On days 14 and 7 before vaccination and days 7, 21, and 49 after vaccination, blood samples were collected from goats via jugular venipuncture into evacuated glass tubes coated with sodium heparin. Time between blood collection and establishment of PBMC cultures was <5 hours.

DNA synthesis was evaluated as described for other ruminant species, with minor modifications. Briefly, after isolation by density gradient centrifugation, PBMCs were resuspended at a concentration of 2×10^6 cells/mL of RPMI-1640 enriched with 25 mM HEPES buffer, 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin/mL, 100 µg of streptomycin/mL, and 0.25 µg of amphotericin B/mL. Triplicate cultures were assayed in 96-well tissue-culture plates. Each culture in the triplicate
-contained 2 × 10^7 PBMCs in 100 µL of enriched RPMI-1640 with an optimal concentration of 1 of the 3 mitogens, which were added separately (ie, phytohemagglutinin [15 µg/mL], concanavalin A [12.5 µg/mL], and pokeweed mitogen [0.2 µg/mL]). Plates were incubated in an atmosphere of 95% air and 5% CO₂ for 48 hours at 39°C. One hundred microliters of the pyrimidine analogue 5-bromo-2’-deoxyuridine (BrdU) in 10 µL of RPMI-1640 was then added to the wells, and plates were incubated for an additional 18 hours. Control wells contained 100 µL of PBMCs suspension without mitogens. Furthermore, additional control wells contained 100 µL of enriched RPMI-1640 without cells or 100 µL of PBMCs suspension without BrdU. Synthesis of DNA was quantitated by use of an ELISA. The assay was performed with a commercial kit, which is based on measurement of BrdU incorporated during DNA synthesis. Values for DNA synthesis were expressed as the optical density (OD) for test wells minus the OD for control wells that did not contain BrdU.

Serologic response—Responses of goats to vaccination were measured in terms of antibody production in serum samples obtained 14 and 7 days before and 7, 21, and 49 days after vaccination. The presence of anti-BTV antibodies in serum was established by use of a competitive ELISA. Results were expressed as mean of inhibition percentage (IP) as follows: IP = 100 - (OD₆₂₀nm sample/OD₆₂₀nm negative control) X 100. As indicated by the manufacturer, an IP of 50% was considered the positive threshold value.

Results—Data of DNA synthesis were analyzed by use of a repeated-measures procedure. Values are expressed as least-square means (± SEM). Values of P < 0.05 were considered significant.

During the 7 weeks following vaccination, PBMCs obtained from vaccinated goats had a significantly decreased response to mitogens in terms of DNA synthesis, compared with PBMCs from the same goats before vaccination (Figure 1). Conversely, during the experiment, no significant change was found in the response of the PBMCs obtained from unvaccinated goats (Figure 2).

No anti-BTV antibodies were found in the serum of vaccinated goats 14 and 7 days before and 7 days after vaccination (Figure 3). However, at 21 and 49 days following vaccination, anti-BTV antibodies were detected in the serum of vaccinated goats. In both situations, the calculated IP was greater than positive threshold value. No anti-BTV antibodies were detected in the serum of unvaccinated goats.

No noteworthy reactions were observed at the injection sites. No clinical signs of BTV infection or
not be used in animals that may already be immunovalent live attenuated BTV serotype 2 vaccine should
unexpected reactions.
under field conditions to establish safety and detect all veterinary biological products should be tested
indicates that before being authorized for general use,
fors diagnostic tests and vaccines for terrestrial animals
with immunosuppression or increased risk for infec-
tions. Information provided in the
expressed as means (± SD). Day 0 indicates the day of vaccina-
tion or injection of sterile PBS solution in vaccinated goats and unvaccinated goats, respectively.
other diseases were observed during the experiment. Rectal temperatures did not change significantly dur-
ing the experiment, and values were not significantly different between vaccinated and unvaccinated goats (data not shown).

Discussion
Results of our study indicate that the vaccine that
has been widely used in the Mediterranean basin in
campaigns against BTV serotype 2 caused discernible
and prolonged immunosuppression, as was established
by evaluating nonspecific lymphocyte blastogenesis.
This finding is novel and is not the same as that from other studies.12,13 MacLachlan et al12 reported that BTV
infection did not consistently suppress mitogen-
induced lymphocyte blastogenesis in bovine fetuses. Stott et al15 reported that the addition of mitogens to
bovine lymphocyte cultures infected with virulent BTV
was responsible for negligible blast transformation.
In a study in calves, Ódeón et al8 hypothesized that
the inhibition of lymphocyte proliferation as a result of
BTV serotype 11 infection might be explained by the
synthesis of suppressive factors (eg, histamine,
prostaglandins, or interferon) that are induced in vivo
or in vitro by virus challenge. Whether the virulent
virus also has these effects in lymphocytes obtained
from goats and whether the same properties are lost or
maintained during the attenuation passages on cell cul-
tures is not known at this time.
Large scale epidemiologic studies performed con-
comitantly with future vaccination campaigns would
be useful to help establish whether vaccination against
BTV by use of live attenuated viruses is associated, as
reported for other viral diseases9,14,15 or vaccines,16-18
with immunosuppression or increased risk for infec-
tions. Information provided in the Manual of standards
for diagnostic tests and vaccines for terrestrial animals9
indicates that before being authorized for general use,
all veterinary biological products should be tested
under field conditions to establish safety and detect unex-
pected reactions.
Results of our study also indicate that the mono-
valent live attenuated BTV serotype 2 vaccine should
not be used in animals that may already be immuno-
suppressed for other reasons (eg, caprine arthritis-
encephalitis virus infections,20 parasitic infections,21 or
metabolic diseases22). Therefore, inoculation of goats
with the monovalent live attenuated BTV serotype 2
vaccine might exacerbate immunosuppression caused
by other infections or poor management.
Results of our study on serologic responses indicate
that vaccination elicits antibody production that is quite
similar to that recorded for Lacaune sheep inoculated
with the same vaccine.9 Antibody production coupled
with a decreased response of PBMCs to mitogens has
been described for dogs inoculated with live vaccines.22
Those authors suggested that inoculation with live vac-
cine may cause a transient shift in the balance between
cell-mediated and humoral (ie, T-helper 1 and T-helper 2
cells) immunity and concluded that this recognized
alteration of immune function should be considered
and live vaccines should be administered only to healthy ani-
mais. Serologic data from Saanen goats infected with
BTV serotype 4 indicates that infection was responsible
for a strong antibody response, which started by the
sixth day after infection.27 It can be hypothesized that
the early detection of anti-BTV antibodies in that study25
may be the result of the serotype of BTV used. In agree-
ment with the study of Hammoumi et al,6 the finding of
immunosuppression in our study, along with other
problems that may develop as the result of the use of the
live vaccine,2 indicates that the development of killed
vaccines or vaccines based on recombinant virus-like
particles may be more advantageous for use in the pro-
tection of animals against BTV infections.2,9

References
1. MacLachlan NJ, Conley AJ, Kennedy PC. Bluetongue and
equine viral arteritis viruses as models of virus-induced fetal injury
3. Schultz G, Dyce AL, Muller MJ. Losses in newborn lambs
4. Young S, Cordon DR. An ovine fetal encephalopathy caused by
5. Hunter P, Modumo J. A monovalent attenuated serotype 2
bluetongue virus vaccine confers homologous protection in sheep.
and immunogenicity of the South African bluetongue virus serotype 2
monovalent vaccine: specific detection of the vaccine strain genome by
7. Jacobson RM, Zabel KS, Poland GA. The overall safety profile
of currently available vaccines directed against infectious diseases.
8. Ódeón AC, Schore CE, Osburn BL. The role of cell-mediated
immunity in the pathogenesis of bluetongue virus serotype 11 in the
experimental infection of vaccine-sensitized calves. Comp Immunol
9. Eskra L, Splitter GA. Bovine herpesvirus-1 infects activated


