Behavioral responses to two intranasal vaccine applicators in horses and ponies

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Objective—To evaluate behavioral compliance of horses and ponies with simulated intranasal vaccination and assess development of generalized aversion to veterinary manipulations.

Design—Clinical trial.

Animals—28 light horse mares, 3 pony geldings, 2 light horse stallions, and 3 pony stallions that had a history of compliance with veterinary procedures.

Procedure—Behavioral compliance with 2 intranasal vaccine applicators was assessed. Compliance with standard physical examination procedures was assessed before and after a single experience with either of the applicators or a control manipulation to evaluate development of generalized aversion to veterinary manipulation.

Results—In all 30 horses, simulated intranasal vaccination or the control manipulation could be performed without problematic avoidance behavior, and simulated intranasal vaccination did not have any significant effect on duration of or compliance with a standardized physical examination that included manipulation of the ears, nose, and mouth. Results were similar for the 2 intranasal vaccine applicators, and no difference in compliance was seen between horses in which warm versus cold applicators were used. For 3 of the 6 ponies, substantial avoidance behavior was observed in association with simulated intranasal vaccination, and compliance with physical examination procedures decreased after simulated intranasal vaccination.

Conclusions and Clinical Relevance—Although some compliance problems were seen with ponies, neither problems with compliance with simulated intranasal vaccination nor adverse effects on subsequent physical examination were identified in any of the horses. Further study is needed to understand factors involved in practitioner reports of aversion developing in association with intranasal vaccination. (*J Am Vet Med Assoc* 2005;226:1689–1693)

In recent years, vaccines intended for intranasal administration to horses and ponies have become commercially available. However, clinicians have complained that some horses with a history of general compliance with veterinary procedures appeared to resent intranasal vaccination. In addition, some horses reportedly have become head-shy or noncompliant with veterinary procedures in general in association with their first intranasal vaccination. Such reports are anecdotal, however, and objective information on behavioral responses to intranasal vaccination in horses is lacking. The present study, therefore, was developed to evaluate possible effects of intranasal vaccination on behavior of horses. Specifically, the purposes of the study reported here were to evaluate behavioral compliance of horses and ponies with simulated intranasal vaccination and assess compliance with standard physical examination procedures before and after a single experience with simulated intranasal vaccination. In addition, to examine particular aspects of the intranasal vaccination procedure that might be aversive to horses, we compared responses to the 2 types of applicators currently available for intranasal vaccine administration in horses and ponies, responses of horses versus ponies, and responses to use of warm versus cold applicators.

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Materials and Methods

Animals—Thirty-six horses and ponies were used in the study. This included 28 light horse mares (5 to 25 years old), 2 light horse stallions (15 and 20 years old), 3 pony geldings (2, 13, and 13 years old), and 3 pony stallions (3, 14, and 30 years old). All horses and ponies were from the reproduction and behavior teaching and research herds of the University of Pennsylvania School of Veterinary Medicine, New Bolton Center. Horse mares, pony geldings, and pony stallions were maintained at pasture (with supplemental grass hay fed during the winter) in same-sex groups throughout the study. Horse stallions were pastured individually with supplemental grass hay fed during the winter. Mares were used primarily for teaching of veterinary reproduction procedures, including teasing, palpation per rectum, and embryo flushing. Stallions were used for teaching teasing, breeding, and semen collection. Geldings were used in a behavior study of cognition. Physical examinations other than reproductive procedures were done as needed for health care, and on up to 6 occasions/y, horses were used for 1-day teaching laboratories that involved ophthalmic and lameness examinations. All animals were also used for blood sampling by jugular venipuncture. They all received quarterly foot care, annual equine infectious anemia testing, and vaccinations. Twitches were applied rarely to the stallions or geldings and occasionally (2 to 3 times/y) to some of the mares. Two of the horse mares served as mount mares for semen collection (1 to 30 times/mo), and a twitch was applied for this procedure. Seventeen of the 28 mares, 4 of the 5 stallions, and 2 of the 3 geldings were known to have had no previous experience with intranasal vaccination. Animal care and research procedures were approved by the institutional animal care and use committee of the University of Pennsylvania.

Simulated intranasal vaccination procedure—Two commercially available applicators for intranasal vaccination[a,b] were used. One applicator (applicator 1[a]) was a polypropylene catheter that was 155 mm long with an outside diameter of 6 mm; the applicator had a female luer lock attachment and a nozzle with a rounded tip that delivered a mist of 5-mm droplets. The other (applicator 2[b]) was a polypropylene catheter of similar length with an outside diameter of 2 mm and an inside diameter of 1 mm; it also had a female luer lock attachment but had a bluntly cut tip.

For simulated intranasal vaccination, applicators were filled with sterile water, rather than vaccine. Simulated vaccination was performed according to the manufacturer's instructions. For applicator 1, 1 mL of sterile water was administered over a period of approximately 1 second. For applicator 2, 2.5 mL of sterile water was administered. Because the manufacturer did not specify how quickly vaccines should be administered, sterile water was administered at a rate of approximately 1 mL/sec. The control manipulation consisted of placing the applicator externally, alongside the muzzle, and expressing the sterile water onto the lateral aspect of the face.

Fifteen light horse mares were evaluated during the summer of 2001, with mares randomly assigned to receive simulated intranasal vaccination with applicator 1 (n = 5), simulated intranasal vaccination with applicator 2 (5), or the control manipulation (5). The remaining 13 light horse mares and the 2 light horse stallions were evaluated during the winter of 2003–2004, with horses randomly assigned to receive simulated intranasal vaccination with applicator 1 (n = 6), simulated intranasal vaccination with applicator 2 (6), or the control manipulation (3).

Because there is a perception among some equine practitioners that horses may experience greater aversion to intranasal vaccination during the winter than during the summer (possibly because applicators are more likely to be cold and more rigid during the winter than during the summer, which may be more aversive[c]), applicators and contents used for 6 horses evaluated during the winter of 2003–2004 were maintained at 33° to 35°C (91.4° to 95°F) until immediately before use, whereas applicators and contents for the other 6 horses were maintained at ambient temperature (5° to 15°C [41° to 59°F]) until used.

The 6 ponies were evaluated during the winter of 2003–2004. Three were randomly assigned to receive simulated intranasal vaccination with applicator 1, and the other 3 were assigned to receive simulated intranasal vaccination with applicator 2. Applicators and contents were maintained at ambient temperature (10° to 15°C [50° to 59°F]) until used.

Horses and ponies remained in their usual pastures for the simulated vaccination procedure. For 28 of the 36 animals, a handling assistant applied a lead shank to the halter and stood on the right side of the animal with 1 hand holding the lead rope and the right side of the halter's noseband. The individual performing the simulated vaccination calmly approached the head from the left side, placing 1 hand on the animal's nose and using the other hand to insert the applicator. For the remaining 8 animals, a single individual restrained the animal with a lead shank and administered the simulated vaccination. All procedures were videotaped for subsequent evaluation of behavior measures.

Videotapes of all simulated intranasal vaccination procedures were reviewed, and compliance with the procedure was scored on a scale from 1 to 10 (application compliance score), with 1 representing complete intolerance of the procedure, 5 representing moderate avoidance but eventual tolerance so that the procedure could be completed, and 10 representing excellent compliance. Intermediate scores represented graduated judgments made on the basis of number and severity of avoidance responses together with the amount of additional restraint applied by the handler to achieve compliance. Time from when the animal was first approached to when the procedure was completed (application duration) was also recorded.

Neither the handling assistant nor the individual performing the simulated intranasal vaccination was familiar to any of the horses before the start of this study, but both were familiar to the ponies. During the study period, these individuals provided care to the ponies, which included daily walk-by inspections at pasture and delivery of supplemental grass hay.

Behavioral compliance with physical examination procedures—For all 36 horses and ponies, behavioral compliance with a simulated physical examination was assessed before and after the simulated intranasal vaccination procedure. Baseline evaluations were conducted 12 hours to 4 days before the simulated intranasal vaccination, except that in 1 animal, the baseline evaluation was performed 25 days before the simulated intranasal vaccination. Follow-up evaluations were conducted 12 hours to 5 days after the simulated intranasal vaccination.

For the simulated physical examination, 26 of the 28 light horse mares were individually hand-led from their pasture to clinical examination stocks located in a nearby barn with which they were familiar, as these stocks were used for routine teaching procedures. The remaining 2 mares were not familiar with stocks and were examined in a 4 X 4-m box stall with which they were familiar. The 2 light horse stallions and the 6 ponies were examined in their pasture or paddock or in a nearby holding paddock or stall. For each animal, baseline and follow-up examinations were conducted under similar conditions and at the same time of day.

The simulated physical examination included 7 specific steps conducted in the same order each time. These included a hand-sweep over the left side of the abdomen and back with a stethoscope, firm manipulation and cleaning of each ear, wiping of each eye with a moist cotton disposable towel, palpation of each nostril with insertion of a finger approximately 2 to 3 cm, examination of the mouth and teeth, oral administration of 1 mL of sterile saline (0.9% NaCl) solution with a dose syringe, and jugular venipuncture with a 20-gauge disposable needle. The examination was preceded by application of fly repellant from a spray bottle over the body and legs in a systematic manner. The individual performing the simulated examination recorded the duration of the examination as the time from approach to completion of all procedures and recorded any avoidance responses. All procedures were videotaped, and videotapes were reviewed to obtain detailed quantitative measures of behavior, including specific avoidance responses for each element of the examination.

During review of the videotapes, compliance with each of the 7 elements of the simulated physical examination was scored on a scale from 1 to 10 (examination compliance score), with 1 representing extreme avoidance behavior making it impossible to safely complete the specific procedure, 5 representing some avoidance attempts but completion of the specific procedure with normal head restraint, and 10 representing completion of the procedure with minimal or no restraint required and no avoidance attempts. Intermediate scores represented graduated judgments made on the basis of number and severity of avoidance responses together with the amount of additional restraint applied by the handler to achieve compliance. Problems with catching the animal, leading it to the barn, entering the stocks, standing until examination, standing until turnout, and returning to the pasture were recorded and characterized as mild, moderate, or severe.

Both the individual who performed the simulated physical examinations and the individual who reviewed videotapes of the procedures were blinded to group assignment of the animals. Simulated physical examinations were done by an experienced veterinary technician who was known to the ponies but not to the horses before the start of the study. This individual also served as the handling assistant during simulated intranasal vaccination of the light horse mares.

Statistical analyses—One-way ANOVA followed by the least significant difference procedure was used to compare application durations among groups (applicator 1, applicator 2, and control manipulation), and the Kruskal-Wallis test was used to compare application compliance scores among groups. Repeated-measures ANOVA was used to compare examination durations before and after the simulated intranasal vaccination procedure among groups, and the Friedman test was used to analyze examination compliance scores for the 7 physical examination procedures. For each group, examination durations before and after simulated intranasal vaccination were compared with dependent *t* tests, and examination compliance scores before and after simulated intranasal vaccination were compared with the Kruskal-Wallis test. Application durations in horses that received warm and cold applicators were compared with an independent *t* test, and application compliance scores were compared with Mann-Whitney *U* tests. All analyses were performed with standard software.[d] For all analyses, values of $P < 0.05$ were considered significant.

Results

Behavioral compliance with simulated intranasal vaccination—For the 22 horses receiving simulated intranasal vaccination and the 8 horses receiving the control manipulation, the procedure was performed without problematic avoidance behavior. Several mares emitted a snort or sneeze immediately following withdrawal of the applicator. One mare (applicator 2 group) pulled its head back, tossing it and holding it out of reach, and stomped in place. However, these avoidance behaviors appeared to be elicited by initial contact of the hand with the nostril before insertion of the applicator. The handling assistant placed a hand over the bridge of the nose with the other hand still on the halter when the individual performing the simulated intranasal vaccination approached a second time. On this second attempt, the procedure was completed without eliciting avoidance behavior.

Mean ± SE application compliance score for horses in which applicator 1 was used (n = 11; 5.5 ± 0.37) was not significantly different from the score for horses in which applicator 2 was used (11; 5.8 ± 0.52), but scores for both applicator groups 1 and 2 were significantly lower than the score for horses that received the control manipulation (8; 8.5 ± 0.32). Mean application duration for horses in which applicator 1 was used (27.8 ± 4.05 seconds) was not significantly different from mean duration for horses in which applicator 2 was used (31.8 ± 7.47 seconds), but values for both applicator groups were significantly longer than mean duration for horses that received the control manipulation (7.2 ± 1.13 seconds).

Mean application compliance score and application duration for horses in which warm applicators were used (n = 6; 5.0 ± 0.45 and 23.8 ± 6.30 seconds, respectively) were not significantly different from values for horses in which cold applicators were used (6; 5.2 ± 0.54 and 31.5 ± 7.23 seconds, respectively).

Three of the ponies (1 in applicator 1 group and 2 in applicator 2 group) tolerated simulated intranasal vaccination; mean ± SE application compliance score was 7.7 ± 0.89, and mean application duration was 28.3 ± 13.98 seconds. Substantial avoidance behavior was encountered with the remaining 3 ponies (2 stallions and 1 gelding). For 2 of these, attempts were terminated after 90 and 120 seconds when it was judged that resistance was worsening with each approach. For the remaining pony, for which improvement was noticed with each successive approach, attempts were continued until the procedure was completed after 5.5 minutes. Two of the 3 ponies that exhibited substantial avoidance behavior had moderately or considerably lower follow-up examination compliance scores, compared with baseline scores, for manipulation of the ears (scores of 9 before and 7 after simulated intranasal vaccination in 1 pony and scores of 5 before and 4 after in the other), palpation of the nostrils (scores of 8 before and 2 after simulated intranasal vaccination in 1 pony and scores of 8 before and 5 after in the other), examination of the mouth (scores of 5 before and 3 after simulated intranasal vaccination in 1 pony and scores of 8 before and 5 after in the other), and oral administration of saline solution (scores of 5 before and 4 after simulated intranasal vaccination in 1 pony and scores of 9 before and 8 after in the other).

The proportion of ponies in which simulated intranasal vaccination was difficult or not tolerated (3/6) was significantly ($P < 0.05$) greater than the proportion of horses (0/30; Fisher exact test, $P < 0.05$). Similarly, the proportion of ponies that showed increased aversion to physical examination procedures following the intranasal treatment (3/6) was significantly greater than the proportion of horses (3/22; Fisher exact test, $P < 0.05$.)

Compliance with physical examination procedures—For horses in the applicator 1 group and horses in the control group, examination durations before and after simulated intranasal vaccination were not significantly different **(Table 1)**, whereas for horses in the applicator 2 group, examination duration before simulated intranasal vaccination was significantly longer than duration after simulated intranasal vaccination. No significant differences in examination duration were found among groups.

For all 7 physical examination procedures, examination compliance scores were not significantly different among groups. Similarly, within each group, values obtained prior to simulated intranasal vaccination were not significantly different from values obtained afterwards.

Too few specific avoidance behaviors were observed to warrant statistical evaluation. Only a few minor problems were noticed in regard to catching the animals, leading them to the barn, entering the stocks, standing until examination, standing until turnout, and returning to the pasture. Specifically, 3 mares pawed a few strokes while standing in the stocks before or after examination, and 2 other mares pulled back on their tethers when loading into the stocks before examination.

Table 1—Duration of a standardized physical examination and compliance with physical examination procedures before and after simulated intranasal vaccination with 1 of 2 commercially available applicators or a control manipulation in horses.

	Applicator 1 (n = 11)		Applicator 2 (n = 11)		Control (n = 8)	
Variable	Before	After	Before	After	Before	After
Examination duration (min)	2.1 ± 0.46	2.0 ± 0.35	2.3 ± 0.50*	1.9 ± 0.44*	2.4 ± 0.73	2.2 ± 0.95
Examination compliance score						
Hand sweep	9.1 ± 0.83	9.1 ± 0.70	9.1 ± 1.22	9.2 ± 0.87	9.1 ± 0.64	9.2 ± 0.89
Manipulation of ears	5.7 ± 2.00	6.4 ± 1.43	5.4 ± 2.38	5.8 ± 1.47	7.1 ± 1.96	6.6 ± 1.77
Wiping of eyes	7.2 ± 1.25	7.0 ± 0.89	6.8 ± 1.08	7.3 ± 0.65	7.1 ± 1.13	7.7 ± 1.04
Palpation of nostrils	7.4 ± 1.43	7.0 ± 1.00	7.1 ± 1.30	7.4 ± 1.03	7.5 ± 1.60	7.0 ± 0.93
Examination of mouth	6.4 ± 1.21	6.4 ± 1.75	6.9 ± 1.30	6.9 ± 1.14	6.9 ± 1.36	6.4 ± 1.60
Oral dosing	7.1 ± 1.51	6.8 ± 1.54	8.2 ± 0.75	8.1 ± 1.14	7.7 ± 1.12	7.4 ± 1.51
Jugular venipuncture	9.1 ± 0.70	8.9 ± 0.83	8.7 ± 1.00	8.6 ± 1.12	8.5 ± 0.76	8.2 ± 1.75

*Values were significantly ($P < 0.001$) different.

Data are given as mean ± SD. The control manipulation consisted of placing the intranasal vaccine applicator externally, alongside the muzzle, and expressing sterile water onto the lateral aspect of the horse's face. Compliance was scored on a scale from 1 to 10, with 1 representing extreme avoidance behavior making it impossible to safely complete the specific procedure, 5 representing some avoidance attempts but completion of the specific procedure with normal head restraint, and 10 representing completion of the procedure with minimal or no restraint required and no avoidance attempts.

Discussion

In the horses enrolled in the present study, simulated intranasal vaccination could be accomplished with minimal restraint in < 90 seconds, with a mean time of approximately 30 seconds between initial approach to the horse and completion of the procedure. This was approximately 20 seconds longer, on average, than the time required for the control manipulation. We found no difference in behavioral compliance for the 2 applicators that are currently commercially available and did not identify any adverse effects on subsequent physical examination of the horses, including palpation of the nostrils and examination of the mouth in association with either applicator.

These findings are not consistent with complaints of some veterinarians that following even a single experience with intranasal vaccination, otherwise well-trained and compliant horses become averse to intranasal vaccination or to veterinary procedures in general. One factor to consider is experience and expertise of the individual performing intranasal vaccination. The individual performing simulated intranasal vaccinations in the present study was chosen because she had general experience with handling horses, although she did not have any previous experience with administering intranasal vaccinations to horses. She simply read and followed the package instructions. It is possible that variations from the label instructions or in the general manner of restraining the horse and administering the vaccination may yield different results. Another factor that should be considered is the study population. Animals used in the present study were highly experienced with certain veterinary reproduction procedures, including loading into stocks, palpation per rectum, and IV and IM injections. However, they were not considered highly experienced with the physical examination procedures performed in this study. Twenty-three of the 36 animals did not have any history of previous intranasal vaccination. Because complete histories were not available for the remaining 13 animals, it is possible that some had been exposed to intranasal vaccination. None had undergone nasogastric intubation while at our facility (mean, 2 years; range, 2 weeks to 15 years), but 18 had been at this facility for < 1 year. In sum, we believe that in regard to intranasal and general physical examination manipulations, our study population likely reflected the generally compliant, well-trained horses about which practitioners had complained were developing aversions to intranasal manipulation and other veterinary procedures after their first intranasal vaccination.

Other factors particular to the research, as opposed to the practice, setting may have contributed to our results. In the research environment, the individual performing intranasal vaccination could proceed in an unhurried manner, with standard relaxed restraint of the horses provided by an experienced coworker. Practice conditions would likely vary in this regard. In addition, sterile water as opposed to vaccine products was used. Perhaps the vaccine products themselves may be more aversive than sterile water. Material safety data sheet specifications for these vaccines indicate no obvious characteristics that might be more aversive than sterile water.

Use of cold applicators in winter has been suggested to influence compliance with intranasal vaccination of horses.[c] In the present study, we did not find any significant differences in application compliance score or application duration between horses in which cold applicators were used and horses in which warm applicators were used. However, the small numbers of horses in each group limited our ability to identify significant differences, and the difference in application duration suggests that it may be worth testing this factor with a larger sample size. It was the impression of the individual performing the simulated vaccinations that the longer application duration in horses in which cold applicators were used was attributable to kinking of applicator 2 during insertion, and review of the videotapes appeared to substantiate this impression. Kinking also appeared to increase the frequency with which the applicator came into contact with the nasal mucosa, which appeared to provoke avoidance behavior. This applicator seemed less likely to kink when warm.

Greater resistance to simulated intranasal vaccination was observed among the ponies in the present study than among the horses. Cold applicators were used in both ponies in which the procedure could not be completed, but what role this played could not be determined. It was the impression of the individual performing the procedure that substantial avoidance behavior in ponies was elicited when the applicator contacted the nasal passage, which was considerably smaller and shorter than in the horses and is known to be highly sensitive.[1] No differences in behavioral responses between ponies in which applicator 1 versus applicator 2 was used were evident, but again the sample size was small. For ponies with substantial aversion to simulated intranasal vaccination (2 stallions and 1 gelding), some increased aversion to manipulation of the head and nose was evident during the physical examination following the procedure. However, study of a greater number of subjects would be required to understand the importance of applicator size in ponies.

In conclusion, no problems with compliance with simulated intranasal vaccination or evidence of subsequent aversion to manipulation of the head and nose were found in the 22 horses receiving simulated intranasal vaccination in the present study. However, a sample of 22 horses is too small to conclude that aversion cannot occur. To better understand anecdotal practitioner reports of aversion in horses, further study might be more productively directed at observing a variety of technicians and techniques and at evaluating what effect, if any, temperature of the applicator might have.

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b. Pinnacle IN, Fort Dodge Animal Health, Fort Dodge, Iowa.
c. Holland R, Intervet, Millsboro, Del: Personal communication, 2003.
d. Statistix, version 8, Analytical Software, Tallahassee, Fla.

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