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Brainstem Auditory Evoked Responses in Foals: Reference Values, Effect of Age, Rate of Acoustic Stimulation, and Neurologic Deficits

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Background: Age and rate of acoustic stimulation affect peak latencies in brainstem auditory evoked responses (BAER) in humans. Those effects are unknown in foals.

Hypothesis/Objectives: Our goals were to (1) establish reference values for BAER in foals by using 3 different stimulation protocols, (2) evaluate the effects of age and stimulation frequencies on BAER tracing in foals up to 6 months old, and (3) compare the data with BAER obtained from foals with central nervous system (CNS) disorders.

Animals: Thirty-nine neurologically normal foals and 16 foals with neurologic diseases.

Methods: Prospective observational clinical study. BAER recorded by using 3 protocols of stimulation (11.33 repetitions per second [Hz]/70 decibel normal hearing level [dBNHL]; 11.33 Hz/90 dBNHL; 90 Hz/70 dBNHL).

Results: No effect of age was observed in normal foals ($P > .005$). No significant difference was observed for latencies and interpeak latencies (IPL) when comparing foals with neurologic diseases and normal foals ($P > .05$), but 78.6% of foals with neurologic diseases had an asymmetry in their tracing, reflecting a difference in conduction time between the left and right side of the brainstem. Increasing the stimulation rate did not improve detection of CNS disorders.

Conclusions and Clinical Importance: We propose BAER reference values for foals up to 6 months of age by using 3 protocols. Most foals with neurologic deficits had abnormal BAER tracing.

Key words: BAER; Brainstem auditory evoked potentials; Equine neonatology.

Brainstem auditory evoked responses (BAER) evaluate the electrophysiologic activity of the auditory pathway in response to externally applied acoustic stimulation. This noninvasive, highly repeatable technique provides objective measurements of the function and integrity of the auditory system.[1] In healthy subjects, it consists of up to 7 waves labeled with Roman numerals recorded during the first 10 ms after acoustic stimulation.[2–4] Waves represent summated neuronal activity at different sites in the brainstem. Wave I is generated by the cochlear nerve, wave II originates from the cochlear nucleus, waves III and IV are generated in the olivary nucleus and the lateral lemniscus, respectively, and wave V in the midbrain (caudal colliculus).[5,6] Physiologic factors such as age[7] and head size[5] affect BAER. Stimulus frequency (clicks/s or Hz) also have clinically relevant effects on tracing.[1] In human infants and premature babies, higher frequencies improve the detection of brainstem abnormalities from hypoxic/ischemic encephalopathy (HIE).[8,9]

In human neonatal intensive care units, BAER testing became a necessary tool in the diagnosis, prognosis, and follow-up of central nervous system (CNS) disorders to evaluate the integrity of the brainstem in various conditions.[10] In veterinary medicine, it is currently the method of choice to evaluate auditory function, but, its use in diagnosing central neurologic dysfunction in horses, especially neonates, remains infrequent.[4,11,12]

Abbreviations:

AG	affected group
BAER	brainstem auditory evoked responses
CG	control group
CNS	central nervous system
dBNHL	decibel normal hearing level
HIE	hypoxic/ischemic encephalopathy
Hz	stimulus frequency
IPL	interpeak latencies

Neurologic disorders of foals in the neonatal period represent a diagnostic challenge. They include HIE, bacterial meningitis, trauma, and other acquired or inherited disorders,[13] which often may involve the brainstem. If BAER testing proves to be as useful in foals as in human infants, it will allow more accurate diagnosis and prognosis of neurologic disorders and monitoring of treatment efficacy in this species. Before it can be used in the evaluation of neurologic disorders of foals, the influence of age and head size should be evaluated for different protocols in the reference population. The aim of our study was to provide reference values for BAER in foals <6 months of age and to evaluate the effects of age on tracing by using 3 stimulation protocols. We also wanted to determine if increasing the frequency of acoustic stimulation improved detection of conduction abnormalities in foals with neurologic disorders.

Materials and Methods

Study Animals

For this prospective study, BAER were performed on foals <6 months old hospitalized at the Centre Hospitalier Universitaire Vétérinaire, Saint-Hyacinthe, Canada, from April 2008 to

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Table 1. Mental status score for foals.

Score 0	Normal mental status	Standing. Alert. Nurses properly. Normal interaction with the dam and environment
Score 1	Mild obtundation	Standing. Calm. Reduced affinity for the dam. Do not find udder. Decreased interaction with environment. Restlessness
Score 2	Severe obtundation	Standing. Obtundation. Few or no interaction with the dam or environment. Compulsive walking. Head pressing
Score 3	Stupor	Recumbent. No spontaneous interaction with environment. Inappropriate reactions when stimulated
Score 4	Coma	Recumbent. No reaction with environment. No reaction when stimulated

April 2010. Client-owned foals were included after owners' consent. This study was approved by the Animal Care Committee.

Before BAER testing, physical and neurologic examinations were performed. To evaluate mental status, foals were observed in their stalls for 30 minutes before manipulating them. Evaluation of mental status in standing foals included affinity for the mare, interaction with the mare and environment, number of times and capacity to nurse, number of attempts to find the udder, response to loud noise, observation of abnormal posture ("star-gazing", head pressing), seizures or some combination of these findings. In recumbent foals, the number of spontaneous movements, number of attempts to stand, responses to external (eg, opening stall door) and physical stimuli as well as interactions with caregivers were recorded. The mental status score used is presented in Table 1. Based on the neurologic examination, foals were divided into a control group (CG) and an affected group (AG) (neurologic deficits). Control foals were used to establish the reference values and were separated into 4 subgroups according to age. Control group 1 included foals 0–7 days old; CG2, foals >1 week to 1 month old; CG3, foals >1–3 months old; CG4, foals >3–6 months old.

Recording of BAER

Recordings were performed with an electromyogram/evoked potential system.[a] Depending on their size and cooperation, foals were restrained either in lateral recumbency or standing. Sedation (xylazine hydrochloride[b] 0.2 mg/kg, IV) was administered when needed, with the owner's consent. An alternating (rarefaction and condensation) click stimulation was delivered via internal earplugs[c] with disposable ear tips[d] introduced into the external auditory meatuses and secured in place with gauze. Three different stimulation protocols were used. Protocols 1 and 2 used a stimulus frequency of 11.33 Hz at intensity level of 70 decibel normal hearing level (dBNHL), and a stimulus frequency of 11.33 Hz at 90 dBNHL, respectively.[3,5] Protocol 3 used a stimulus frequency of 90 Hz at 70 dBNHL.[14] Ipsilateral stimulations were recorded for both ears. A masking noise (30 dBNHL below stimulation intensity) was applied to the controlateral ear. To assess repeatability, traces were always recorded twice.

Disposable subdermal stainless steel needle electrodes[e] were used to acquire data. The reference electrode (positive) was placed at the vertex, over the parietal suture, rostral to the site where the temporalis muscles diverge. The recording electrode was placed over the zygomatic process of the stimulated ear (ipsilateral). The ground electrode was placed over the zygomatic process of the contralateral ear and this site provided an upward inflection of the waves. Amplifier sensitivity was set at 1 μv/division, sweep speed at 1 ms/division with a bandpass filter of 100 Hz/3 KHz with automatic artifact rejection. Each trace represented the average of a minimum of 500 responses over the first 10 ms after acoustic stimulation. Data were analyzed by a computer-based program.[f] Waves I through V were identified using manually directed cursors. Absolute latencies for peaks I, III, and V as well as I–III, III–V, and I–V interpeak latencies (IPL) were calculated for each tracing.

Data Analysis

A repeated measures multivariate linear model, with ear and stimulation protocol as within-subject factors and sex and age as cofactors, was used to evaluate the effects of the 3 protocols on waves I, III, and V latencies, and I–III, III–V, and I–V IPL in normal foals.

The same model, with ear and stimulation protocol as within-subject factors and mental status as between-subject factor, was used to compare the effects of the 3 protocols on waves I, III, and V latencies, and I–III, III–V, and I–V IPL between normal and foals with neurologic disorders. Tukey's posthoc tests were used to compare pairs of means.[g] Significance level was set as $P < .05$. Reference values were obtained using 3 standard deviations, representing 99.7% of the population.

Results

Study Animals

Thirty-nine neurologically normal foals were included in the CG, 26/39 males (67%) and 13/39 females (33%). Breeds included Warmbloods (13/39), Quarter/Paint Horses (9/39), Standardbreds (7/39), Thoroughbreds (6/39), and others (4/39). Reasons for hospitalization included: healthy foals accompanying their dam (10/39), musculoskeletal problems (13/39; myopathies, fractures, angular limb deviations, septic arthritis, osteomyelitis), urinary problems (6/39; omphalophlebitis, patent urachus, umbilical hernia), digestive problems (3/39; colic, meconium impaction, diarrhea), and other nonneurologic complaints (7/39; pneumonia, anemia, neonatal isoerythrolysis, eye problems, failure of passive transfer of immunity). Age of foals at time of testing ranged from 2 to 139 days (mean, 37.2 days; median, 21 days). Control group 1 included 9/39 foals, 17/39 in CG2, 7/39 in CG3, and 6/39 in CG4. Brainstem auditory evoked response tracing obtained from 1 normal foal are shown in Figure 1.

Sixteen neurologically abnormal foals were included in the AG, 12/16 (75%) males and 4/16 (25%) females. Fourteen were <1 week old, 1/16 was 1 month old, and 1/16 was 5 months old. Breeds included Quarter/Paint Horses (3/16), Warmbloods (3/16), Standardbreds (2/16), Thoroughbreds (2/16), Clydesdales (2/16), and others (4/16). Reasons for presentation included hypoxic-ischemic encephalopathy (4/16), failure of passive transfer of immunity (1/16), meconium impaction

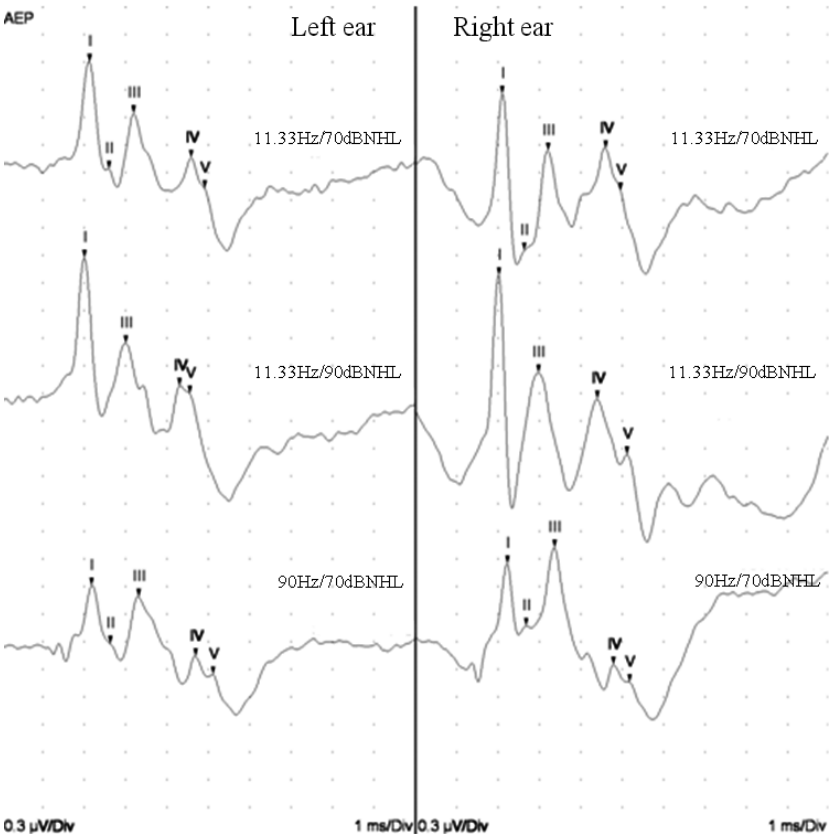

Fig 1. Brainstem auditory evoked responses (BAER) tracing obtained from the left and right ears of a healthy foal by using 3 protocols. All waves are not identifiable in all tracing. Wave V is partially fused with wave IV on 11.33 Hz/70 dBNHL tracing and on the left 11.33 Hz/90 dBNHL tracing. Wave II is undetectable on 11.33 Hz/90 dBNHL tracing.

(2/16), bladder rupture (2/16), diarrhea (2/16), isoerythrolysis (1/16), nutritional myopathy (1/16), septicemia (1/16), orphan foal (1/16), and musculoskeletal diseases (2/16).

All AG foals had abnormal mental status. Twelve suffered from mild to severe obtundation (score 1–2), 3 were in a stuporous state (score 3), and 1 was comatose (score 4). One presented with seizures. Most had no menace response but this finding was attributed to their young age (<1 week old). Two foals had head tilts that were not associated with deafness, but 1 had concurrent unilateral facial paralysis.

Unilateral or bilateral deafness (isoelectric line recorded in response to the highest intensity level of stimulation; 90 dBNHL) was found in 7.1% (4/55) of all foals tested (CG and AG). None of the 55 foals tested showed clinical signs of deafness. The foal with bilateral deafness was excluded from analysis because no tracing could be obtained. The other 3 were used in the control group based on clinical and neurologic examination findings. None of them were Paint Horses.

BAER Testing

Brainstem auditory evoked response tracing were easily obtained without sedation in most foals (49/55). Forty-three foals (78.1%) had a complete set of tracing for the 3 protocols. All tracing were not available for both ears in all foals because of either lack of cooperation of nonsedated foals (4/55), deafness (4/55), or low amplitude of the tracing (impossible to place cursors reliably in 5 of the 90 Hz/70 dBNHL traces). A total

Table 2. Wave I, III, and V latencies (mean ± SD) and I–III, III–V, and I–V interpeak latencies (IPL, mean ± SD) at different stimulation frequencies and intensities in normal foals <6 months old.

	Stimulus (frequency/intensity)		
	11.33 Hz/70 dBNHL (n = 107)	11.33 Hz/90 dBNHL (n = 102)	90 Hz/70 dBNHL (n = 98)
Wave I (ms)	1.15 (±0.08)[a]	1.04 (±0.59)[b]	1.21 (±0.08)[c]
Wave III (ms)	2.37 (±0.1)[a]	2.25 (±0.15)[b]	2.49 (±0.12)[c]
Wave V (ms)	4.06 (±0.19)[a]	3.97 (±0.24)[b]	4.28 (±0.23)[c]
I–III IPL (ms)	1.22 (±0.09)[d]	1.21 (±0.14)[e]	1.29 (±0.11)[f]
III–V IPL (ms)	1.70 (±0.20)[d]	1.69 (±0.25)[e]	1.79 (±0.22)[f]
I–V IPL (ms)	2.91 (±0.20)[d]	2.93 (±0.25)[e]	3.07 (±0.24)[f]

Different letters represent statistical differences among different protocols (a, b, c, d and f: $P < .0001$; e: $P = .0016$). n = number of ears.

Table 3. Suggested reference values for waves I, III, and V latencies and I–III, III–V, and I–V IPL at different stimulation frequencies and intensities in foals <6 months old.

	Stimulus (frequency/intensity)		
	11.33 Hz/70 dBNHL	11.33 Hz/90 dBNHL	90 Hz/70 dBNHL
Wave I (ms)	0.9–1.39	0–2.21	0.97–1.45
Wave III (ms)	2.07–2.67	1.80–2.70	2.13–2.85
Wave V (ms)	3.41–4.63	3.25–4.69	3.59–4.97
I–III IPL (ms)	0.95–1.49	0.79–1.63	0.96–1.62
III–V IPL (ms)	1.10–2.30	0.94–2.44	1.13–1.45
I–V IPL (ms)	2.31–3.51	2.12–3.68	2.45–3.75

of 107/110 tracing (right and left ears) were available for protocol 1, 102/110 tracing for protocol 2, and 98/110 tracing for protocol 3.

Reference Values and Effect of Age on BAER Tracing

There were no significant differences between mean latencies and interpeak latencies (IPL) for sex, age, or ear tested (all $P > .05$) in CG, therefore, measures were pooled to calculate reference values. The ear was used as statistical unit. A significant difference between mean latencies and IPL was found among the stimulation protocols. Latencies decreased with increasing level of stimulus intensity with peaks I, III, and V of 90 dBNHL tracing appearing significantly earlier than for 70 dBNHL tracing (all $P < .0001$). Latencies increased with increasing stimulation rates with peaks I, III, and V appearing significantly later for 90 Hz tracing than for 11.33 Hz tracing (all $P < .0001$). Interpeak latencies followed the same pattern (all $P < .0016$). Latencies and IPL for each protocol are presented in Table 2. Reference values (mean $\pm$ 3 SD) are listed in Table 3.

Foals with Neurologic Deficits

All latencies and IPL of AG foals were within normal values obtained from CG foals for all protocols. No significant differences were found between the 2 groups even at higher frequencies (90 Hz). When left and right tracing were available (CG: 30/39; AG: 14/16), both ears were compared for symmetry of the traces. As opposed to normal foals, a statistically significant effect was seen between ears in the AG foals for latencies of wave V ($P = .01$) and I–III, III–V, and I–V IPL ($P = .04$, .04, and .005, respectively) for all protocols. Eleven AG foals (78.6%) had increased latencies and IPL of >0.2 ms in 1 ear compare to the other. This threshold was used because it is currently the maximal accepted difference to validate the repeatability of the test.[1] Wave V latencies and III–V and I–V IPL were the most frequently affected measurements.

Discussion

We provide reference values of BAER in foals from birth to 6 months of age by using 3 stimulation protocols. The only other references available were based on a limited number of foals <1 week old and used only low stimulation frequencies.[11,12] Our report represents the first time that the effects of higher stimulation frequencies have been described in normal and neurologically affected foals. Brainstem auditory evoked response testing is not affected by the state of arousal or therapeutic dosages of most CNS depressants.[15] Age is reported to alter latencies and IPL in humans and small animals,[9,16,17] reflecting maturational changes in the CNS. We could not show a significant effect of age with any of the protocols used, and tracing obtained were similar to those of adult horses. This finding differs from what is observed in humans and small animals,[4,10] but correlates with histopathology of the brain and spinal cord showing that gyral development and myelin maturation are more advanced at birth in foals than in other species.[18,19]

Head size is another factor that might influence latencies.[4,5,20] When comparing adult Thoroughbreds and ponies, head size was found to correlate with BAER latencies.[5] Another study demonstrated that larger horses had faster conduction times (shorter I–V IPL).[15] Controversial results also have been published in humans and dogs.[1,4] To assess differences in head size within our population of foals, we divided them into 4 subgroups but could not detect any significant difference among them. This finding is similar to what was reported by Munro et al[21] when they compared 2 breeds of dogs (Dalmatians and Jack Russell Terriers). A significant difference in latencies and I–V IPL was found among breeds, but not within a given breed despite the considerable scatter in head sizes within each group. They concluded that although the larger breed of dogs had increased latencies, there was no specific correlation with head size.[21] In horses, differences observed between ponies and Thoroughbreds[5] also might have been because of breed rather than size differences. We did not measure head sizes, but the lack of differences observed among subgroups suggests that this parameter does not contribute to variations in BAER tracing in foals. Ponies were not included in our study, but it would be interesting to see if the observations made by Mayhew and Washbourne apply to younger animals. Differences in breeds eventually could be evaluated in horses too, but we did not have enough foals to evaluate this parameter.

Brainstem auditory evoked response testing was well tolerated and easily obtained from most foals without sedation showing that this noninvasive, repeatable, quantitative method can be used in foals <6 months of age. Data presented here could be compared with responses recorded from clinical patients to assess the integrity of their auditory pathways. However, variations among laboratories can be observed because of technical differences. Keeping that in mind, when comparing our reference values with data from Mayhew

and Washbourne,[5] I–V and III–V IPL are longer in foals than in adults. This could represent a delay in maturation of the central auditory pathway in foals <6 months of age. In human infants, a decrease in wave V latencies and III–V IPL is seen with increasing age and represents maturational changes (eg, myelination, axon diameter, synaptic efficacy) in the brainstem auditory pathway.[19]

Neurologic disorders in foals remain a diagnostic challenge. Changes in behavior (eg, decreased affinity for the dam, restlessness, head pressing, compulsive walking) and arousal, with or without cranial nerve abnormalities, are related to alterations of the cerebrum or brainstem.[13] In our study, BAER testing was used to assess integrity and function of the brainstem auditory system in foals with neurologic deficits. No significant differences were detected between affected and control foals. All data acquired from AG foals were within our reference range. However, when for a given foal, tracing from left and right ears were compared to each other, a significant difference in wave V latencies and I–V IPL was observed in 78.6% (11/14) of the neurologically abnormal foals and not in normal foals. This finding demonstrates a difference in brainstem conduction time between the 2 sides of the auditory pathway and thus reflects suboptimal function of the central auditory pathway in these foals.

In most of the AG foals, the only abnormality of the neurologic examination was an altered mental status. Altered mental status is nonspecific for neurologic diseases and can be triggered by other causes such as metabolic derangements including hypoglycemia, electrolyte, and acid-base disturbances or even sepsis.[18] We intended to compare foals with abnormal mental status and concurrent debilitating diseases with foals with altered mental status related only to CNS diseases. Unfortunately, only 4 foals with HIE met these criteria, preventing statistical analysis between these 2 subgroups.

In human medicine, considerable improvements have been made in the diagnosis of neonatal neurologic diseases using magnetic resonance imaging (MRI).[22] We intended to compare BAER results with MRI images, but only 2 owners gave consent to perform this test. In 1 foal, focal hyperintense areas on T2-weighted images of the latero-caudal cortex of right and left parietal lobes were noticed. No lesions were observed in the brainstem. The foal was euthanized because of unrelated problems. Gross necropsy findings included multifocal discoloration of the parietal cortex at the junction of the gray and white matter. Microscopically, numerous foci of slight variability in the thickness of the cortical gray matter were observed, associated with scattered single neurons within the white matter (neuronal heterotopia). The other foal was part of the CG and MRI was unremarkable.

Increasing stimulation frequencies improves detection of BAER abnormalities in human infants with neurologic deficits[8] but not in experimental cats.[23] In horses, the effects are unknown. In humans and cats, higher repetition rates trigger decreased amplitudes and increased latencies. In our foals, the waves followed the same pattern, but it did not improve detection of CNS abnormalities and lessened the resolution of the waveforms preventing analysis of a few tracing, impeding reliable positioning of the cursors. The rate of 90 Hz represented the maximum rate possible with our equipment. The stimulation may have been too slow to trigger an abnormal response. In 1 study, significant differences in IPL were demonstrated only at much higher rates (455 and 910 Hz).[24]

As in other species, BAER testing has been used in horses to evaluate auditory function.[12,25] The prevalence of deafness in the equine population is unknown and would require systematic screening as performed in humans and dogs.[26,27] Recently, auditory dysfunction was associated with specific pigmentation patterns in Overo Paint Horses.[12] We report a prevalence of 7.1% of deaf foals in our study population. Unilateral deafness was identified in 3 foals and bilateral deafness in 1. None showed clinical signs of deafness. Two were Clydesdales from the same sire such that a genetic factor cannot be ruled out. The others were bay foals of unrelated origins (Warmblood and Standardbred). Obstruction of the external ear canal and pathology of the middle ear (otitis media) were associated with diagnosis of deafness in other species.[1,28] In human infants tested early in life, isoelectric tracing can be recorded in normal hearing subjects because of vernix caseosa accumulation in ear canal or amniotic fluid in the middle ear.[29] Gross observation of the external ear canal did not identify any abnormalities and none of the foals had clinical signs compatible with middle ear disease. Radiographs or MRI were not performed and thus conduction disorders cannot be completely ruled out. All deaf foals were tested at least twice during their hospitalization and tracing remained abnormal.

In conclusion, BAER testing is a reliable and easily performed technique that can be useful in the diagnosis of neurologic disorders in foals. The age did not affect BAER results in foals up to 6 months of age. However, when values for foals and adult horses were compared, mild conduction delay in the most central portion of the brainstem was detected in foals. This could be attributed to differences in CNS maturity. Changes in head size associated with age do not seem to influence BAER tracing in foals. The prevalence of deafness among the study population was higher than expected. There was no benefit in using a higher repetition rate of acoustic stimulation. It lengthened the duration of examination and made tracing more difficult to read. Nonetheless, other studies are needed before concluding that higher repetition rates are not useful in foals. A most interesting finding is that the majority of foals with neurologic deficits had asymmetry in their BAER tracing, reflecting a difference in conduction time between left and right sides of the brainstem. This indicates that BAER might be useful in the diagnosis of neurologic disorders in foals <6 months of age.

Footnotes

[a] Cadwell Sierra WedgeII®, Cadwell Laboratories, Kennewick, WA
[b] Xylazine, Rompun, Bayer Health Care animal division, Toronto, ON, Canada
[c] Cadwell insert headphones, Cadwell Laboratories, Kennewick, WA
[d] Adult foam tips, 202710-000, Elk Groove Village, IL
[e] Ambu®Neuroline Subdermal, 12 mm/27G needles, 2022354-000, Ballerup, Denmark
[f] Sierra Waves8.0, Cadwell Laboratories, Kennewick, WA
[g] SASv.9.2., Cary, NC

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Conflict of Interest: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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