Computed tomographic assessment of brain tissue disruption and skull damage in equine cadaveric heads caused by various firearm-ammunition combinations applied as potential gunshot methods for euthanasia of horses

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Received December 6, 2019.
Accepted April 20, 2020.

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OBJECTIVE
To evaluate with CT the characteristics of brain tissue disruption and skull damage in cadaveric heads of adult horses caused by each of 6 firearm-ammunition combinations applied at a novel anatomic aiming point.

SAMPLE
53 equine cadaveric heads.

PROCEDURES
Heads placed to simulate that of a standing horse were shot with 1 of 6 firearm-ammunition combinations applied at an aiming point along the external sagittal crest of the head where the 2 temporalis muscles form an inverted V. Firearm-ammunition combinations investigated included a .22-caliber long rifle pistol firing a 40-grain, plated lead, solid-core or hollow-point bullet (HPB); a semiautomatic 9-mm pistol firing a 115-grain, jacketed HPB; a semiautomatic .223-caliber carbine firing a 55-grain, jacketed HPB; a semiautomatic .45-caliber automatic Colt pistol firing a 230-grain, jacketed HPB; and a 12-gauge shotgun firing a 1-oz rifled slug. Additional heads placed in a simulated laterally recumbent position were shot with the semiautomatic 9-mm pistol–HPB combination. All heads underwent CT before and after being shot, and images were evaluated for projectile fragmentation, skull fracture, and cerebrum, cerebellum, and brainstem disruption.

RESULTS
Computed tomography revealed that all firearm-ammunition combinations caused disruption of the cerebrum, cerebellum, and brainstem that appeared sufficient to result in instantaneous death of a live horse. Hollow-point ammunition was as effective as solid-core ammunition with regard to brain tissue disruption. Brain tissue disruption was not affected by head positioning.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that the examined firearm-ammunition combinations, when applied at a novel aiming point, appear to be reasonable options for euthanasia of horses. (*Am J Vet Res* 2021;82:28–38)

Under the Federal Food, Drug and Cosmetic Act, horses euthanized by injection of barbiturate solution are not allowed to enter the food supply chains for humans or pets.[1] In accordance with US federal, state, and local law, animals euthanized with barbiturates must be disposed of in a manner that does not contaminate ground water or allow the consumption of a carcass by scavenging wildlife.[2,3] Composting was evaluated as an alternative for carcass disposal after euthanasia of horses by IV injection of pentobarbital sodium, but the drug was still detectable in carcass-derived compost material at the end of the study (day 367), with no clear indication of concentration reduction.[4] Historically, many rendering facilities accepted horses euthanized by chemical means. In light of recent contamination of dog food with pentobarbital and an increase in the general public's awareness that carcasses of horses euthanized with pentobarbital have entered the pet food supply chain, many rendering facilities are no longer accepting horses euthanized by chemical means or sometimes even horses euthanized by any method.[5,6] Furthermore, situations requiring near-contemporaneous euthanasia of many horses, such as large-scale disease outbreaks and natural disasters, necessitate a need for an economically feasible method for mass euthanasia. According to the AVMA Guidelines for

ABBREVIATIONS

ACP	Automatic Colt pistol
HPB	Hollow-point bullet
KE	Kinetic energy
LR	Long rifle
SCB	Solid-core bullet

Animal Euthanasia 2020 edition,[7] euthanasia by gunshot is an acceptable method when certain conditions are met. These conditions are that the individuals performing the euthanasia are well trained in firearm safety, safety measures are in place to prevent human injury, and an appropriate anatomic aiming point is used.[7,8] Euthanasia by gunshot has been used routinely for horses in other parts of the world and should be given more consideration as a reasonable alternative method of equine euthanasia in the United States.

There is limited consensus among published data regarding the appropriate caliber of firearm and ammunition along with the extent of brain tissue disruption that must occur for appropriate euthanasia of horses. The caliber of the firearm and the various properties associated with the ammunition being fired have multifactorial roles in whether a projectile achieves sufficient penetration of the skull and causes lethal brain tissue disruption.[9] Different types of ammunition have different characteristics that may make them more or less desirable for euthanizing horses. Full metal jacket and semijacketed ammunition maintain their shape upon impact and typically achieve a greater depth of penetration.[10] Because the frontal bones in horses are thinner than those in cattle, full metal jacket ammunition recommended for use in cattle may pose a greater risk of exiting a horse's head and injuring individuals nearby. Hollow-point projectiles are designed for maximum expansion and fragmentation upon impact,[11] which can lead to increased tissue disruption and decreased risk of the projectile exiting a horse's calvarium. Given this projectile expansion and associated initial release of KE, it has been postulated that hollow-point ammunition may not have the appropriate KE to penetrate the skull of adult horses.[7,8]

There are disadvantages to the use of firearms for euthanasia, such as limitations on firearm use by local laws, potential danger to bystanders and the firearm operator, and required education of the firearm operator on using the particular firearm and knowledge of species-specific anatomic features of the head. Postmortem diagnostic tests, such as histologic examination, may not be able to be performed or may be limited because of local, regional, or extensive tissue disruption.[7] Additionally, there may be societal concerns with the presence and use of firearms.

The purpose of the study reported here was to assess, through the use of CT, the extent and patterns of skull damage and penetration and disruption of brain tissue in adult cadaveric equine heads achieved by use of various combinations of firearms and ammunition. In addition, instead of use of the AVMA-recommended aiming point (ie, the intersection of 2 diagonal lines extending from the outer corner of an eye to the base of the opposite ear[7]), the use of a novel easily palpable anatomic aiming point was evaluated.

Materials and Methods

Sample

The heads of 53 cadavers of horses that were ≥ 2 years of age were purchased from rendering facilities. The heads were free of any external trauma, such as gunshot wounds. The causes of death of these horses were unknown and presumed to be by injectable euthanasia solution or natural causes. The heads were disarticulated at either the first or second cervical vertebra and stored at –20°C. Institutional animal care and use committee approval was not necessary for this study because no live animals were involved. At the time of use, each equine cadaveric head had been thawed at room temperature (approx 20°C) for 24 to 36 hours.

Firearms

The firearms used in the study were chosen because they were readily available to law enforcement officers and veterinarians. All firearms were owned by study personnel, and standard factory-loaded ammunition was used. Firearm operators were appropriately trained through various organizations including the armed forces, law enforcement, and hunter safety groups or were certified firearm instructors. Eye and ear safety protection was used by the firearm operators at all times during all periods of firearm discharge.

Study design and procedures

Before the start of the study, each equine cadaveric head underwent CT with a multislice helical CT scanner machine[a,b] by use of standardized institutional protocols with a maximum slice thickness of 3.8 mm to determine that all heads were free of preexisting trauma-induced damage. Each head was randomly assigned for testing of 1 of 6 firearm-ammunition combinations by selection of numbered pieces of paper from a bag. Projectiles were noted to be exiting the cadaver heads with the shotgun; because of safety concerns, the number of cadaver heads in this group was limited.

Firearm-ammunition combinations included the following: 12-gauge shotgun[c] firing a 2.75-inch 1-oz rifled slug[d] (designated as shotgun-slug), semiautomatic .223/5.56 carbine[e] firing a .223-caliber 55-grain jacketed HPB[f] (designated as .223-caliber carbine–HPB), semiautomatic .45-caliber ACP[g] firing a jacketed HPB[h] (designated as .45-caliber ACP–HPB), semiautomatic 9-mm pistol[i] firing a 147-grain jacketed HPB[j] (designated as 9-mm pistol–HPB), .22-caliber LR pistol[k] firing a 40-grain plated lead SCB[l] (designated as .22-caliber LR pistol–SCB), and .22 caliber LR pistol[k] firing a 40-grain plated lead HPB[m] (designated as .22-caliber LR pistol–HPB). The use of 2 types of .22-caliber LR pistol bullets was elected to allow comparison of SCBs and HPBs.

The experimental procedures were performed at outdoor private shooting ranges. Firearm-ammunition combinations were used on equine cadaveric heads that were positioned to simulate a horse standing with its head in a natural position. To accomplish this positioning, the head was placed in a custom-made stanchion so that the frontal bone was angled at approximately 70° to the ground. In addition, for the 9-mm pistol–HPB combination, some equine cadaveric heads were positioned to simulate a horse in lateral recumbency with the head horizontal and lying flat on the ground.

The firearm operators used a traditional grip to hold the firearm, and no stabilizing devices were used. Nine firearm operators were randomly assigned to the testing protocols on the basis of personnel availability. All firearm operators had similar aiming ability. The KE of the projectile of each firearm-ammunition combination generated during testing was calculated with the following equation:

$$KE = mv^2 \div 2$$

where m is the mass of the projectile (in kg) and v is the muzzle velocity (in m/s) provided by the firearm. All equine cadaveric heads were shot at a distance of approximately 20 to 60 cm and at an angle directed toward the greater foramen of the occipital bone and vertebral column approximately 70° from the plane of the frontal bone with use of a novel anatomic aiming point. This novel aiming point was palpated along the external sagittal crest of the head where the 2 temporalis muscles formed an inverted V **(Figure 1)**. Following weapon discharge, the head and its immediate area were searched for evidence of the projectile exiting the head. The equine cadaveric heads were scanned with a multislice helical CT scanner again after being shot, and the images were stored in an electronic medical database.

All pre- and postshooting images were reviewed by a board-certified veterinary radiologist (KW) and a second-year veterinary radiology resident (JRL) with imaging viewing software.[n] These reviewers were able to reconstruct images using a bone and soft tissue reconstruction algorithm. Transverse images were reconstructed in sagittal and dorsal planes. For the postshooting images, both the radiologist and radiology resident were blinded to the firearm-ammunition combination used. The images were subjectively scored for the following variables: projectile fragmentation, skull fracture, and structural disruption of the cerebrum, cerebellum, and brainstem. A grading scale of 0 to 5 was used for each variable **(Appendix)**. Additionally, the distance from the AVMA recommended aiming point (the intersection of 2 diagonal lines extending from the outer corner of the eye to the base of the opposite ear) and the authors' preferred aiming point (the location where the 2 temporalis muscles formed an inverted V on the external sagittal crest) was measured with 3D reconstruction software.[n]

Statistical analysis

With regard to CT assessments, inter-rater agreement between the board-certified veterinary radiologist and second-year veterinary radiology resident was evaluated with intraclass correlation coefficients, as described by Shrout and Fleiss.[12] Two-sample *t* tests were used for the comparison of data for the .22-caliber LR pistol HPB versus SCB and data for the 9-mm firearm-HPB combination with heads in the simulated standing position versus the simulated laterally recumbent position. An ANOVA was used for data analysis to examine the differences among the firearm-ammunition combinations. Post hoc pairwise comparisons were performed with the Holm adjustment to account for multiple pairwise comparisons.[13] All analyses were performed with commercially available software[o] for statistical computing. Two-sided significance was set at a value of $P \leq 0.05$.

Results

Most horses were similarly sized with the exception of 3 draft horses. All heads used were free of trauma-induced damage, as determined by CT prior to commencement of the study. The novel anatomic aiming landmark was easily palpated and identified on all equine cadaveric heads by all firearm operators. Use of this novel aiming point also resulted in a consistent amount of brain disruption within each study group. Although the amount of disruption varied among groups, the disruption was considered sufficient to induce insensibility and death, regardless of the firearm-ammunition combination. The novel aiming point and the AVMA recommended aiming point were very similar. Among the 53 heads, the palpated landmark at the inverted V of the 2 temporalis muscles was ≤ 0.5

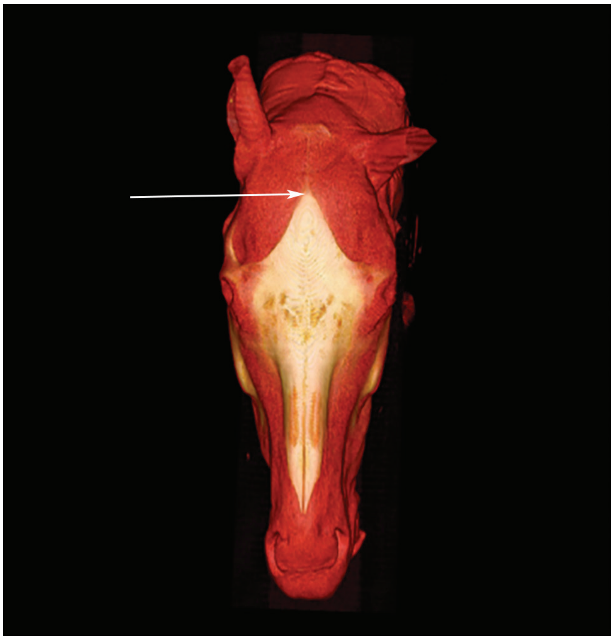

Figure 1—Three-dimensional CT reconstruction of the dorsal aspect of a cadaveric horse head to illustrate the novel anatomic aiming point used in a study of brain tissue disruption and skull damage in equine cadaveric heads caused by various firearm-ammunition combinations. The novel aiming point was palpated along the external sagittal crest of the head where the 2 temporalis muscles form an inverted V (arrow).

Table 1—Firearm-ammunition combinations and calculated projectile KE that were evaluated in a study of gunshot-related brain tissue disruption and skull damage in 53 equine cadaveric heads.

Firearm	Ammunition	Simulated position	No. of equine cadaveric heads	Projectile KE (J)
.22-caliber LR pistol	.22-caliber 40-grain plated lead SCB	Standing	9	173
.22-caliber LR pistol	.22-caliber 40-grain plated lead HPB	Standing	8	197
Semiautomatic 9-mm pistol	9-mm 147-grain jacketed HPB	Standing	8	434
Semiautomatic 9-mm pistol	9-mm 147-grain jacketed HPB	Lateral recumbency	8	434
Semiautomatic .223-caliber carbine	.223-caliber 55-grain jacketed HPB	Standing	8	1,738
Semiautomatic .45-caliber ACP (.45)	.45-caliber 230-grain jacketed HPB	Standing	8	625
12-gauge shotgun	12-gauge 2.75-inch 1-oz rifled slug	Standing	4	1,897

Kinetic energy was calculated by use of the following equation: $KE = mv^2/2$, where m is the mean mass of the projectile (in kg) and v is the mean muzzle velocity of the firearm (in m/s), based on manufacturer-provided information.

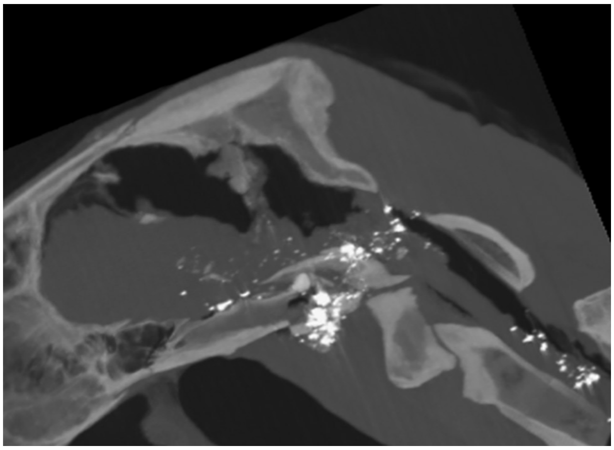

Figure 2—Representative maximum-intensity CT image of an equine cadaveric head with the cranial portion of the neck that was positioned to simulate the head of a standing horse and subsequently shot with a semiautomatic .223-caliber carbine firing a 55-grain HPB. Notice the cerebral, cerebellar, brainstem, and spinal cord disruption along with severe fragmentation of the HPB extending down the spinal cord. Rostral is to the left in this image.

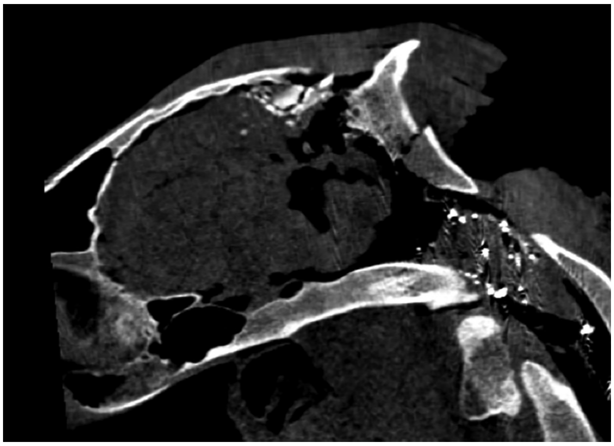

Figure 3—Representative sagittal CT reconstruction of an equine cadaveric head that was positioned to simulate the head of a standing horse and subsequently shot with a semiautomatic .45-caliber ACP firing a 230-grain, jacketed HPB. Notice the moderate cerebral, cerebellar, brainstem, and spinal cord disruption. Rostral is to the left in this image.

cm from the AVMA-recommended aiming point in 45 (85%) heads; the 2 aiming points were within 2 cm of each other in 51 (96%) heads and within 2.5 cm of each other in all 53 (100%) heads. The calculated KE for the projectiles released during use of the various firearm-ammunition combinations varied from 173 to 1,738 J **(Table 1)**. Several firearm operators reported a sense of unease when shooting the cadaveric heads. It was found that marking the targeted spot with white tape helped firearm operators focus on the target and less on the aesthetics of the situation. It is our experience that a marker of some type at the targeted spot on cadaveric heads and heads of live animals to be euthanized assists with the process and increases the firearm operators' comfort with use of an aiming point. Neither appreciable backspatter nor movement of any cadaveric head was observed; however, no additional measures beyond immediate observation were used.

With regard to CT assessments, the board-certified veterinary radiologist and the second-year veterinary radiology resident had overall good (0.4 to 0.6) to excellent (> 0.8) agreement in scoring of the images in all categories. The only significant difference in mean scores were noted for cerebral disruption; the board-certified veterinary radiologist's scores were higher than those of the second-year veterinary radiology resident. The board-certified veterinary radiologist's scores were used for further comparative analysis of the tested firearm-ammunition combinations.

CT assessment of projectile fragmentation

The highest mean (SD) score for projectile fragmentation was associated with the .223-caliber carbine–HPB combination (4.9 [SD, 0.4]), and the mean lowest scores were associated with the .22-caliber LR pistol–SCB combination (2.9 [0.6]) and semiautomatic 9-mm pistol–HPB combination (2.9 [1.1]). There were significant differences in projectile fragmentation across the firearm-ammunition combinations. The .223-caliber carbine-HPB combination caused significantly more projectile fragmentation, compared with that for the .22-caliber LR pistol–HPB combination (3.7 [SD, 0.5]; $P = 0.038$) or the .22-caliber LR pistol–HPB combination ($P < 0.001$) and the semiautomatic 9-mm pistol–HPB combination ($P < 0.001$). The shotgun-slug combination (4.5 [SD, 0.6]) and .45-caliber ACP–HPB combination (4.2 [0.9]) were associated with significantly (all $P < 0.01$) more projectile fragmentation than were the .22-caliber LR pistol–SCB combination and semiautomatic 9-mm pistol–HPB combination. The remaining 2-way comparison of firearm-ammunition combinations after Holm adjustment indicated no significant difference.

The HPB fragments fired from the .223-caliber carbine were found up to the level of the cranial aspect of the second cervical vertebra **(Figure 2)**, which would likely cause severe damage to the spinal cord. Similar projectile fragmentation and extension into the cranial portion of the vertebral column were also seen in 3 heads shot with HPBs fired from the .45-caliber ACP **(Figure 3)** and in 1 head shot with the shotgun. Such projectile fragmentation and extension were not evident for any other firearm-ammunition combinations tested.

CT assessment of skull fracture

All skulls were penetrated with the tested firearm-ammunition combinations. The highest mean score for skull fracture was associated with the shotgun-slug combination (4.8 [SD, 0.5]) and the lowest mean score was associated with the .22-caliber LR pistol–SCB combination (1.8 [0.7]). The skull fracture score for the shotgun-slug combination was significantly greater than that for the .22-caliber LR pistol with the HPB (2.4 [SD, 0.5]; $P < 0.001$) or SCB ($P < 0.001$) or the semiautomatic 9-mm pistol–HPB combination (3.2 [0.7]; $P = 0.021$). The shotgun-slug combination caused extreme destruction of the skull; moreover, the slugs exited out of 2 of the 4 shot heads **(Figure 4)**. Projectiles used in the other tested firearm-ammunition combinations did not exit any skull. Therefore, the number of equine cadaveric heads shot with the shotgun and slug was limited to 4 because of the undesirable possible consequence of a slug exiting the skull and increasing the risk of potential injury to bystanders. The second highest mean skull fracture score was associated with the .223-caliber carbine–HPB combination (4.2 [SD, 0.7]), which was significantly ($P \leq 0.001$) greater than the score for the .22-caliber LR pistol, regardless of bullet type. The semiautomatic .45-caliber ACP–HPB combination was associated with a significantly ($P = 0.048$) higher skull fracture score (3.5 [SD, 0.9]) than was the .22-caliber LR pistol–SCB combination. Lastly, skull fracture score for the semiautomatic 9-mm pistol–HPB combination was significantly ($P = 0.002$) higher than that for the .22-caliber LR pistol–SCB combination.

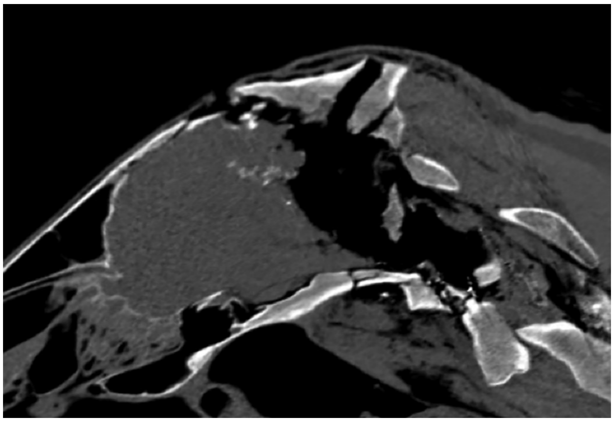

Figure 4—Representative sagittal CT reconstruction of an equine cadaveric head that was positioned to simulate the head of a standing horse and subsequently shot with a 12-gauge shotgun firing a 1-oz rifled slug. Notice the severe cerebral, nearly complete cerebellar and brainstem, and severe spinal cord disruption. Rostral is to the left in this image.

CT assessment of cerebral disruption

All tested firearm-ammunition combinations resulted in cerebral disruption. The shotgun-slug combination (4.2 [SD, 0.5]), was associated with the most cerebral disruption and the .22-caliber LR pistol–SCB combination (2.9 [0.8]) was associated with the least cerebral disruption. Mean scores associated with the

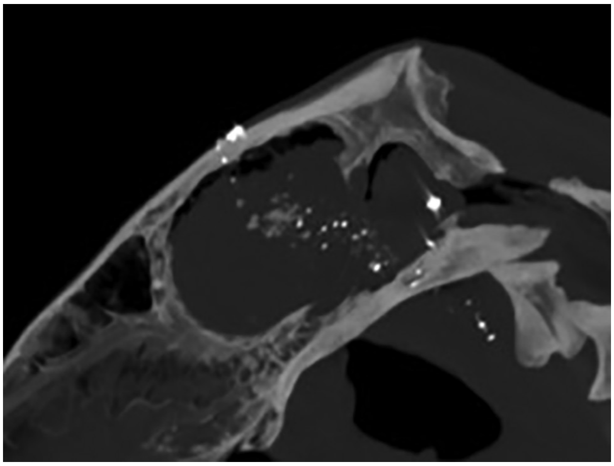

Figure 5—Representative maximum-intensity sagittal CT reconstruction of an equine cadaveric head that was positioned to simulate the head of a standing horse and subsequently shot with a .22-caliber LR pistol firing a 40-grain SCB. Notice the cerebral, cerebellar, brainstem, and spinal cord disruption. Rostral is to the left in this image.

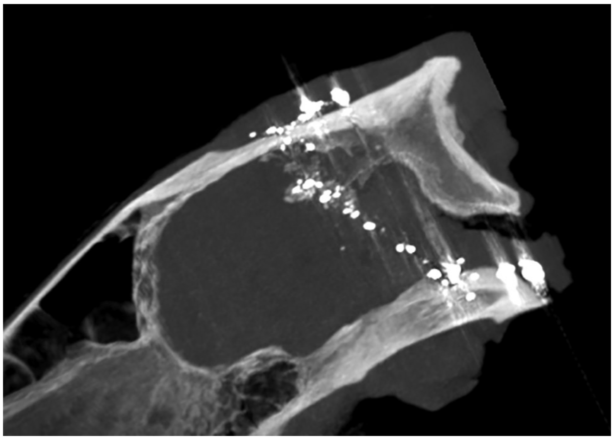

Figure 6—Representative maximum-intensity sagittal CT reconstruction of an equine cadaveric head that was positioned to simulate the head of a standing horse and subsequently shot with a .22-caliber LR pistol firing a 40-grain, jacketed HPB. This firearm-ammunition combination also resulted in cerebral, cerebellar, brainstem, and spinal cord disruption. Rostral is to the left in this image.

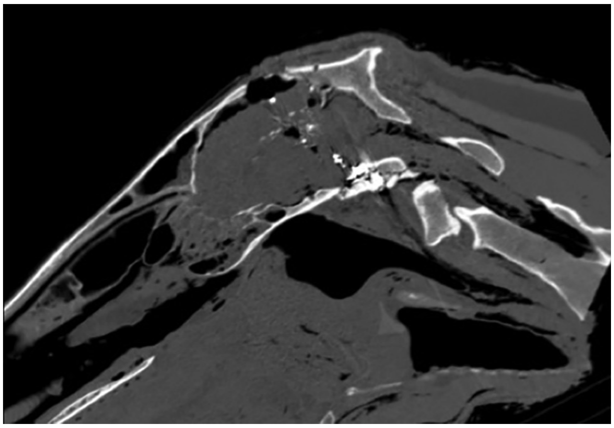

Figure 7—Representative sagittal CT reconstruction of an equine cadaveric head that was positioned to simulate the head of a standing horse and subsequently shot with a semiautomatic 9-mm pistol firing a 147-grain, jacketed HPB. Notice the cerebral, cerebellar, brainstem, and spinal cord disruption. Rostral is to the left in this image.

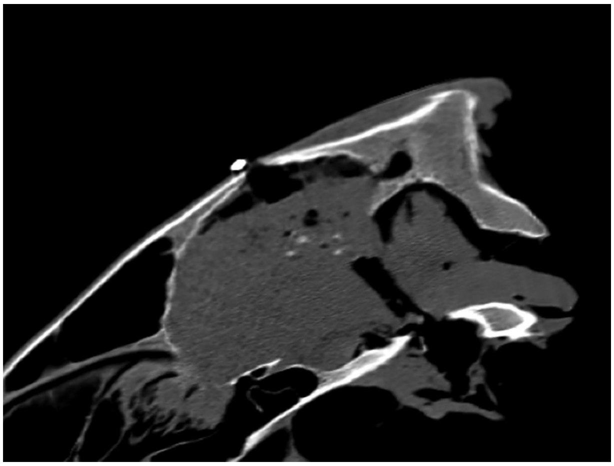

Figure 8—Representative sagittal CT reconstruction of an equine cadaveric head that was positioned to simulate the head of a laterally recumbent horse and subsequently shot with a semiautomatic 9-mm pistol firing a 147-grain, jacketed HPB. With the head in this position, the firearm-ammunition combination has resulted in cerebral, cerebellar, brainstem, and spinal cord disruption. Rostral is to the left in this image.

semiautomatic .45-caliber ACP–HPB, .22-caliber LR pistol-HPB, .223-caliber carbine-HPB, and the semiautomatic 9-mm pistol–HPB combinations were 3.3 (SD, 0.5), 3.0 (0.0), 3.8 (0.7), and 3.1 (1.1), respectively. Although the ANOVA suggested a significant (P = 0.030) difference among combinations, no significant differences in cerebral disruption scores were evident when firearm-ammunition combinations were tested with the pairwise Holm adjustment.

Cerebellar disruption

All tested firearm-ammunition combinations resulted in cerebellar disruption. The shotgun-slug combination (5.0 [SD, 0.0]) had the highest score for cerebellar disruption, and the .22-caliber LR pistol with the HPB (2.3 [0.5]) or SCB (2.9 [1.3]) had the least disruption among the firearm-ammunition combinations. The shotgun-slug and the .223-caliber carbine-HPB (4.6 [SD, 0.5]) combinations were associated with more cerebellar disruption, compared with findings for the .22-caliber LR pistol–HPB or .22-caliber LR pistol–SCB combinations (all P < 0.025). No other significant differences were noted between the scores for the tested firearm-ammunition combinations. Mean scores for semiautomatic .45-caliber ACP-HPB and semiautomatic 9-mm pistol–HPB combinations were 3.4 (SD, 1.6) and 3.4 (0.7), respectively.

Brainstem disruption

All tested firearm-ammunition combinations caused brainstem injury. The shotgun-slug combination (5.0 [SD, 0.0]) was associated with the most brainstem disruption, and the .22-caliber LR pistol–HPB combination (3.0 [0.6]) was associated with the least brainstem disruption. The scores for the .223-caliber carbine–HPB (4.9 [SD, 0.4]; P = 0.002) and shotgun-slug (P = 0.009) combinations differed significantly from that for the .22-caliber LR pistol–HPB combination. There were no significant differences between other firearm-ammunition combination scores. Mean scores for the semiautomatic .45-caliber ACP–HPB, .22-caliber LR pistol–HPB, and semiautomatic 9-mm pistol–HPB were 3.9 (SD, 1.5), 3.7 (1.0), and 3.6 (0.5), respectively.

Comparison of the .22-caliber LR pistol–HPB combination versus the .22-caliber LR pistol–SCB combination

In unadjusted 2-sample t tests, the .22-caliber LR pistol–HPB combination was associated with higher scores for skull fracture (P = 0.048) and projectile fragmentation (P = 0.009), compared with findings for the .22-caliber LR pistol–SCB combination. There was no significant difference between those 2 firearm-projectile combinations with regard to cerebral, cerebellar, or brainstem disruption **(Figures 5 and 6)**.

Comparison of the semiautomatic 9-mm pistol–HPB combination with the equine cadaveric heads in positions to simulate standing horses versus laterally recumbent horses

Eight equine cadaveric heads were positioned to simulate standing horses, and 8 heads were positioned to simulate laterally recumbent horses. The heads were shot with the semiautomatic 9-mm pistol–HPB combination. There were no significant differences in cerebral, cerebellar, or brainstem disruption between heads shot after placement in the standing and laterally recumbent positions **(Figures 7 and 8)**.

Discussion

To our knowledge, this was the first study to use CT to assess brain damage associated with different firearm-ammunition combinations used on heads of cadaveric horses. The results indicated that the novel anatomic aiming point and shooting distance used with each of the various firearm-ammunition combinations caused brain tissue disruption that was deemed sufficient to likely cause death of a living horse.

In the veterinary medical literature, recommended shooting distances from the target for euthanasia of large animals range from 5 cm up to 25 m.[7,14,15] In the study reported here, a distance of 20 to 60 cm from the targeted location on the cadaveric heads was chosen to simulate a comfortable distance for standing animals that are accustomed to being handled with a lead line held by the firearm operator. It is our opinion that the firearm operator should hold the horse and that having a horse held by an additional animal handler increases the risk of injury to the animal handler, especially among individuals unfamiliar with gunshot-related euthanasia. A distance of 20 to 60 cm from the head allows accuracy of shot placement owing to the firearm's proximity to the target while avoiding direct contact. It is not recommended to hold a firearm flush to an animal's body and discharge the projectile (a contact shot). When a firearm is fired flush against a target, the barrel of a firearm may become damaged by overpressurization, placing the firearm operator and surrounding observers at greater risk of injury or death.[7] However, as a result of modern firearm manufacturing, such barrel damage is highly unlikely. When a short distance is created between the firearm and a horse's head, there is a decreased risk that the horse could contact the firearm if the animal moves prior to euthanasia. Nevertheless, it is possible that an animal could move and bump the firearm, thereby disrupting shot placement and resulting in unsuccessful euthanasia or a missed shot, or cause unintentional discharge of the firearm that increases the risk of injury to individuals in the area. A potential factor to consider when choosing a muzzle-to-target distance is backspatter. The caliber of firearm also affects the amount of backspatter. Gross backspatter was not observed in the present study, but a firearm operator should consider the possible effects of backspatter and wear protective eyewear.

With regard to gunshot-related euthanasia of horses, current recommendations for a projectile's point of entry on a horse's head vary somewhat. The AVMA Guidelines for Euthanasia of Animals 2020 edition[7] describes the correct anatomic site for application of gunshot as the intersection of 2 diagonal lines each running from the outer corner of 1 eye to the base of the opposite ear. Other aiming points are described as 2.5 cm above the intersection of 2 lines originating from the medial canthus of each eye to the base of the contralateral ear or 2 cm above the intersection of lines drawn from the middle of each eye to the base of the contralateral ear.[7,8,14] The consensus is that passage through the frontal sinuses should be avoided and that the muzzle of the firearm should be tilted to direct the shot through the cerebral cortex toward the brainstem along a line toward the vertebral column.[7,14–16] In the present study, the targeted area was located by palpation of the external sagittal crest of the head to identify where the 2 temporalis muscles formed an inverted V; at this location, the firearm was aimed toward the brainstem and foramen magnum and along the vertebral column. This novel anatomic aiming point was chosen because it is easily palpable and identifiable in horses. This aiming point and that recommended by the AVMA were not dissimilar; the 2 points were located within 0.5 cm of each other in 84% of the heads used in the study. It is our opinion that use of a palpable anatomic feature increases the ease with which an appropriate aiming point can be located by firearm operators, especially individuals unfamiliar with gunshot-related euthanasia. It is important for the firearm operator to aim toward the brainstem and the vertebral column to ensure that the fired projectile causes sufficient brain tissue damage for the shooting to be considered humane. The method used in the present study achieved considerable disruption of the cerebrum, cerebellum, and brainstem in equine cadaveric heads, regardless of the firearm-ammunition combination tested or the familiarity of the firearm operator with equine anatomy. In 1 report,[16] an individual with limited experience in gunshot-related euthanasia of horses fired a .22-caliber rifle to euthanize 4 horses and 4 mules; most shots penetrated the nasal cavity, and > 1 shot was required to euthanize 5 of the 8 animals. Projectile trajectories were mildly variable in the present study and did not affect the extent of brainstem disruption. Among experienced individuals who use a firearm to euthanize horses, projectile trajectories can vary.[15,17] In a study[17] of 15 horses that were euthanized by means of a .32-caliber dispatch pistol with 5.51-mm ammunition fired by an experienced individual, damage to the hindbrain differed as a result of variation in projectile angle and, therefore, trajectory.

In the present study, projectile fragmentation extended through the vertebral canal to the level of the rostral aspect of the second vertebra, resulting in severe damage to the spinal cord, in association with the .223-caliber carbine–HPB combination; for 3 heads shot with HPBs fired from the .45-caliber ACP and in 1 head shot with the shotgun, projectile fragmentation and extension into the cranial portion of the vertebral column were also seen. The .223-caliber carbine, .45-caliber ACP, and shotgun had more muzzle energy, compared with the other firearms used, which could lead to more tissue disruption and allow projectile fragments to extend into the vertebral canal. To our knowledge, this extent of projectile fragmentation has not been previously described for gunshot-related euthanasia of large animals.

Another factor that has a role in the efficacy of euthanasia of large animals is positioning of the animal. In the present study, most cadaveric heads were positioned to simulate the level of the head of a relaxed or mildly sedated horse, but not the low head level often observed in moderately or heavily sedated horses. This positioning was specifically chosen because the use of chemical sedation currently reduces the number of options for carcass disposal. Sedation often alters the horse's head position, and the firearm operator must be aware of this to maintain the appropriate muzzle-to-target angle and continue to direct the line of fire toward the brainstem and vertebral column. In the present study, projectile fragmentation, skull fracture, and cerebral, cerebellar, and brainstem disruption scores associated with the semiautomatic 9-mm pistol–HPB combination were similar, regardless of whether the cadaveric heads were in a position that simulated that of the head of a standing horse or a horse in lateral recumbency. The immediate environment of standing horses should be evaluated prior to gunshot-related euthanasia. Consideration should be given to the safety of firearm operators and bystanders as the horse falls during the procedure. During the present study, the authors observed that it was challenging for firearm operators to achieve the correct aiming point and angle for a cadaveric head in lateral positioning on the ground and additional time was spent positioning and repositioning before the firearm was discharged. Additional concerns regarding real-life gunshot-related euthanasia of laterally recumbent horses were expressed; for example, if the animal was moving while on the ground, the safety of the firearm operator and persons in the surrounding environment would be endangered because the shot would have a higher chance of being less accurately placed, and the risks of ricochet and backspatter would likely increase. Further research would be needed to explore whether the findings for cadaveric heads positioned to simulate the head of horses in lateral recumbency in the present study would be similar for all firearm-ammunition combinations. In addition, the firearm operators in the present study all had firearm training, but some had limited to no experience with euthanasia of horses in lateral recumbency, which could have influenced their perceptions and the study results.

Given the diverse firearm types and calibers, levels of propellant used in the cartridges, and projectiles available, it would be impossible to evaluate every potential firearm-ammunition combination that could be used in the euthanasia of horses. Firearm-ammunition combinations tested in the present study were selected on the basis of their availability to law enforcement personnel and the public within the United States. The firearms were selected to evaluate the potential of a variety of firearms while more closely examining the effectiveness of HPBs for gunshot-related euthanasia of horses. The projectile KE required for gunshot-related euthanasia of large animals has been debated and is variable among species.[7] The AVMA Guidelines for Euthanasia of Animals 2020 edition[7] states that the firearm and ammunition selected must achieve a muzzle energy of at least 407 J for animals weighing up to 400 lb and at least 1,356 J for animals weighing > 400 lb. In the present study, the 12-gauge shotgun firing a 1-oz rifled slug and the .223-caliber carbine firing a 55-grain HPB were the only 2 firearm-ammunition combinations that achieved projectile KE > 1,356 J; however, all firearm-ammunition combinations used in the study caused considerable disruption of the cerebrum, cerebellum, and brainstem. As further discussed in the AVMA Guidelines for Euthanasia of Animals 2020 edition,[7] an accurate aiming point likely has an important role in the success of euthanasia performed with firearm-ammunition combinations that have projectile KE < 1,356 J. On the basis of the results of the present study, projectile KE as low as 173 J was sufficient to penetrate the frontal bone and cause disruption of the cerebrum, cerebellum, and brainstem when applied to equine cadaveric heads in a controlled environment. The shotgun-slug combination had the highest projectile KE and caused the most brain tissue damage. However, we consider this firearm-ammunition combination for euthanasia of horses less desirable than other methods for multiple reasons. With the shotgun-slug combination, large entry and exit wounds were created in the equine cadaveric heads, which would likely be considered unsightly by observers. With the creation of the exit wounds, the risk of slugs or slug fragments exiting the head would be high (as illustrated in 50% of the equine cadaveric heads used in the present study). The possibility of the shotgun slug exiting a horse's head generates additional concerns for the safety of the firearm operator and other individuals within the immediate area as well as potential damage to the surroundings. Projectile KE alone is not sufficient for determining whether a firearm-ammunition combination is appropriate for euthanasia because other combinations with similar KE may result in variable CNS tissue disruption depending on the type of ammunition used.[18,19] However, an understanding of muzzle energy remains critical when choosing an appropriate firearm-ammunition combination for purposes of gunshot-related euthanasia.

In nearly all studies of gunshot-related euthanasia of large animals, the firearms have been used with SCB ammunition. Hollow-point bullets have not been considered sufficiently effective for euthanasia of large animal species on the basis of the amount of brain tissue disruption.[7,8] Those types of bullets fragment on impact and lose KE, which could potentially result in less damage to brain tissue as indicated in a report[18] of euthanasia of 12- to 18-month-old beef steers by means of a .22-caliber rimfire rifle firing a 30-grain HPB. However, another study[15] revealed that a .22-caliber LR with a 40-grain HPB did result in reliable, instantaneous insensibility (prior to exsanguination) with 1 shot in each of 46 horses and ponies. The frontal bone of cattle is much thicker than the frontal bone of horses, which could explain the differences with regard to the effects of HPBs in the 2 species. The results of the present study were more consistent with the latter study[15] given that HPBs fired by a .22-caliber LR pistol consistently penetrated the frontal bone and caused brain tissue damage in the equine cadaveric heads. There was more projectile fragmentation and more extensive skull fracturing associated with the HPBs, compared with the SCBs, fired from the .22-caliber LR pistol. The findings were consistent with these bullets fragmenting on impact. All the firearm-ammunition combinations used in the present study, with the exception of one of the two .22-calliber LR pistol–SCB combinations, involved HBPs and none of the combinations failed to penetrate the frontal bone and cause brain tissue disruption in the cadaveric heads.

The findings of the previous studies and the study of the present report further confirm the importance of the proper choice of a firearm-ammunition combination that will penetrate the optimal anatomic location in the species being euthanized so that the procedure will result in instantaneous death. Although no 1 firearm-ammunition combination is correct for all potential applications, a firearm operator's familiarity with the firearm-ammunition combination and identification of the correct anatomic aiming point is critical for a successful euthanasia outcome with the least potential risks to the animal handler, firearm operator, and observers.

The main limitation of the present study was the fact that equine cadaveric heads and not live animals were used. As a consequence, the effect of hemorrhage, fluid pressure alteration, and the minimum extent of brain damage required to ensure humane death could not be evaluated. Other variables, such as the interval from gunshot administration to loss of reflexes and heartbeat, changes in stress-related hormone concentrations, and electroencephalographic response, were not measured. The minimum amount of brain tissue disruption necessary to ensure a humane death has not been clearly quantified. It has been reported that destruction with a free projectile of the medulla oblongata, which controls breathing, along with concurrent disruption of the cerebrum and cerebellum will result in insensibility and death in horses.[17] In a study[18] of gunshot-related euthanasia procedures performed on cadaveric cattle heads, it was determined that injury causing ≥ 30% disruption of brain tissue would result in instantaneous death. However, it has also been reported that most of the brain must be disrupted for instantaneous loss of consciousness and death.[7]

In addition, in the present study, heads in the simulated lateral recumbent position were only exposed to the semiautomatic 9-mm pistol–HPB combination, and the comparison of the use of HPBs versus SCBs was only evaluated for the .22-caliber LR pistol. The findings of such comparisons may have been different if data were available for all types of firearms used in this study.

The number of heads exposed to each firearm-ammunition combination was small, which may have influenced the integrity of the statistical analyses. However, we believed that the comparison of various firearm-ammunition combinations would provide useful information regarding euthanasia of horses, despite the small sample sizes.

Lastly, the level of firearm training and experience in shooting equine cadaveric heads was variable among the firearm operators involved in the present study, and the cadaveric heads varied in size. The study was designed to simulate a realistic scenario with midrange horse head sizes, readily available firearms and ammunition, variable levels of firearm training and anatomic knowledge among firearm operators, naturally achievable head positioning, and realistic shooting distance. With all these variables taken into account, results were considered consistent and should be reproducible beyond the limitations of this study.

In the study of the present report, all firearm-ammunition combination caused considerable disruption of the cerebrum, cerebellum, and brainstem. In our opinion, all combinations would, most likely, cause sufficient damage to result in successful euthanasia of a horse. The semiautomatic 9-mm pistol–HPB combination caused similar brain tissue disruption when used on cadaveric heads placed in a position that simulated the heads of standing horses and laterally recumbent horses and was our preferred combination. For 3 of the 5 measures of damage, scores associated with this combination did not differ significantly from scores for the shotgun-slug combination (the most destructive combination), and yet overpenetration of the skull was not observed. The 9-mm pistol is a handgun and is easier to maneuver, compared with a long-barreled firearm, such as a carbine or shotgun. A handgun can also be holstered before and after euthanasia, thereby allowing the firearm operator the opportunity to secure the firearm quickly and safely. Although there was no significant difference in the amount of cerebral, cerebellar, or brainstem disruption between use of the .22-caliber LR pistol and the 9-mm pistol or semiautomatic .45-caliber ACP, there was a pattern of more tissue damage with increasing muzzle energy. The semiautomatic .45-caliber ACP fired projectiles with more KE than did the 9-mm pistol, but we have had more experience with a 9-mm pistol for successful euthanasia of multiple horses, which likely influenced our preference.

The .22-caliber LR pistol produces less noise, which may be less startling to onlookers; has little chance of skull overpenetration; and is relatively inexpensive. Owing to the decreased penetration and decreased overall tissue damage as well as a reduced muzzle energy associated with the .22-caliber LR pistol, its use requires a greater degree of accuracy. Given the reduced latitude for error with a .22-caliber LR pistol in a less controlled situation, use of that firearm may not result in instantaneous death, which is of concern.

The combination of the .223-caliber carbine with 55-grain HPBs was highly effective for tissue disruption, but there is a greater chance of overpenetration of the projectile if aiming is errant. Owing to the size of the firearm, it is more challenging to handle and secure immediately prior to and after euthanasia.

The 12-gauge shotgun with a 1-oz slug was also highly effective for tissue disruption, but bullet fragments exited some of the cadaveric heads, which was a safety and aesthetic concern, making it a less optimal option. The long barrel and size of the shotgun pose problems similar to those associated with use of the .223-caliber carbine.

The results of the present study should be interpreted with caution because there is no best firearm, ammunition, and operator combination that would result in successful euthanasia in all situations. Selection of an appropriate firearm-ammunition combination with sufficient muzzle energy to produce the desired disruption of the cerebrum, cerebellum, and brainstem with the least risk of backspatter or projectile exit would be the most judicious. Further research is necessary to determine the optimal method of euthanasia for all animals, especially in situations involving depopulation of high numbers of large animals.

Acknowledgments

The project was supported by the Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR002373.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

The authors thank David Ergun for helping with CT scanning of the horse heads, John Palmer for assistance with the firearms and firearm safety, and Dr. Keith Poulsen for help with disposal of equine cadaver heads.

Footnotes

a. GE LightSpeed CT scanner, 8 slice, GE Co, Fairfield, Conn.
b. Equina system, Asto CT LLC, Madison, Wis.
c. Remington model 870 express, Remington Arms, Madison, NC.
d. Winchester Ranger, 2 3/4-inch length, 1-oz slug, Winchester Ammunition, Alton, Ill.
e. Ruger model mini-14 Sturm, Ruger & Co, Inc, Southport, Conn, and Yankee Hill Machine model AR-15a2, Yankee Hill Machine Co, Easthampton, Mass.
f. .223 Remington ammunition, Hornady Frontier, Huntsville, Ala.
g. 1911a1 Mil-Spec stainless, Springfield Armory, Geneseo, Ill.
h. Federal 45 Auto +P HST 230 gr JHP, Lake City Army Ammunition Plant, Independence, Mo.
i. Glock model 19, Glock Ges mbH, Deutsch-Wagram, Austria, and 22 Beretta model 92fs stainless, Beretta, Accokeek, Md.
j. Winchester Ranger 9-mm grain T-series, Winchester Ammunition, Alton, Ill.
k. Ruger model super single-six, Sturm, Ruger & Co Inc, Southport, Conn.
l. Federal AutoMatch, Lake City Army Ammunition Plant, Independence, Mo.
m. Winchester Super-X high velocity ammunition, Winchester Ammunition, Alton, Ill.
n. Intellispace, Koninklijke Philips, NV, Netherlands.
o. R Core Team, R: A language and environment for statistical computing, version 2013, R Foundation for Statistical Computing, Vienna, Austria. Available at: www.R-project.org/. Accessed Nov 24, 2019.

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Appendix

Scoring system that was used to determine the extent of projectile fragmentation, skull fracture, and structural disruption of the cerebrum, cerebellum, and brainstem in equine cadaveric heads shot with various firearm-ammunition combinations.

Score	Description
0	No change; no physical tissue disruption caused by the projectile and no fragmentation of the projectile or osseous structures
1	Minimal (approx < 10%) tissue disruption caused by the projectile and minimal fragmentation of the projectile or osseous structures
2	Mild (approx ≥ 10% to < 30%) tissue disruption caused by the projectile and mild fragmentation of the projectile or osseous structures
3	Moderate (approx ≥ 30% to < 50%) tissue disruption caused by the projectile and moderate fragmentation of the projectile or osseous structures
4	Severe (approx ≥ 50% to < 70%) tissue disruption caused by the projectile and severe fragmentation of the projectile or osseous structures
5	Nearly complete or complete (approx ≥ 70%) tissue disruption caused by the projectile and nearly complete or complete fragmentation of the projectile or osseous structures