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Differentiation of Hydrocephalic Calf and Human Calvariae

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ABSTRACT: Occasionally, partial calvariae of hydrocephalic calves are found in forensic contexts and mistakenly identified as human. Such specimens can be properly identified through immunological assessment of associated soft tissue, microscopic analysis of associated hair, and morphological comparison with documented museum specimens. Morphological comparison should focus on the form of the occiput, supraorbital grooves, and bulbous vault and presence of coronal processes.

KEYWORDS: physical anthropology, human identification, musculoskeletal system, calf calvaria

Frequently, forensic anthropologists are called upon to distinguish human skeletal remains from those of nonhuman animals. Usually, such determinations are easily made from gross morphology. Occasionally, the human/nonhuman status is difficult to assess because of fragmentation or modification of the normal bone morphology by exposure to intense heat, taphonomic changes, surgical intervention, or disease.

On 30 June 1988, author Ubelaker received two calvariae (Fig. 1) from Dr. Larry Balding, Office of the Chief Medical Examiner, Oklahoma City, Oklahoma. The partial cranium labeled 73-3 had been found in 1973 near a freshwater lake in eastern Oklahoma by two fishermen. The calvaria labeled 8801249 had been found in 1988 in southern central Oklahoma, apparently brought into a yard by a family dog. Examinations of both specimens by others had suggested origins ranging from a pathological child to a variety of animals, including an owl and a dolphin. Specimen 73-3 apparently had been chewed by carnivores and lacked any bones of the face or cranial base. The bones of the vault were very thin and displayed a large fontanelle-type opening in the center but no apparent sutures. Some desiccated soft tissue adhered to most surfaces. Indentations for the superior margins of the orbits were present but were approximately 10 cm apart. The bones of the vault in Specimen 8801249 were very similar to those in Specimen 73-3, with the orbital margins separated by 10 cm. Although the vault was very high and bulbous in a humanlike pattern, the occipital was present and showed a distinctly nonhuman pattern.

In 1987, author Berryman had examined a similar cranium from Mississippi that likewise

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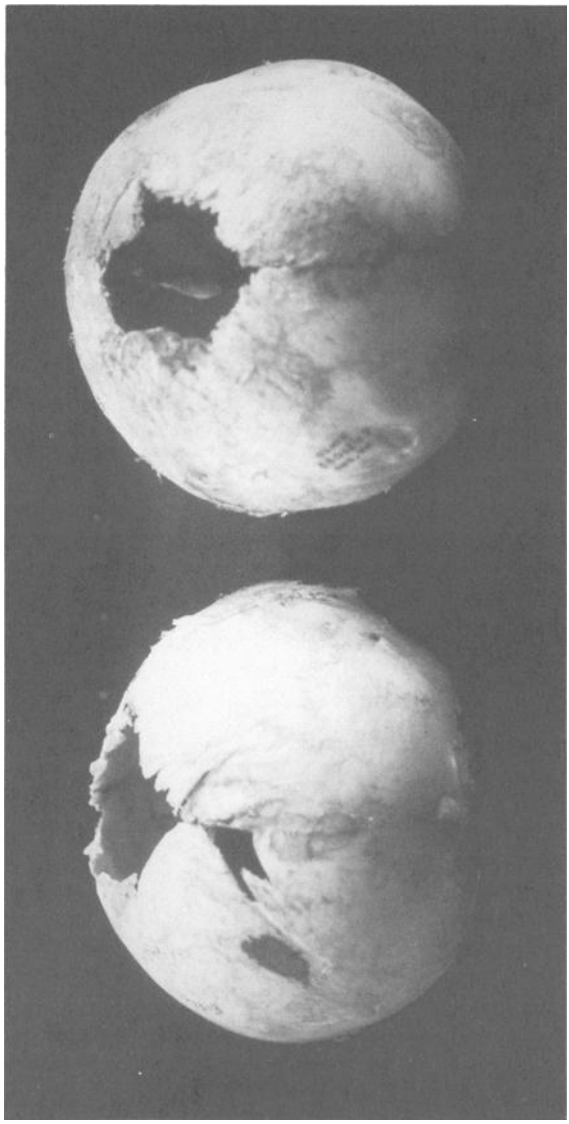

FIG. 1—*Two calvariae from Oklahoma, 73-3 and 8801249.*

had been submitted through the medical examiner system as a possibly human forensic case (Fig. 2). This specimen was substantially smaller than either of the two from Oklahoma but also displayed a bulbous vault with wide separation of the orbits (approximately 7 cm). The frontal, parietal, and occipital bones were present but exhibited damage from carnivore activity.

Comparisons of these three specimens with vertebrate collections in the National Museum of Natural History at the Smithsonian Institution initially failed to find an exact match, although the greatest similarity seemed to be with calf crania. A bovine origin was suggested, especially by the form of the occipital condyles, the presence of what appeared to be coronal processes (horn buds) on the specimen from Mississippi, and the very characteristic supraorbital grooves [*1*] (Fig. 3). However, none of the known calf specimens in our collections displayed the bulbous vault or wide separation of the orbits shown in the forensic specimens.

Immunological Assessment of Soft Tissue

To test the hypothesis that the specimens had, in fact, originated from calves, small samples of desiccated soft tissue were removed from the two Oklahoma calvariae and submitted for immunological evaluation to author Sutton at the University of Tennessee Toxicology and Chemical Pathology Laboratory, Memphis, Tennessee.

Although species origin determination is a commonly requested assay in the forensic science laboratory, the testing method of choice may be altered depending upon the individual sample characteristics. The first step in this instance was to devise a test procedure to accommodate these rather unusual samples. Sample A was extremely dry and brittle (total weight, 0.095 g), while Sample B (total weight, 0.496 g) was slightly pliable. Due to the obvious extent of desiccation, an aggressive extraction process, alternating the use of a stomacher and passive extraction into phosphate-buffered saline (PBS) at pH 7.2 over a period of five days, was employed. The resultant extracts were only nominally clarified prior to use.

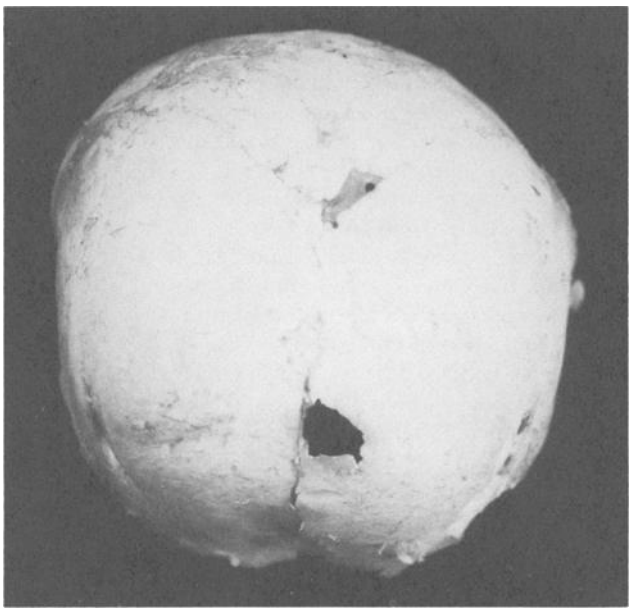

FIG. 2—*Calvaria from Mississippi.*

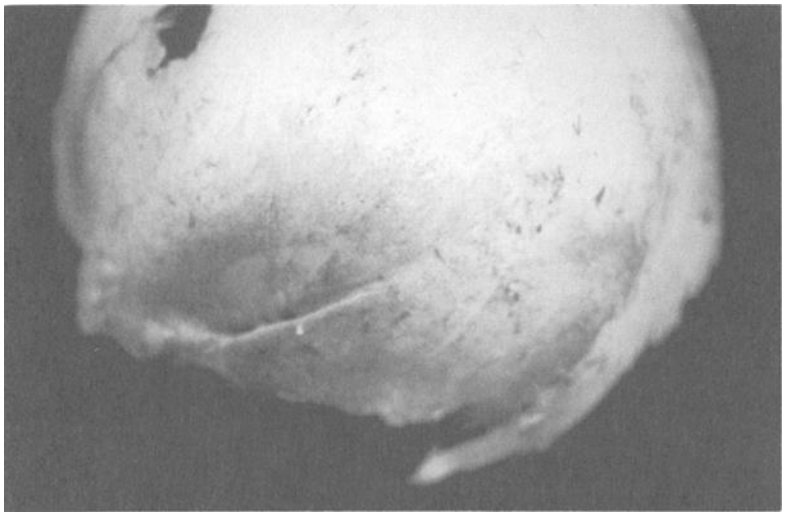

FIG. 3—*Detail of coronal processes and supraorbital grooves on the calvaria from Mississippi.*

The condition of the extracts, coupled with the fact that the potential antigen concentration present was entirely unknown, led to the choice of a double diffusion test method. Type I agar (1%) in PBS at pH 7.2, with the addition of polyethylene glycol 8000 (3.5%) to enhance precipitin lines [*2,3*], was prepared and stored at 0 to 4°C. Trial and error indicated that the optimum conditions were a 1-mm agar depth, sample wells of 2-mm diameter, and well distances of 5 mm. The pattern employed was circular with a single centrally located well. The wells were charged with 3 μL of extract or antibody.

Precipitating antibodies (produced against whole serum) were selected with the morphological characteristics of the calvaria in mind. The species chosen were bovine, deer, horse, sheep, swine, and human. The antibodies were reconstituted according to manufacturer's directions, aliquoted, and stored at −20°C until needed to avoid deterioration over the testing period. Since the test methodology chosen to accommodate the sample restrictions of this particular case is relatively primitive in comparison with methods normally employed, duplication of the manufacturer's sensitivity and specificity testing for each antibody was necessary. The sheep, swine, horse, deer, and human antisera were found to be specific to their corresponding whole sera. The antibovine antibody, however, was found to cross-react with deer and sheep serum (maximum dilutions of 1:1024 and 1:2048, respectively). This cross-reactivity would, of course, have to be eliminated as a possible source of any positive reaction with the bovine antibody.

Sample A was not reactive with any of the antibodies tested. Although this may indicate that the tissue did not originate from any of the animal species tested, it more probably reflects the extreme desiccation of the sample.

Sample B, which had initially appeared the more pliable of the two, produced a precipitin line in the presence of antibovine serum. An identity pattern was developed when Sample B was placed in a well adjacent to whole bovine serum, indicating that the antibody recognized a protein common to Sample B and the known bovine serum. No precipitin was produced in the control situation (Sample B in PBS at pH 7.2), thereby eliminating nonspecific precipitation or artifact. Sample B also did not react with antisheep or antideer antisera, eliminating the possibility that the precipitin line was due to cross-reactivity.

The species identification of skeletal remains is, traditionally, limited to comparisons of gross morphology. In these particular cases, morphological characteristics alone were

inconclusive. The immunological identification of dried tissue from one of these skulls as being bovine in origin indicates a potential for including such techniques in similar cases. The failure to obtain conclusive results with the more desiccated specimen is probably attributable to the sample condition alone. Had it been economically feasible, testing with additional antisera might have been conclusive in establishing species identification.

Microscopic Hair Analysis

Detailed examination of the specimen from Mississippi revealed hair adhering to the bone. Microscopic examination of the hair identified the nonhuman characteristics of centrally distributed pigment granules, large medulla diameters, and abundant ovoid bodies. Ovoid bodies are solid oval structures larger than pigment granules which occasionally occur in human hair but are frequent and diagnostic characteristics of bovine hair [*4*].

Morphological Comparison of Human and Hydrocephalic Calf Crania

A calf cranium with very widespread orbits and a humanlike, vertically bulbous vault most probably originates from a calf with congenital hydrocephaly. Hydrocephalus is well known in both human and nonhuman vertebrates. Basically, the condition is defined as the distention of the ventricles of the brain, with an increase in volume of the cerebrospinal fluid [*5*]. Cerebrospinal fluid is produced by the choroid plexus and then travels through the ventricles, foramina of Monro, aqueduct of Sylvius, fourth ventricle, and cerebellar foramina, finally to reach the subarachnoid space. At that point, cerebrospinal fluid is resorbed, mostly by the arachnoid villi, and transferred to the venous system.

Hydrocephalus results from either (1) overproduction of cerebrospinal fluid or, more commonly, (2) some sort of blockage of the pathway to the venous system. If cerebrospinal fluid passes normally into the subarachnoid space but is prevented from being absorbed by the arachnoid villi, the hydrocephalus is classified as the communicating type [*6*]. When the blockage is in the pathway within the brain, the condition is classified as noncommunicating or obstructive.

In immature humans, hydrocephalus may produce a dramatic enlargement of the head if it occurs before fusion of the cranial sutures. The frequency of this condition in humans varies in different populations from 0.2 to 5 per 1000 births [*6*]. In nonhuman animals, the mechanism is similar. The condition occurs most commonly in calves and foals but is known in all domestic and laboratory animals. Hydrocephalus is known to be both congenital and acquired in origin, although the congenital condition shows the most pronounced effects and is more likely to affect bone. Congenital hydrocephaly appears to represent an autosomal recessive trait [*7,8*], although there is some evidence that it could also be linked to nutritional deficiencies of Vitamin A, folic acid, Vitamin B 12, niacin, and zinc [*9*]. Most congenital conditions involve blockage of the aqueduct of Sylvius and can result in extensive cranial malformation if the sutures are open [*10*].

One veterinary pathology text [*11*] notes:

> Dilatation of the ventricle often produces enormous expansion and enlargement of the skull (macrocephaly), a condition never observed in association with acquired hydrocephalus. The circumference of the calf's head may exceed one meter. The skull is spherical and distended like a balloon, or more pointed and steeple-like, or flat and expanded laterally. By contrast, the facial part of the head appears small and insunken. . . . The bones, and particularly the temporal, parietal, and frontal bones, are generally expanded superficially and exhibit larger defects which are closed by membrane. The wide gaps which still remain between them, however, are bridged by connective tissue membranes. New bones (wormian bones) often develop in these membranes. The sutures are often closed and present rough ridges on the

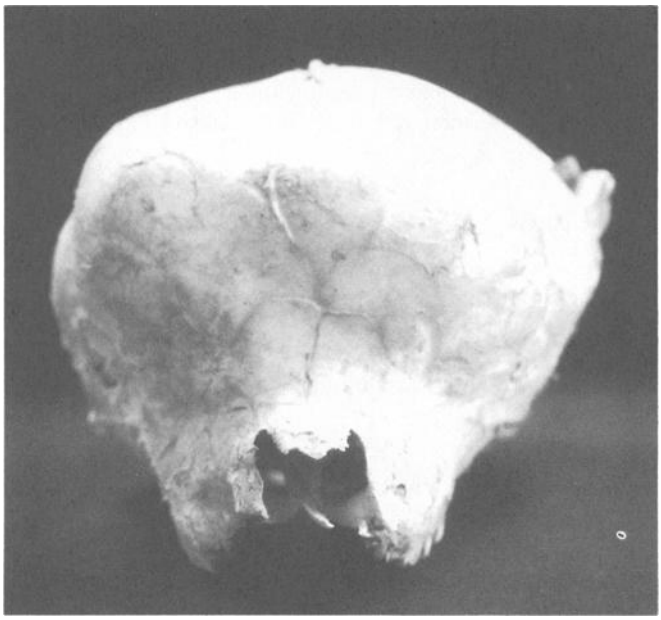

FIG. 4—*Hydrocephalic calf from Missouri, collections of Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution.*

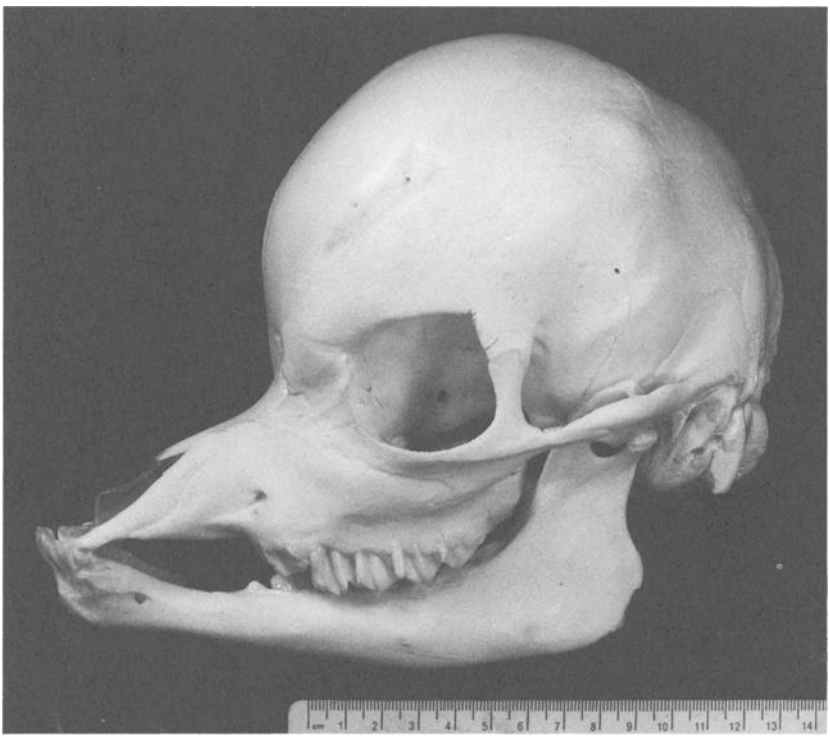

FIG. 5—*Hydrocephalic calf cranium from Germany, lateral view (Department of Anthropology, National Museum of Natural History, Smithsonian Institution).*

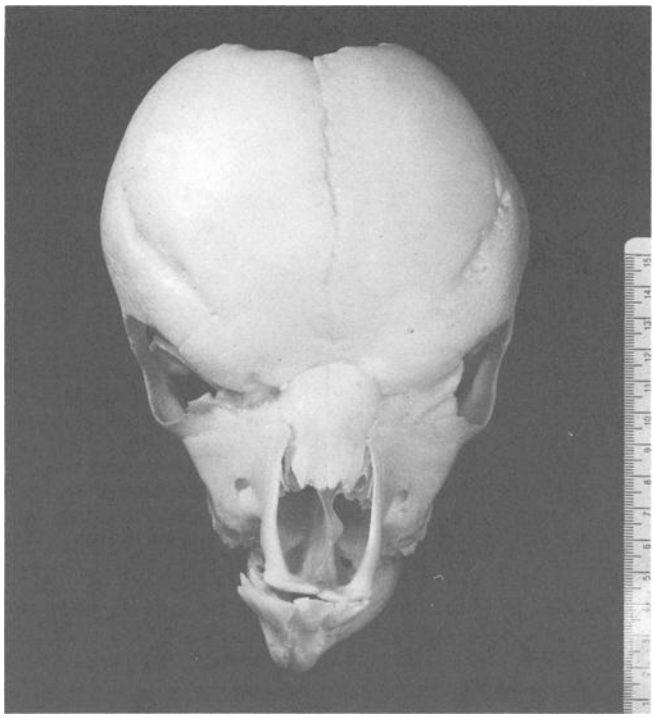

FIG. 6—*Hydrocephalic calf cranium from Germany, frontal view (Department of Anthropology, National Museum of Natural History, Smithsonian Institution).*

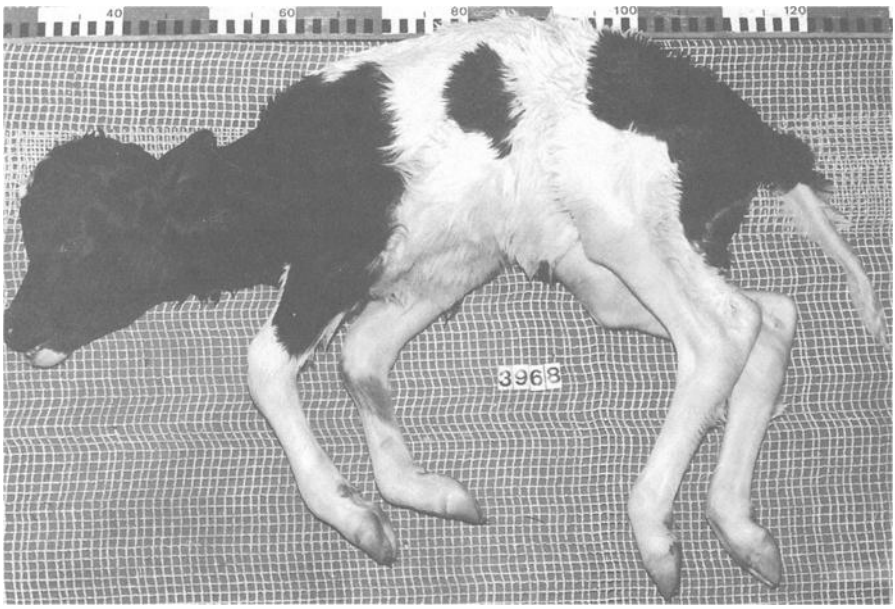

FIG. 7—*Hydrocephalic calf from Germany prior to maceration.*

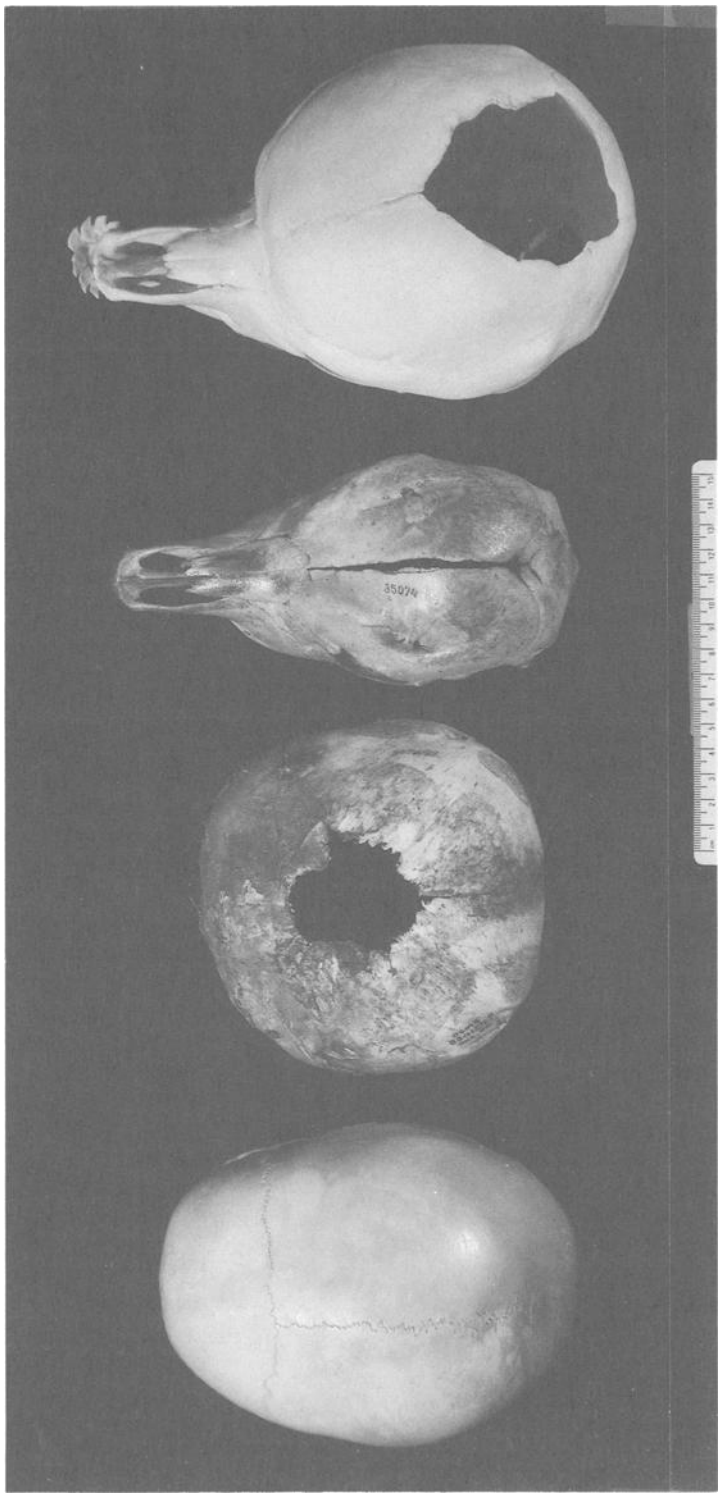

FIG. 8—*Comparison of crania from a human child, a forensic specimen from Oklahoma, an immature normal calf, and an immature hydrocephalic calf (superior view).*

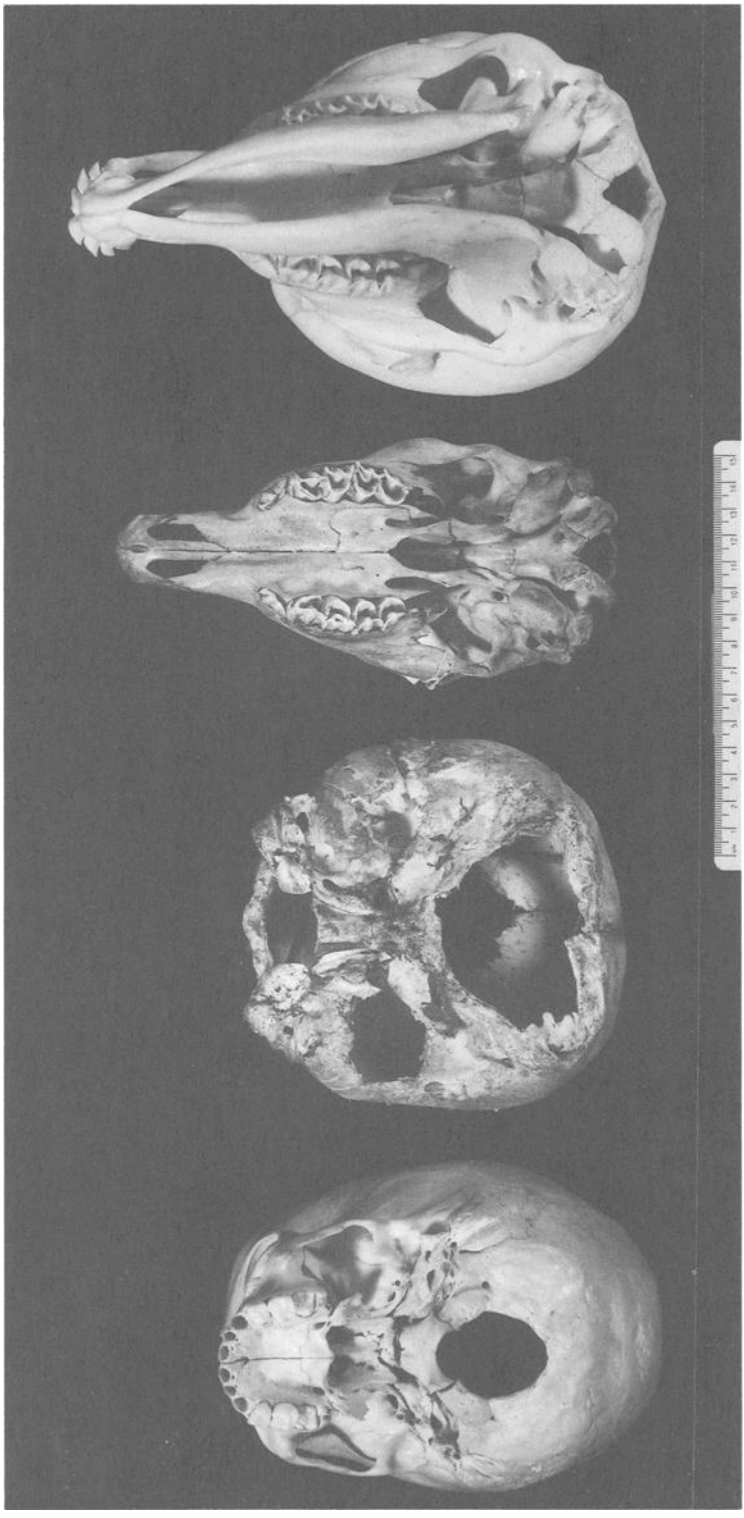

FIG. 9—*Comparison of crania from a human child, a forensic specimen from Oklahoma, an immature normal calf, and an immature hydrocephalic calf (basilar view).*

inner side. The extreme stretching causes pressure atrophy of the base of the skull, broadening and flattening of the fossae, atrophy of the zygomatic arches and narrowing of the orbits so that the eyes bulge forward. The eyes and ears are also seen to be wider apart.

The literature presents contradictory accounts of the viability of calves with congenital hydrocephalus. Some reports indicate that affected calves usually are born dead. However, Blood and Henderson [*12*] claim that congenitally affected animals are usually alive at birth but are unable to stand, and most die within 48 h. In 1963, Gibbons noted that such calves are "unable to rise or coordinate movements, but many are able to stand, walk, and even nurse. Body movements usually are awkward. Falling occurs frequently, and in a few animals, cleft palate is present" [*13*].

An early text by Law [*14*] suggests that extreme congenital hydrocephaly in calves usually prevents a natural birth and requires intervention to save the cow. He noted:

The head back of the eyes rises into a great rounded ball which proves to be an insuperable obstacle to parturition. The forefeet and nose being the parts presented, no progress can be made, and even if the feet are pulled on, the nose cannot by any means be made to appear. The oiled hand introduced into the passages will feel the nose presenting between the forelegs, and on passing the hand back over the face the hard, rounded mass of the cranium is met with. A sharp-pointed knife or a cannula and trocar should be introduced into the palm of the hand and pushed into the center of the rounded mass so as to evacuate the water. The hand is now used to press together the hitherto distended but thin and fragile walls, and the calf may be delivered in the natural way. If the enlarged head is turned backward, it must still be reached and punctured, after which it must be brought up into position and the calf delivered.

The literature clearly indicates that many, if not most, cases of hydrocephalus in animals do not produce noticeable alterations in cranial morphology. When such alterations occur, they are nearly all caused by congenital hydrocephalus, involving some blockage of the normal pathway for the flow of cerebrospinal fluid [*15*]. Affected crania can assume a variety of shapes but usually involve a bulbous cranial vault with exaggerated separation of the eyes.

Macerated examples of hydrocephalic calves for comparison appear to be very rare. After a considerable search, one specimen was located in the collections of the Smithsonian Institution (Fig. 4). Although its history is not well documented, it carries the label "hydrocephalic calf, *Bos taurus* from Washburn, Missouri." It displays the typical bulbous vault but appears to lack orbits entirely.

A prepared cranium and mandible from a clinically documented hydrocephalic calf were identified in the collections of Prof. G. W. Rieck of the Institut für Tierzucht und Haustiergenetik in Giessen, Germany (Figs. 5 and 6). The specimen is quite similar to the specimens of this study and originates from a Holstein-Frisian male calf that died 12 Dec. 1974 (Fig. 7). The calf was diagnosed as having congenital hydrocephaly, as well as hypoplasia of the thyroid. The Institut has a series of such crania; thus, Dr. Rieck kindly donated the specimen [now National Museum of Natural History (NMNH) Catalog No. 387001] to the Smithsonian for comparative purposes. Note the bulbous vault, the relatively diminutive face, and the characteristic supraorbital grooves on the prepared specimen.

Figure 8 shows the superior views of, from left to right, a normal human cranium (four-year-old child from California, Catalog No. 385737), the forensic specimen from Oklahoma (OCME 8801249), a normal immature calf (Catalog No. 35074), and the documented hydrocephalic calf from West Germany (Catalog No. 387001).

Figure 9 shows the basilar views of the same crania, arranged in the same order as in Fig. 8. Here, the human cranium is distinctly different, not only in the morphology of the foramen magnum and the occipital condyles, but also in the positioning of the foramen magnum relative to the cranial vault.

Conclusions

In all likelihood, immature bovine crania with congenital hydrocephaly will continue to appear as forensic cases, mistaken for crania of pathological human infants. Pathologists, vertebrate zoologists, and physical anthropologists have, in the past, experienced difficulty in properly identifying these specimens. Identification should concentrate on the morphology of the occipital area, wide separation of the orbits, the likely presence of the characteristic wide, irregular supraorbital groove on the frontal bone, and evidence of the development of coronal processes (horns). Note that many modern calves are polled (from stock genetically bred to lack horns). Even these crania will show the supraorbital grooves and vestiges of the coronal processes that should differentiate them from human crania. Immunological examination of associated soft tissue, microscopic analysis of associated hair, and morphological comparison with clinically documented specimens should make correct identification possible, even when the forensic specimens are incomplete or damaged.

Acknowledgments

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