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Successful Separation of *Craniopagus parasiticus* in an Egyptian Buffalo Calf

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Abstract: This report presents a case of *Craniopagus parasiticus* in an Egyptian female buffalo calf born in a village in the Nile Delta in September 2005 that has been successfully separated by the current team of neurosurgeons. The aim of this work was to preserve the developed twin by sacrificing the parasite. Magnetic resonance image (MRI) was done to determine the anatomical features and vascular supply of the parasitic head. Blood sample was collected from the buffalo calf for chromosomal analysis. Surgical separation was done when the twins were three months old. The cytogenetic analysis of the buffalo calf revealed normal female with 100 % XX karyotypes in cultures of blood lymphocytes. The diploid chromosome number was found to be 25 pairs. The percentage of structural chromosomal aberration reached 18 %; including deletions (14%) and fragments (4%). No postoperative neurological deficit was observed after the operation. Three years after surgery, the calf is still alive, in reasonably good health and produces a normal birth. In conclusion, this is a successful separation of *Craniopagus parasiticus* in Egyptian buffalo calf.

Key words: Craniopagus · Conjoint twins · Craniopagus parasiticus · Joint head

INTRODUCTION

Conjoined twins are one of the rarest and most challenging congenital malformations with an estimated incidence of about 1 in 50,000 pregnancies, but around 60% of them are stillborns, giving an overall true incidence of about 1 in 200,000 live births with a male-female ratio of 1:3 [1]. They are classified according to the most prominent site of attachment into craniopagus, thoracopagus, ischiopagus, omphalopagus and pygopagus [1].

The word "craniopagus" refers to twins joined only at the head. The term was derived from Greek (*kranio* meaning "head" and *pagus* meaning "together"). The plural is craniopagi [2]. In humans, craniopagus represents 2 to 6% of conjoined twins and is the rarest type of this disorder [3]. Conjoined twins are always genetically identical and share the same sex. Females are more commonly affected with a male to female ratio of 1:4[4].

Craniopagus parasiticus is an anatomic malformation in which conjoined twins are united at the crowns of their crania, with one twin being underdeveloped and forming a parasite [4]. *Craniopagus parasiticus* in humans is an extremely rare anomaly. Bondeson and Allen [4] reviewed six cases of *Craniopagus parasiticus*. The first case was reported in 1790 in Bengal, the second case was reported in 1828 in France. The third and fourth were reported in Germany in 1866 and the fifth case was reported also in France in 1939. The sixth case was reported in Brazil in 1940.

Aquino *et al.* [5] published a report on the seventh case in North America. The eighth was operated upon in the Dominican Republic. The ninth case was reported in Egypt by Lotfy *et al.* [6]. Seven of the ninth reported patients were males. Attempts at surgical separation have not been commonly performed because the majority of these twins have traditionally been stillborn. Recently a case of craniopagus conjoined twins was recorded in Africa by Ble *et al.* [7], but the surgical treatment was not considered owing to the absence of appropriate technological conditions and means.

In animals, conjoint twins were reported in cattle [8, 9], buffalo [10] and pigs [11] but craniopagus is

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extremely rare phenomenon in animals. Inspite of no statistics are available in animals; to our best of knowledge, this is the first case for surgical separation in buffalo. The literature on the surgical separation of craniopagus parasiticus is totally non-existing in animals. This is owing to the fact that this anomaly is extremely rare and because surgical separation was not attempted in animals before.

Congenital abnormalities arise from adverse factors affecting the fetus in the early stage of development. From in-vitro fertilization work in human beings, a break in the zona, an old egg and delay in fertilization or ovulatory drugs that lead to the hardened zona pellucida could all produce monozygotic twinning by physically separating the conception into two cell masses [12]. Familial monozygotic twinning could be associated with an inherited abnormality of the zona pellucida or some other mechanism, leading to failure of early blastocytes to stay together [13]. Moreover, abnormalities may result from genetic or environmental causes. Environmental or non-genetic causes have the same economic results as genetic causes, but are far easier to rectify. Several environmental factors have been implicated in chromosomal abnormalities, those include age [14, 15], season [16], nutrition [17] and environmental pollution [18].

The aim of this work was to interpret surgical separation of *Craniopagous parasites* in buffaloes with emphasis on examination of the chromosomal picture.

MATERIALS AND METHODS

The Case Report: A case of *Craniopagus parasiticus* Egyptian buffalo calf born in a village related to Hussaineya Centre in Al-Sharkiya District in the Nile Delta in September 2005 was investigated. The case was clinically examined and no abnormality was detected except an attached parasitic head to the normal head at the occiput. The case was weighing 80 kg.

Pre-Operative Investigations

Blood sample was taken for complete blood picture. Ultrasonography was done using apparatus model 100 SL, Biomedical, Netherlands and proved normal viscera, chest & heart and genitalia.

Magnetic resonance imaging was done (MRI, G.E. Open 0.2 Tesla, Signa Profile, USA) to show the anatomical features and vascular supply of the parasitic head.

Chromosomal Analysis: Blood samples were collected using sterile syringes. Blood cells were cultured for 72 hrs at 38° C in 5 ml TCM-199, 1 ml fetal calf serum and 0.1 ml phytohaemagglutinin (PHA). After incubation, cells were treated with colchicine (0.05%) for 2 hrs, then with a hypotonic (0.075 M KCL) for 30 min. After fixation in acetic acid: ethanol (1: 3) solution, the cells suspension were dropped on wet slides then flamed to dry. The slides were stained with Giemsa stain and covered with DPX mounting media for chromosomal analysis. Chromosomal abnormalities were recorded in at least 100 metaphase spreads.

Aneasthesia for the MRI: Food and water were withheld for 24 hrs before the examination. An IM injection of 0.02 mg/kg atropine sulphate, was given15 min. before the procedure. 0.15 mg/kg IM xylazine was given followed by 15 mg/kg IM ketamine HCl 10-15 min. later. This provides anesthesia for 45 min.

Anesthesia for Surgery: A dose of 0.02 mg/kg atropine sulphate was given s/c, 15 min before anesthesia. IM injection of 20µg/kg medetomidine was then given followed by IM injection of 15 mg/kg ketamine Hcl 15 min later. IM 0.15 mg/kg xylazine Hcl provided anesthesia for 120-150 min.

Surgical Procedure: The separation was done on 15/11/05 and lasted for approximately 3 hrs. The head was first shaved and the feeding vessels and terminal end of the parasitic head were identified.

The animal was first put on its right side and then the position changed during surgery to be on the left side. The surgical incision was planned by making skin flap from the parasitic head this facilitated coverage of the surgical field at the end of the procedure.

After the skin flaps were done, the dissection was deepened between the parasitic head and the occipital region of the main head till the blood supply of the parasitic head was controlled. Then, skin flaps over the occiput were made to allow separation of the skull from the occipital region. Bone flaps were made and the dura was opened. A cyst was encountered separating the two brains. The saggital sinus was controlled and the dissection carried out till behind the left horn. The position of the calf was then changed to its left side and the same procedure was repeated. Bleeding was controlled using bone wax and the saggital sinus was ligated. The two incisions were joined together from

below and a gigli saw was used to completely separate the two heads. Hemostasis was done and the skin was closed.

During surgery, with the commencement of blood transfusion, the buffalo developed severe transfusion reaction in the form of pallor and shock. The blood transfusion was immediately stopped and a volume expander (Histril) as well as intravenous injection of 8 mg corticosteroids were used in order to reverse the shock state. Blood transfusion was attempted

Postoperative Care: The surgery was followed by a smooth post-operative course of antibiotics treatment. A course of IM antibiotics (neobiotic 4g/day) was given for 7 days. Wound infection was encountered which necessitated repeated dressing till the wound healed.

RESULTS

Clinical Investigation: The clinical investigation revealed that, the parasitic head showed deficient part of the lower jaw. Secretions were coming out of the nostrils and mouth as there was no esophagus. A stump was present in the lower part of the parasitic head representing its body. Sensations and corneal reflexes were present in the parasitic head. Accordingly, the parasite received all its blood supply through the area at which it was connected with the normal twin. The arterial blood supply to the parasite was found to arise from the external carotid circulation.

The size of the parasitic head was almost equal to that of the normal head. The conjoined calvaria had a circumferential base of 25 cm and was connected at the occipital region of the twins. The inter-axis angle between the two heads was 20 degrees with a small rotational angle. The two eyes of the parasite were normal, apart from conjunctival infection of the left eye. The nostrils were normal. The mouth of the parasite was normal down to the pharynx and salivation was evident (Fig1).

Fig. 1: Crainiopagus in buffalo calf

MRI Findings: In MRI findings, there were changes related to complete cranial duplication. The main calvarium measures 14x9x9 cm. where was the accessory calvarium measures were 9.2x10.5x8.5cm. The main calvarium was defected at its occipital region, where it gave rise to the accessory calvarium which appears directed posteriorly and to the right 20 degree from the middle line. The main calvarium was including the well-formed brain with its supra and infra-tentorial structures swith the brain stem appearing continuous with the spinal cord through the foramen magnum. The accessory calvarium was presenting arachnoid cyst replacing the occipital lobes and the posterior fossa structures. There was no identifiable infra-tentorial compartment or communication with the main cervical canal of the animal. The accessory calvarium and its intra-cranial structure were fed from the external carotid system on either side of the neck, where the internal carotid arteries were feeding the main calvarium and its structures (Fig 2).

Surgical Intervention: In the present case, the time limit and blood loss were the two most crucial challenging factors the surgical team faced. The entire surgery had to be completed in a maximum of three hours that the sedation provided. The unknown nature of blood groups

Fig. 2: MRI Findings showing incomplete cranial duplication with accessory head filled with two cerebral hemispheres and an occipital arachnoid cyst

Fig. 3: The calf after surgery

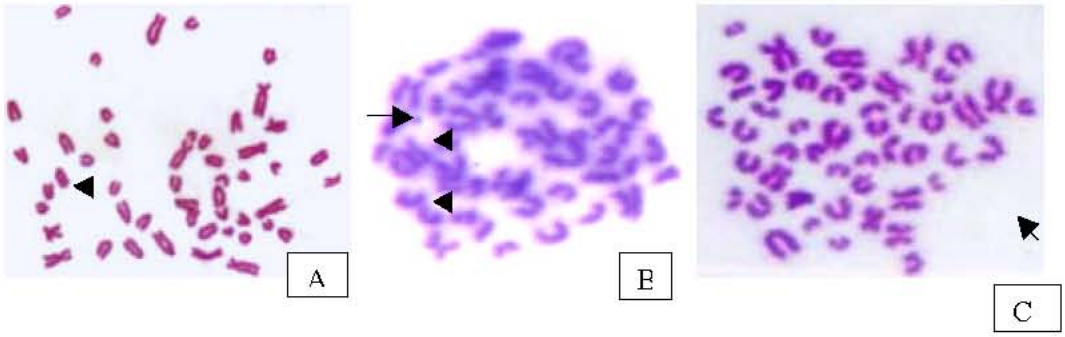

Fig. 4: Metaphases spread from blood cultured cells of conjoint buffalo twins showing normal metaphase (A), fragment (B) and chromatid deletion (C)

in buffaloes meant that the transfusion was a rather risky procedure in spite of the precautions taken. The anatomy of the calf's skull and differences between it and the human skull were demonstrated to our team by a veterinarian preoperatively. As bleeding from the dural sinuses is the most dangerous cause for surgical failure, the arterial supply to the parasite was ligated first and then the dural sinuses were dealt with. Adequate skin and bone flaps taken from the parasitic twin helped in closing the surgical defect in one-stage. The calf recovered immediately after surgery (Fig 3).

Chromosomal Analysis: Conjoint buffalo twins were judged to be a normal female with 100 % XX chromosome in cultures of blood lymphocytes (Fig.4A). The diploid chromosome number was found to be 25 pairs. Five pairs were submetacentric and the remaining 20 pairs were acrocentric including X chromosome, who was the largest acrocentric. The percentage of structural chromosomal aberration reached 18 %; they include 14% deletions (Fig.4C) and 4% fragments (Fig.4B).

DISCUSSION

Craniopagus parasiticus is an extremely rare condition in animals. The first attempt to separate such twins in humans was performed in the Dominican Republic in 2004. The infant died 7 hours after surgery. The second was done successfully in Egypt in Feb 2004 by our team. In this case report we discuss a successful surgical separation of parasitic head attached to the occiput of a buffalo calf. This anomaly has been seen several times in cats and rabbits but, in buffaloes this congenital abnormality is generally rare.

The procedure was the first to be attempted. Preparing for the surgery included many steps that were done for the first time. This included the MRI that was not reported to have been used for a buffalo before.

The neurosurgical team met several times to discuss possible surgical procedures. The team reviewed the literature on the surgical separation of craniopagus in general. The most important cause of failure in published cases was death of the patient during surgery. This occurred most commonly because of bleeding from the

sinuses and major veins at the end of the operation. By the time, the members of the previous neurosurgical teams were exhausted and improper decisions may have been made. The parasitic twin is naturally a good donor of skin and bone to the autosite; accordingly, the separation could potentially be accomplished with a one-stage surgery.

The results of chromosomal analysis agree with those reported on normal Egyptian water buffalo-cows [19, 20] and the karyotype reported for the Indian River buffaloes [21]. The common abnormalities in this study are chromatid deletions. Similar structural abnormalities were found to be associated with reduced fertility [22, 23]. These structural abnormalities could not be traced to any specific cause, since such abnormalities can be induced by certain viruses, drugs and pesticides. However, these aberrations are indicative of the generalized susceptibility of the animals for chromosomal derangement; it represents either a deficiency or alteration of portion of DNA and could lead to animal anomalies. In this respect, Farin *et al.* [24] indicated that errors in development in the fetuses were the potential genetic mechanisms that may contribute to abnormal phenotypes of the embryos. Moreover, It is well known that chromosome abnormalities affect developmental potential of early embryos and may be potential predictors of developmental outcome [25]. In addition, chromosomal abnormalities were recorded as a cause of lower fertilization *in vitro* [26] and early embryonic death [27]. The definitive aetiological causes of conjoined twins are unknown [9]. However, many researchers have attributed this phenomenon to either genetic or environmental factors, or both [8]. In this work the increased incidence of structural chromosomal aberrations may explain the environmental factor in this abnormality.

In conclusion, this is successful separation of *craniopagus parasiticus* in Egyptian buffalo calf. No postoperative neurological deficit was observed after the operation. Three years after surgery, the calf was still alive with good health and produce normal birth.

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