

Cephalothoracopagus (monocephalic dithoracic) conjoined twins in a pig (*Sus scrofa f. domestica*): a case report

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ABSTRACT: In this report, we present a rare case of cephalothoracopagus (monocephalic dithoracic) conjoined twins with anencephaly in pig. Conjoined Polish large white piglets were born at term after an uncomplicated birth. The litter consisted of 11 piglets. The conjoined twins were born alive, but died shortly after birth and were subjected to further investigation. Blood vessels of the heart were filled with LBS 3060 latex, and then the twins were fixed in 10% non-buffered formalin. Necropsy revealed the external and internal anatomy of the affected twins. Examinations demonstrated abnormalities of skeletal, digestive, cardiovascular, respiratory and nervous systems. The twins had a single head, neck and chest and were separated from the umbilicus caudally. They had four forelimbs and four hindlimbs. Examination of the skeleton revealed two complete vertebral columns connected with one skull. Two tongues and a cleft palate were present in the oral cavity. The conjoined twins had a single pharynx, oesophagus, stomach, duodenum, part of jejunum, spleen, liver and pancreas. The remaining part of the digestive system was doubled. Each piglet had a separate urogenital apparatus. The examination revealed only one heart with structural abnormalities. Two larynxes and tracheas were identified. The right twin had the right lung while the left twin had the left lung. To the authors' knowledge, this is the first detailed report of this type of conjoined twins in the pig.

Keywords: Polish large white; anencephaly; cleft palate; congenital malformation; skull hypoplasia; parturition

Conjoined twins or Siamese twins represent rare congenital malformations of monozygotic twins. This defect has been observed in humans (Singh et al. 2003; Sharma et al. 2013) and in different species of animals, e.g., cow (Vanderzon et al. 1998), black rat, European hedgehog (Kompanje 2005), sheep (Samuel et al. 2014), mouse (Hynd et al. 2013), ostrich (Mazzullo et al. 2007), leopard (Kompanje and Hermans 2008) and dog (Nottidge et al. 2007). Numerous classifications of conjoined twins are available, based on anatomy, site of union, symme-

try level of twins and embryological development (Chen 2012).

Abnormalities in the anatomy of conjoined twins arise during prenatal development. The implications of these abnormalities can lead to organ dysfunction, organ failure or even death. Genetic and environmental factors are thought to be main causes of the development of conjoined twins. At present, two hypotheses (fusion and fission) are used to explain the mechanisms behind the formation of conjoined twins (Spencer 2000a; Spencer 2000b).

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The aim of this study is to describe a case of cephalothoracopagus (monocephalic dithoracic) conjoined male twins in the pig. According to Herring and Rowlatt (1981), cases of cephalothoracopagus are fused at the head and chest. The conjoined head can have two faces opposite each other (Janiceps) or side by side (Herring and Rowlatt 1981). Cephalothoracopagus cases were previously documented in the literature in man (Sharma et al. 2013), domestic cat (Mazzullo et al. 2009), mouse (Hemm et al. 1977; Hynd et al. 2013), sheep (Cazabon and Adogawa 2003; Monfared et al. 2013), goat (Shojaei et al. 2015), hamster (Willhite et al. 1985) and dog (Nottidge et al. 2007).

Various types of conjoined twins in the pig were reported by Partlow et al. (1981), McManus et al. (1994) and Mostafa et al. (2005). However, cephalopagus in this species was not thoroughly investigated and described.

Case description

A three-year-old Polish large white sow gave birth to a litter of 11 piglets. The dam had two previous parturitions. The birth happened at term, with no complications. Nine of the 11 piglets were healthy and normal, while two were diagnosed as conjoined cephalothoracopagus. There was no history of previous congenital malformations in piglets originating from the same parents. The male twins were born alive but died soon after birth.

Specimens were prepared to investigate specific features, such as vasculature of the heart. Blood vessels of the heart were filled with LBS 3060 latex, followed by fixing in 10% non-buffered formalin. External and internal structures (skeleton, vital organs) were examined and documented.

The weight of the conjoined twins was 1750 g and they were joined from the top of the head to the umbilicus. Body parts in the two twins were symmetric (Table 1).

Table 1. Body measurements of Cephalothoracopagus twins in pig at birth

	Left twin	Right twin
Crown – rump length (mm)	267	265
Head – tail length (mm)	333	332
Tail length	66	67
Chest width (mm)	124	

The twins had a common head, neck and chest. An umbilical cord was attached to the partially fused abdomen. Parts of the abdomen, pelvis and tail were doubled. The piglets had four forelimbs and four hindlimbs. All limbs were properly developed. On the basis of anatomical observations this case was classified as cephalothoracopagus (monocephalic dithoracic). The severe asymmetry in this case was associated with anencephaly and the complete absence of one face (Figures 1 and 2).

The skull of the cephalothoracopagus was abnormally flattened with hypoplasia. The rudimentary cranial cavity was covered by fragments of soft tissue (Figure 2). After removal of this soft tissue, it became apparent that the foramen magnum, sella turcica, cribriform plate and crista galli were all present in duplicate. The basilar artery was passing through the right foramen magnum (Figure 3). The occipital bone had four occipital condyles. Examination showed one fused skull with an abnormal contour and two complete, separate vertebral columns. The fused thorax had two sternums and two sets of ribs.

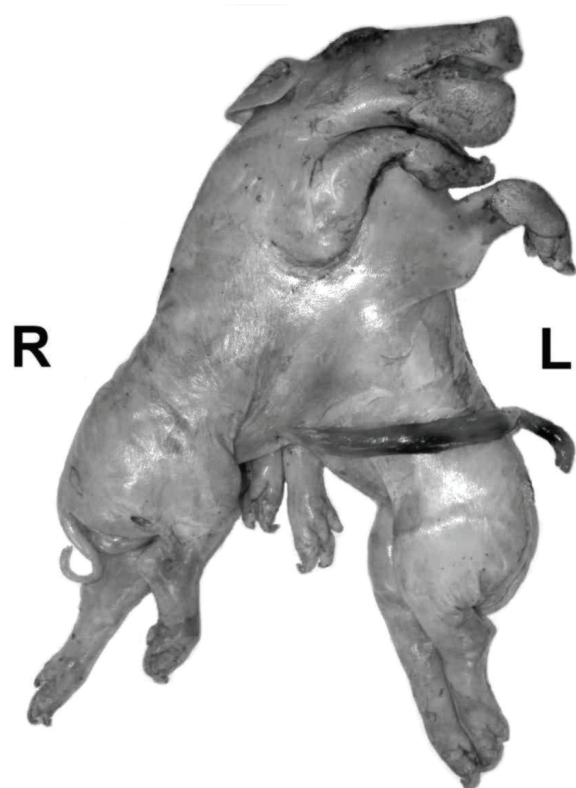


Figure 1. Ventral aspect of cephalothoracopagus conjoined twins
L = left individual, R = right individual



Figure 2. Dorsal aspect of cephalothoracopagus conjoined twins

L = left individual, R = right individual

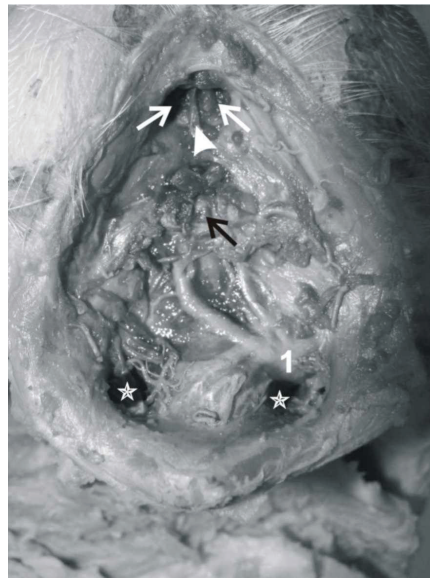


Figure 3. Cranial cavity with hypoplastic skullcap of cephalothoracopagus conjoined twins

★ = foramen magnum, 1 = basilar artery, arrowhead = crista galli, black arrow = sella turcica, white arrow = cribriform plate

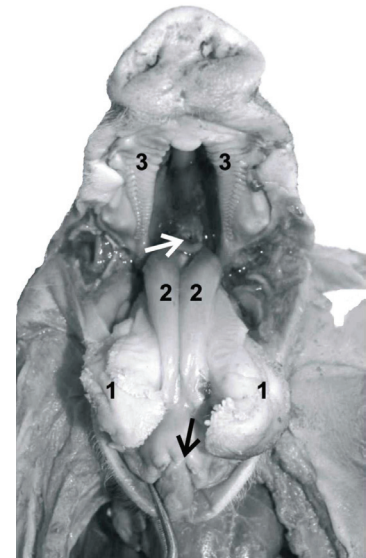


Figure 4. Oral cavity and pharynx of cephalothoracopagus conjoined twins

1 = tongue, 2 = frenulum of tongue, 3 = palate, black arrow = teeth, white arrow = larynx first

Autopsy of the conjoined twins revealed the arrangement and structure of the internal organs. The oral cavity contained two separate tongues, which were connected with a single hyoid bone. Two frenula of the tongue were strongly pronounced, while the incisors of the upper dental arch were displaced. The cleft palate was present, and the palatine rugae formed two separate systems (Figure 4). The twins shared one pharynx, oesophagus, stomach, duodenum and about 89 cm of jejunum. Further, the jejunum separated into two parts. Each of the two parts of the jejunum ended in ileum, which then passed into the large intestine ending with an anus (Figure 5). The twins had a common liver, gall bladder, bile duct, pancreas, accessory pancreatic duct and spleen. The liver of the twins consisted of a number of lobes, typical for a pig. The chest cavity was separated from the abdominal cavity by a large diaphragm.

Internal examination revealed the presence of a common heart. The right ventricle of the heart was enlarged. Two portions of the heart were observed as well as the absence of the interatrial septum. The thickness of the walls in the two cardiac ventricles was similar. Severe abnormalities of the great blood vessels were also observed and the twins had two aortas. The right coronary artery was originating

from the right ascending aorta. A short right pulmonary artery and the right subclavian artery originated from the right aortic arch. The right common carotid artery, the left common carotid artery, the left subclavian artery and the left pulmonary artery originated directly from the left aortic arch (Figure 6).

Two inferior caval veins (left and right) were observed. The right inferior caval vein entered the common vestibule on its right side, while the left inferior caval vein merged with the superior caval vein before entering the vestibule at its left side (Figure 6). All vessels were normal-sized and fully-formed; further they filled easily with latex during specimen preparation showing that they efficiently supplied the organs of the body.

The conjoined twins had two larynxes and tracheas. The first larynx consisted of only one cartilage; the epiglottis and its cavity was covered by mucosa (Figures 5 and 7). This larynx had a blind ending. The entry to the oesophagus was localized above the first larynx. The oesophagus widened, then entered the cavity of the second larynx and was common to both the gastrointestinal and respiratory tracts. The entry to the left trachea which then led to the left lung was observed in the broadened part of the oesophagus. The second larynx comprised

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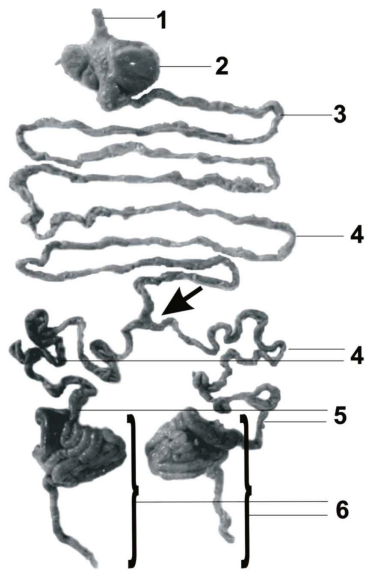


Figure 5. Digestive system of cephalothoracopagus conjoined twins
1 = oesophagus, 2 = stomach, 3 = duodenum, 4 = jejunum, 5 = ileum, 6 = large intestine, arrow = area where the jejunum is bifurcated

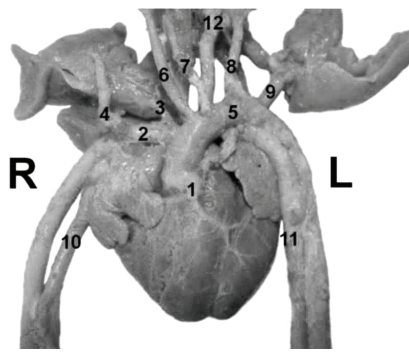


Figure 6. Heart and its vessels
1 = left coronary artery, 2 = left aortic arch, 3 = right pulmonary artery, 4 = right subclavian artery, 5 = right aortic arch, 6 = right common carotid artery, 7 = left common carotid artery, 8 = left subclavian artery, 9 = left pulmonary artery, 10 = right inferior caval vein, 11 = left inferior caval vein, 12 = superior caval vein

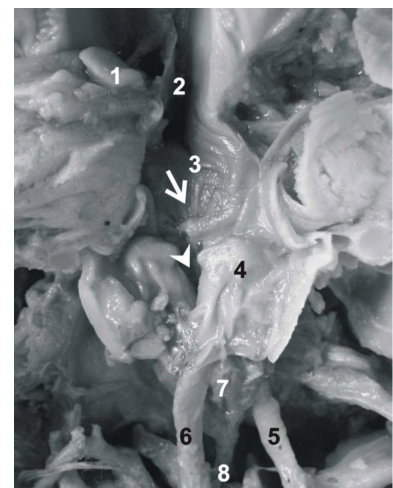


Figure 7. Respiratory and digestive apparatus
1 = epiglottis of the first larynx, 2 = oesophagus, 3 = wide part of oesophagus, 4 = second larynx, 5 = left trachea, 6 = right trachea, 7 = second widening of the oesophagus, 8 = structurally normal oesophagus, arrow = entry to the left trachea, arrowhead = the entrance to the distal oesophagus

of five cartilages. The cavity of this larynx gave rise to the right trachea and the oesophagus. The right trachea led to the right lung. Abnormal widening of the oesophagus was observed shortly after it left the larynx. Further, the oesophagus presented with the correct course and structure (Figure 7). A normal bilobed thyroid gland was associated with the second larynx. The lungs had normal lobation patterns.

The urogenital system was developed normally in both twins. Each twin had its own independent urogenital tract and adrenal glands which were correctly arranged.

DISCUSSION

Cephalothoracopagus is a rare case of conjoined twinning. The piglets described here were delivered at term and their size was similar to that previously described by Evans and Sack (1973). A number of head abnormalities were observed, such as ancephaly and cleft palate. It has been reported that ancephaly and other defects of the neural tube can be characteristic of conjoined twins (Herring and Rowlett 1981; Willhite et al. 1985; Ozkur et al. 2006; Deveer et al. 2010). Ancephaly occurs as a result

of a failure to close anterior neural tube regions and leads to subsequent brain degeneration after contact with amniotic fluid. It causes cessation of fetal forebrain development and the formation of the vault of the skull (Gilbert 2006). A large proportion of affected foetuses die during prenatal development or soon after birth. The exact aetiology of neural tube defects is poorly understood. Research suggests that these abnormalities are caused by genetic and/or environmental factors (Padmanabhan 2006; Greene and Copp 2009). The literature on ancephaly in animals is very limited. Willhite et al. (1985) described this defect in the hamster, Huisinga et al. (2010) in the dog and Greene and Copp (2005) in the mouse. Cleft palate is a type of orofacial cleft and is a fissure or longitudinal opening occurring during embryonic development. Syndromic occurrences besides cleft palate can include cleft lip or a combination of both cleft lip and cleft palate (Deveer et al. 2010). Cleft palate (palatoschisis) can be present in both humans and animals (Deveer et al. 2010; Smolec et al. 2010a; Smolec et al. 2010b). Conjoined twins with this defect have previously been reported in the literature (Nottidge et al. 2007; Deveer et al. 2010). Another

common abnormality in skull formation is craniosynostosis, which is characterised by premature fusion of cranial sutures (Senarath-Yapa et al. 2012). The complexity of molecular signaling pathways and tissue interactions involved in craniosynostosis has led to the development of many different animal models to study both normal development and pathological mechanisms (Grova et al. 2012).

During examination of pig cephalothoracopagus some organs in the single head were found in duplicate. This phenomenon can occur both in humans and animals. Mazzullo et al. (2009) found two tongues in conjoined cat twins. Similar findings in man were made by Herring and Rowlett (1981). Nottidge et al. (2007) described a single but enlarged tongue in the dog. A single tongue was also reported in human cephalopagus janiceps by Singh et al. (2003). On the other hand, Sachan et al. (2016) reported four nostrils, two complete jaws and a lack of distinct eyes in the buffalo.

In the present study, macroscopic abnormalities of cephalothoracopagus were limited to the conjoined parts of the body. However, conjoined dysmorphologies are usually syndromic in manifestations, and several systems and organs, not limited to the conjoined parts, can be affected (Binanti and Riccaboni 2012). The separate body parts, i.e., part of the abdomen and pelvis with its viscera, tails and limbs were normally developed. Similar observations were made by Singh et al. (2003). In rare cases, malformations were observed in unconnected parts of the body of Siamese twins in which one forelimb was rudimentary and contained only two fingers (Ozkur et al. 2006).

The pig cephalothoracopagus reported here had two vertebral columns with two sternums. Similar findings were reported by Hemm et al. (1977) in a mouse conceptus. However, Hynd et al. (2013) did not report the presence of a sternum in mice, whereas Kompanje and Hermans (2008) observed two sternums in a leopard cat.

The gastrointestinal tract in our case of conjoined twins in a pig was single up to the level of the mid-jejunum where it doubled. A similar structure of this tract was observed in a human (Herring and Rowlett 1981; Singh 2003), a hamster (Willhite et al. 1985) and a cat (Mazzullo et al. 2009). Interestingly, in mice, doubling was observed only in the colon (Hynd et al. 2013). Similar developmental defects can occur also in other types of Siamese twins, particularly in ventrally conjoined twins.

A common liver with one gallbladder was observed in the conjoined piglets. Ozkur et al. (2006) described a case of conjoined twins where two partially fused livers were present. A fused liver and two pairs of kidneys were reported in a buffalo cephalothoracopagus (Sachan et al. 2016), whereas Herring and Rowlett (1981) observed the presence of two independent livers in a human, where each had its own gall bladder. Common organs of the digestive tract and a common umbilical cord are typical in Siamese twins with ventral fusion (Spencer 2000b).

The conjoined piglets described in this report shared one atypical heart. Abnormalities of the cardiovascular system are common in various types of conjoined twins. The common chest in cephalothoracopagus often contains two hearts, which can have atypical anatomy and vascularisation (Baumgartner 1928; Spencer 2000a; Spencer 2000b; McMahon and Spencer 2006; Hynd et al. 2013; Sharma et al. 2013). Sometimes one of the hearts is rudimentary or only one heart is present (Herring and Rowlett 1981; Spencer 2000b; McMahon and Spencer 2006; Ozkur et al. 2006; Nottidge et al. 2007). The common heart in cephalothoracopagus usually features two aortas (McMahon and Spencer 2006).

Cephalothoracopagus usually presents with an abnormal respiratory tract. One of the anatomical defects is the presence of a common lumen for the trachea and oesophagus (Herring and Rowlett 1981; Spencer 2000b). The piglets described in this case report had two larynxes (one was incomplete) and two tracheas. Another group of researchers reported a single larynx and trachea in a hamster (Willhite et al. 1985). In cephalothoracopagus in the pig, a separate trachea was connecting each lung in the same manner as previously described in human conjoined twins (Ozkur et al. 2006). Interestingly, Hynd et al. (2013) reported two tracheas connected to two sets of lungs in mice. Spencer (2000b) reported that in some cases of conjoined twins, the trachea or bronchi may be atretic or absent. These organs may also arise from the oesophagus as observed in the piglets in this study.

Conjoined twins arise as a consequence of congenital embryonic duplication of the germinal layer arising from a single ovum. Their anatomy can suggest that the cranial ends of the embryos could be closer together than the caudal ends during embryonic development. The process of folding in tail areas can occur normally, while in the head area only

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one fold is possible. Therefore, a single foregut and two hindguts develop. This single foregut then has a critical impact on further stages of embryogenesis and leads to the abnormal positioning of larynges, lungs, livers and gallbladders. The abnormal heart, on the other hand, is a result of an abnormally positioned closure of the foregut, which leads to an abnormal fusion of the cardiogenic cords (Herring and Rowlett 1981).

Modern techniques of prenatal diagnosis allow an accurate determination of the degree of fusion in conjoined twins. These techniques are used in humans in order to detect developmental abnormalities at early stages of pregnancy and to plan for possible separation of Siamese twins in postnatal life (Ozkuur et al. 2006; Sharma et al. 2013). The process of separation of conjoined twins in humans is performed with a high success rates (Rabeeh 2006). However, prognoses for cephalothoracopagus twins are extremely poor, as surgical separation is often not an option due to the large number of developmental disorders affecting the function of organs and systems. Advanced prenatal screening techniques in animals are very rarely used and conjoined twins are often only diagnosed during or after a difficult birth. Difficult births in animals can cause death of a female or/and offspring(s) and contribute to reproductive wastage in animal production.

On the basis of different cases of conjoined twins, a list of frequently recurring anatomical defects was created (Spencer 2000b). Developmental abnormalities are explained based on the theory of fusion or/and fission (Spencer 2000a; Spencer 2000b). However, according to the author of this list some cases of conjoined twins with complex developmental abnormalities are inexplicable.

Proteins such as activin, nodal and Sonic hedgehog form a cascade of secreted signals regulating right-left asymmetry. During embryonic development in conjoined twins, the signalling cascade of the right embryo can influence the cascade of the left embryo resulting in randomisation of its situs and the occurrence of laterality defects (Levin et al. 1996). On the other hand, maternal gene *Vg1* (TGF-beta family) was implicated in dorso-anterior development and left-right axis formation. Disruptions in the expression of this gene can randomise cardiac and visceral left-right orientation (Hyatt et al. 1996; Hyatt and Yost 1998). Moreover, specific actions of *Hox* and *Pax* genes are implicated in very early embryogenesis (Gilbert-Barnes 2007).

Each case of conjoined twins should be treated as unique due to the presence of peculiar anatomical differences. These differences are particularly important during the process of decision making and planning of separation. Animals Siamese twin models exhibit a large variation of developmental abnormalities.

Conjoined twinning is a random event, and the current understanding is that there is no risk of repetitiveness of this malformation. Hence, there is no need to eliminate the parents of conjoined progeny from breeding programs. Sonfada et al. (2010) suggested that developmental abnormalities, including conjoined twins, could be caused by an incorrect diet. The authors based their theory on the analysis of numerous cases of developmental abnormalities in small ruminants taking into consideration the environment in which the animals were grazing. Developmental abnormalities can also be caused by exposure to environmental toxicants, chemicals, pesticides, herbicides and some drugs. Toxic plants can be particularly dangerous, as well as the lack of dietary supplements in the regions where nutrition is poor, which in some cases depends on the season. Similar causes of developmental abnormalities were described by Samuel et al. (2014). Researchers have also identified the diet of pregnant females as a possible cause of developmental abnormalities (Willhite et al. 1985). Greene and Copp (2005) and Padmanabhan (2006) emphasised the importance of folic acid supplementation in the prevention of neural tube defects.

Polish large white sows have an average of eleven piglets per litter (Szulc et al. 2013). The Siamese twins from the present study originated from a typical Polish large white litter, and from an area without a previous history of conjoined twins. It is, therefore, difficult to determine the factors that caused this abnormality. This report presents a rare example of cephalothoracopagus conjoined twins in the pig that has not previously been described in literature. The occurrence of conjoined twins in animal husbandry leads to losses in animal production. Thus, it is very important to investigate and understand what causes the formation of this defect. To facilitate this process, it is necessary for breeders to report each case of Siamese twins, and to conduct strict and improved farm practices including karyotyping and prevention of accidental mating.

In order to allow further scientific investigation, breeders should provide necessary information

about the ancestors of animals, animal welfare conditions, their nutrition, vaccinations, illnesses, etc. Such information could facilitate the identification of pathogens. In some cases, the environments in which livestock live are severely restricted, so identification of teratogenic factors is also crucial. The priority should be to understand the causes of this defect so as to prevent its occurrence.

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