SRY and DelE Detection in Polled Intersex Tangshan Dairy Goat

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ABSTRACT

The sex determining region Y (SRY) gene and deletion element DelE were analyzed in 34 polled intersex syndrome (PIS) or normal Tangshan dairy goats (3 females and 3 males) by PCR technology. The result showed that the special male SRY gene only existed in male goats, and was absent in all PIS goats or normal female goats. The 28 PIS goats were homozygote for DelE, and the other 6 goats were heterozygote for DelE deletion or homozygote for non DelE deletion. It could be concluded that the PIS Tangshan dairy goats were female for its genetic basis and the DelE was partial deletion.

Key words: DelE, PIS, Tangshan dairy goat, SRY.

INTRODUCTION

The polledness intersexual Syndrome (PIS- -) (Capra hircus), deformed individuals with abnormal horn development and intersexuality, could have malformed reproductive organs and loss of reproductive function. In goats, the PIS (polled intersex syndrome) mutation is responsible for both the absence of horns in males and females and sex-reversal affecting exclusively XX individuals (Pailhoux et al., 2001). Most of the reports about intersex goat were the cases of female-to-male sex-reversal (Pailhoux et al., 2001; Pailhoux et al., 2002; Boulanger et al.,2008 and Monteagudo et al., 2008). The genetic sex basis of intersex goats was considered as female by analysis sex ratio for 1660 newborn lambs (Chang et al., 1980). Karyotype analysis also showed that most intersexuality was 60 XX. Vaiman et al (1999) found that the PIS goat was caused by the deletion of 11.7-kb fragment located at chromosome 1q43 which was homologous to human chromosome 3q23. Shinichi et al. (2011) first detected sex determining region Y(SRY) gene in a 5-day-old hornless goat with dysuria (PIS goat) and showed that the SRY gene was absent. Shinichi et al. (2011) also showed that the intersex goat with dysuria was absent for DelE fragment. Unfortunately, Shinichi et al. (2011) only detected SRY gene and DelE fragment in one hornless goat with dysuria (PIS goat). There are rich breeds of goat with PIS in China (E et al, 2015; Zhang, 2014; Yang, 2012; Zhang, 2006). Tangshan dairy goat is a kind of excellent dairy local breed with the descendant of Saanen, and there is a larger proportion of intersex in the dairy goats. To understand the genetic sex basis of PIS in this breed, 34 hornless and intersex Tangshan dairy goats and 6 normal goats were investigated SRY and DelE situation.

MATERIALS AND METHODS

A total number of 40 blood samples of 1-month-old Tangshan dairy goats were randomly selected from farms in Hebei province of China, including 34 polled intersex goats and 6 normal goats (3 females and 3 males). Polymerase chain reaction (PCR) analysis was performed to detect the SRY gene and DelE fragment. Additionally, β-globin was also amplified as an internal control. The primers were designed follow previous reports (Table1). PCR amplification was carried out on a programmable thermal controller (German Biometra) with a total volume of 50µL solution containing 150ng genomic DNA, 5µl 10 × PCR reaction buffer (Mg2+ plus), 400 pmol/µl each forward and reverse primer, 200pmol/µl dNTPs, and 2U Taq DNA polymerase from Tiangen Biotech (Beijing). For the detection of SRY gene, cycling conditions had an initial denaturation at 94°C for 4min, followed by 35 cycles of 30 sec, annealing at 62°C for 30s, and extension at 72°C for 1 min. A final extension was done at 72°C for 10 min (Monteagudo et al., 2008). For the DelE fragment and β-globin, the amplification conditions were the same as above, except that extension at 72°C for 45s while for β-globin was 30s and annealing temperature was at 63°C and 60°C respectively (Monteagudo et al., 2008; Konnai et al., 2006). PCR products were detected on a 1.0% agarose gel including 0.5ng/ml of ethidium bromide, photographed under GEL imaging system (BIO-RAD Company).

RESULTS AND DISCUSSION

The observation and classification for genitalia of polled intersex goats: The genitalia of intersex Tangshan dairy goats were classified typically as follows: Type I showed the female character, with narrow introitus and long perineal.
The masculinization character showed in the large clitoris (Fig. 1A). Type a! was with the masculine phenotypes characterized by a short penis-like structure under the anus, while the normal should be situated in abdominal. Scrotum couldn’t be observed from the appearance (Fig. 1B). Type b! had a middle phenotype between a male and a female type, because both ostium vaginae and male testicle tissue could be seen. Two intra-abdominal testicle-like structures attached to abdominal and anogenital distance was also longer than normal (Fig. 1C). The percentage of three types was 35.3%, 47.1% and 17.6% respectively in Tangshan dairy goat.

**Variation of SRY gene in Tangshan dairy goat:** The PCR results of SRY gene detection was shown in Fig. 2A. It was obvious that an expected 113-bp fragment of the controlling β-globin was correctly amplified in the DNA samples from the PIS, normal male or female goats, indicating the effectiveness of PCR reaction system. The SRY gene (660-bp fragment) was not detected in all of the PIS or female goats. The SRY gene only appeared in normal male goats indicated that the PIS goat was absent with SRY gene and their genetic sex basis was female.

**Variation of DelE fragment in Tangshan dairy goat:** The PCR results of DelE fragment detection in Tangshan dairy goats was shown in Fig. 2B and Fig. 2C. The expected 113-bp fragment of the controlling β-globin was also correctly amplified in the DNA samples from the PIS, normal male and female goats in the process of DelE fragment detection. In 34 PIS Tangshan dairy goats, the 147-bp DelE fragment were amplified in 6 samples (Fig. 2C), no amplification was obtained in other 28 PIS goats (Fig. 2B). It could be inferred that the 28 PIS goats were homozygote for DelE deletion. The genotype of other 6 PIS goats might be in two states, heterozygote for DelE deletion or homozygote for non DelE deletion.

The mode of inheritance is dominant for the polled trait and recessive for sex-reversal, as well as the linkage between hornlessness and intersexuality (Asdell, 1944; Pailhoux et al., 2005). As shown in the previous study, the intersex goats usually were classified as masculine phenotypes and feminine phenotypes (Zhang et al, 2006; Shinichi, 2011). The former characterized by a small penis, epididymis and testicular, no introitus (Zhang et al, 2006). The later had narrow vulvar opening and hypertrophic clitoris and the vagina was short or blind. No testis, penis, or scrotum was detected (Batista et al, 2000; Zhang et al, 2006; Shinichi, 2011). In Tangshan dairy goat, the type I was accorded with the feminine phenotype. The type II had a little difference with the male phenotype, because the epididymis and testicular were not seen in the male

![FIG 1: The external appearance features of the PIS-/- goat.](image)

A: The perineal raphe was narrow (up arrow), and labia was not obvious but it has a large clitoris (below). B: Scrotum was absent, and a small penis was seen near the anus. C: A couple of Scrotum-like structure were seen in the abdominal aspect (below arrows), after a long anogenital distance.
Mammalian sex determination is governed by the presence of Y chromosome. The sex-determining region Y (SRY) gene was considered as the testis determining factor (TDF) (Koopman et al., 1991; Mukherjee et al., 2015). Mutation in this gene causes sex-reversal in many mammalian species. SRY plays a key role in a number of intersex animals. Mutation or loss of function of SRY results in complete male to female sex reversal (Jager et al., 1990; Maier et al., 2003) whereas ectopic expression of SRY in XX individuals due to chromosomal translocation of SRY may result in female to male sex reversal. Similar to our result, Shinichi et al. (2011) first detected SRY gene in a 5-day-old hornless goat with dysuria (PIS goat) to identify the genetic sex basis and showed that the SRY gene was absent. Our result showed that all of the 34 PIS Tangshan dairy goats were absent for SRY gene, indicated that the sex genetic base of 34 polled intersex goats was female. Our finding confirm the sex genetic base of PIS goat.

Pailhoux et al. (2001; 2005) found 11.7-kb deletion triggers intersexuality and polledness in goat by sequencing the amplification product obtained from intersex individuals, and the deleted region was delimited precisely. By detected the deletion in two breed (Alpline and Saanen), a total of 17 studied XX sex-reversed PIS-/- goats were observed in a homozygous state, indicating the complete deletion. In recent years, DelE deletion was usually took as the mark of PIS-/- (Monteagudo et al., 2008; Shinichi et al., 2011; Yang et al., 2012). The result of this study supported the conclusion that the 11.7kb deletion is partial missing in Tangshan dairy goat. This suggested that the DelE fragment was not credible to identify the genotype of PIS. Further research is necessary to study the mutation of PIS in goat intersexuality. The finding of the Chinese indigenous breed of goat could provide a basic data for further understanding the contribution of DelE fragment in the sex-developmental mechanism.

DECLARATION OF INTEREST
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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