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Accuracy of Ultrasonographic Diagnosis of Sex and Effect of Sex and Birth Type on Biparietal Diameter of Saanen Goat Fetuses

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Summary

The effects of sex and birth type on biparietal diameter (BPD) were examined from 6th to 14th weeks of gestation in 29 pregnant Saanen does by ultrasonography and after birth by observing the kids directly. Fifteen does delivered singles, 13 had twins and one goat had quadruplet males. Twelve of the twins were male and 13 were female; 7 singles were male and 8 were female. In twin pregnancies, the most accurate period for diagnosis of sex of the fetus by observation of the position of the genital tubercle was the 9th week of gestation. However in singles, two errors (13 percent) were made at the 9th week. Two-way anova analysis revealed that birth type did not affect BPD of Saanen goat fetuses, and sexes did not differ until the 14th week ($P < 0.05$). Even then, the difference was too small to be useful to predict the sex of the offspring. Chi-square test was applied to compare the success rates of ultrasonography for prediction of fetal sex in different weeks of gestation. Ratio for success of sex determination by ultrasonography was greater from the 9th week of gestation compared with earlier periods in twin pregnancies ($P < 0.001$). On the other hand, there were no significant differences among gestation weeks in terms of ratio for success of sex determination by ultrasonography in single pregnancies ($P > 0.05$). Thus it is concluded that sex of the fetus can be diagnosed directly at the 9th week, but one cannot establish the sex of the fetuses by using only ultrasonographic measurements of BPD in either twin or single pregnancies in Saanen goats.

Keywords: Biparietal Diameter, Birth Type, Saanen Fetal Goat Sex, Ultrasonography

Introduction

Ultrasonography is an important non-invasive technique for fetal-sex assessment. Early fetal sexing provides the best planning for the acquisition and commercialization of animals, and allows the marketing of male and female fetuses, while still in utero and the differentiation of fetuses for use in meat or milk herds (Santos et al., 2007b). In cows, early sex determination permits identification of male and female co-twins, which could lead to a female freemartin. Fetal sex determination by ultrasonography was performed previously in cattle, buffaloes, horses, sheep and goats (Mari et al., 2002; Tainturier et al., 2004; Santos et al., 2007a, b; Ali and Fahmy 2008; Azevedo et al., 2009; Moraes et al., 2009).

The genitalia of the fetus develop from the mesenchyme of the ventral abdominal wall, between the tail, the hindlimbs and the umbilical cord (Yotov et al., 2011). Penis in males and clitoris in females differentiate from the genital tubercle (GT), which is an embryonic structure. Initially, the location of the GT is between the hind limbs, and the sex cannot be identified at this point. The GT later migrates towards the umbilical cord and appears as hyperechogenic points in males, whereas it migrates towards the tail and appears as echogenic genital swellings in females (Mari et al., 2002; Yotov et al., 2011).

Amer (2010) reported evaluation of fetal sex of both single and multiple fetuses in goats during the stages of 40 d to 60 d, 61 d to 70 d and 90 d to 109 d after mating. Optimum results were achieved in the first stage, and the accuracy of sex diagnosis varied among these stages, being 93 percent, 82 percent and 58 percent, respectively.

In goats, Azevedo et al. (2009) reported the presence of a bilobar hyperechoic structure near the umbilical cord that allowed correct diagnosis as a male fetus. When this structure was not observed, the fetus was diagnosed as female at the same period of pregnancy.

Ultrasonography was used in horses and cattle to identify fetal sex by visualizing the penis or scrotum or the location of the genital tubercle (Mari et al., 2002). Fetal sex was determined by visualizing the external genitalia (penis, prepuce, scrotal bag, nipples, and genital swelling) and/or according to the location of the GT. In several studies, the sex of the fetuses was confirmed by visualizing the external genitalia after birth (Santos et al., 2006, 2007a, b; Amer 2008, 2010; Azevedo et al,. 2009; Moraes et al., 2009).

Biparietal diameter (BPD) involves the measuring of the skull of the fetuses when it has achieved an oval shape, and the flax cerebri mid-line is dividing the hemispheres into two equal parts. The aim of this study was to detect effects of sex and birth type on BPD during different stages of gestation in Saanen goat fetuses.

Materials and Methods

Animals and management

Twenty nine pregnant Saanen does aged between 1-½ years and 3 years, kept under standard management conditions, were used for this study. The goats were observed carefully for estrus twice daily, morning and afternoon, and were mated by the buck naturally. The day of mating was designated as day 0 of gestation.

Experimental design

Fifteen of the does were examined transrectally once a week, on the same weekday, from the 6th through 14th weeks of gestation. Another 14 does were examined transrectally only until the 10th week of gestation and then transabdominally until the 14th week of gestation. Ultrasonography was performed with a linear transducer (5.0 mHz to 8.0 mHz) using a B-mode, real-time scanner (Medison SA 600V, South Korea) adapted to a PVC in order to facilitate the manipulation into the animal's rectum. Before transrectal examination, the rectum was evacuated manually and the ultrasound probe was covered by a coupling gel and introduced into the rectum after evacuation while does were standing. For transabdominal ultrasonography the goats were scanned in lateral recumbency or in the standing position, after shaving the ventral abdominal wall beside the udder. In order to detect the genital tubercle easily, the main parts of the fetus were established first.

After visualizing the fetus at the early periods of gestation, sex of the fetuses was identified according to the location of the GT (Fig. 1a). In later periods of gestation, the sex was identified by visualizing the penis, prepuce, scrotal bag (Fig. 1b), nipples, or vulva (Fig. 1c). Images were frozen on the screen and measured with built-in electronic callipers. The BPD was measured when the skull of the fetuses was visualized oval in shape, with the flax cerebri mid-line dividing the hemispheres into two equal parts. The measurements were taken from the outer surface of the proximal calvarium to the inner surface of the distal calvarium (Fig. 1d).

During the last week of ultrasonography, the estimation of the skull became more challenging. The aim was to establish the fetal sex and BPD, so the ultrasound examinations lasted as long as was necessary to obtain optimal scanning conditions, which always took a maximum of 15 minutes.

Statistical analysis

For statistical analysis, the two-way anova method was used to test for effects of sex and birth type on BPD at each week of gestation (6 weeks through 14

Figure Legends: Fig. 1a. Male fetus; genital tubercle is close to the umbilical cord (arrow).
Fig. 1b. Fetal scrotal bag at the 10th week of the gestation (arrows).
Fig. 1c. Vulvar region in a female fetus (arrow).
Fig. 1d. Biparietal diameter of a fetus.

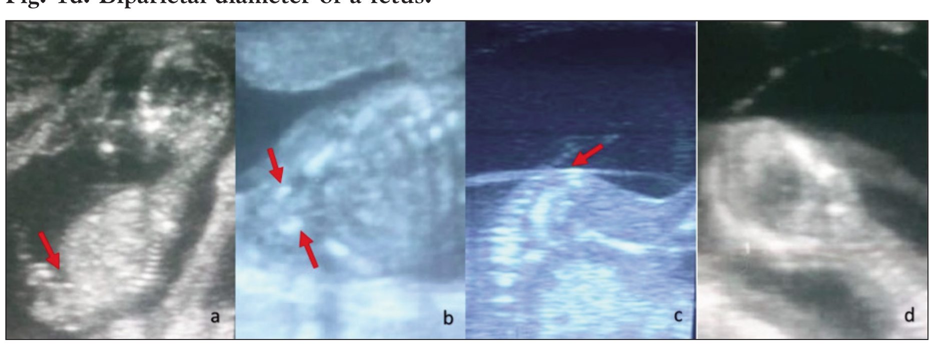

Table 1. Mean biparietal diameters (mm) and and standard errors (SE) from the 6th week to the 14th week of gestation and after birth in relation to sex of the fetuses in singleton and twin Saanen goat fetuses.

	Sex				Birth Type				Significance	
Gestation	Male		Female		Single		Twin			Birth
Period	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Sex	Type
6. Week	12.3	0.4	11.8	0.4	12.0	0.5	12.1	0.3	NS	NS
7. Week	16.1	0.5	15.9	0.5	16.0	0.6	16.0	0.4	NS	NS
8. Week	20.2	0.7	19.1	0.7	19.2	0.8	19.7	0.6	NS	NS
9. Week	24.6	0.7	23.5	0.8	24.3	0.8	23.8	0.6	NS	NS
10. Week	29.7	0.8	28.7	0.9	29.5	1.0	29.0	0.7	NS	NS
11. Week	34.2	0.6	33.2	0.7	34.1	0.8	33.3	0. 6	NS	NS
12. Week	37.3	0.6	36.5	0.6	37.7	0.7	36.1	0.5	NS	NS
13. Week	41.5	0.6	40.3	0.6	41.1	0.7	40.7	0.5	NS	NS
14. Week	45.7	0.4	44.2	0.4	4.5	0.5	44.9	0.4	*	NS
After Birth	83.8	1.0	80.8	1.0	82.8	1.1	81.8	0.9	*	NS

NS: Not significant ($P > 0.05$)
*: ($P < 0.05$)

weeks) in Saanen goat fetuses. Chi-square test was applied to compare the success rates of ultrasonography for prediction of fetal sex at different weeks of gestation.

Results

The results of weekly ultrasound examinations were compared with either identified sex or BPD of the offspring at birth. Of 45 fetuses examined in 29 Sannen does, 44 were born and one was mummified. Fifteen does delivered singles, 13 had twins and one had quadruplets. Twelve of the twins were male and 13 were female (Appendix Table 1, page 14). Seven of the singles were male and eight were female (Appendix Table 2, page 15). One goat gave birth to four males, but during ultrasound examinations only two of the fetuses could be distinguished and these two were evaluated as male (Fig. 1c). Transabdominal ultrasonography was found to be more difficult than transrectal ultrasonography and required more time to examine the fetuses in order to distinguish either BPD or fetal sex.

The mean weekly measurements of BPD and the sex of the fetuses during different stages of gestation are given in Table 1. After birth, the fetal sex was confirmed for each kid. Fetal sex of two of the offspring was evaluated incorrectly; the others were established correctly.

Statistical analysis

According to the two-way anova analyses, the BPD differed between the sexes only at the last (14th) week and after birth ($P < 0.05$). The BPD did not differ with birth type at any stage. Ratio for success of sex determination by ultrasonography was greater from the 9th week of gestation onward compared with earlier periods in twin pregnancies ($P < 0.001$). On the other hand, there were no significant differences between gestation weeks in terms of ratio for success of sex determination by ultrasonography in single pregnancies ($P > 0.05$) (Table 2). In the 15 single pregnancies in this study, two false diagnoses were obtained (13 percent). In one doe, the false diagnosis may be attributed to the ultrasonographic presentation of the fetus at the time of the examination. In this doe, the fetus was recorded as female till the 9th week, then it was recorded as male, but was female at birth, so diagnosis after 9th week was false. In the second doe, from the onset of examination till the end the fetus was assumed as male, but at birth it was distinguished as female. In examination of this fetus, the sex indicators or their locations were unclear or absent. Conversely, results were changed in 5 of 13 does (38 percent) in 9th week in twin pregnancies. When compared with after-birth results, they were subsequently detected correctly through the end of the study. The two false results in single pregnancies may have occurred because there was not as much extra care as was

Table 2: Successful prediction rates (%) for sex of the fetus according to the weeks of ultrasonographic examinations.

Weeks	Single (%)	Twins (%)	All of the data set (%)
6	61.5	80^{b}	73.7^{c}
7	60.0	84^{b}	75^{c}
8	73.3	80^{b}	77.5^{bc}
9	66.7	100^{a}	87.5^{abc}
10	85.7	100^{a}	94.6^{a}
11	85.7	95.7^{ab}	91.6^{ab}
12	86.7	100.0^{a}	95^{a}
13	86.7	100^{a}	94.9^{a}
14	86.7	100^{a}	95^{a}
Chi-square	**8.822 (NS)**	**26.14 (***)**	**23.855 (**)**

NS: Not significant ($P>0.05$).
a, b, c: Differences between the means lacking a common letter in the same column are significant (**= $P<0.01$; ***= $P<0.001$)

taken during ultrasonographic examinations in twin pregnancies.

In the present study, accuracy of ultrasonographic examinations was greatest at the 9th week of gestation, which is in line with recommendations by Santos et al. (2007b). The most frequent variations were observed in the diagnosed sex of the fetuses in Saanen goats after day 63 (9th week) of pregnancy. Oliveira et al. (2005) reported that GT migration took place around 48.9 d ± 1.8 d in Saanen goats. As reported by Santos et al. (2007b), the GT migration time affects the accuracy of fetal sexing in small ruminants. They examined migration of the genital tubercle between the days of gestation 40 d to 60 d with ultrasonography, 55 d to 70 d transrectally, and 100 d to 120 d transabdominally. They found that migration of GT occurred around day 50 of the pregnancy in goat fetuses and suggested that examinations should take place between 55 d to 70 d of pregnancy to avoid false diagnosis of the sex of the fetuses. Santos et al. (2007a) recommended multiple examinations for fetal sexing in triplet pregnancies, as more fetuses increased the risk of failures, which agrees with our results. In the present study, we could not visualise the sex of two fetuses nor even their presence in one quadruplet pregnancy.

It was very difficult to determine the sex of the fetus with an examination lasting less than 15 minutes at only one plane. It has been reported that the appearance of the genital tubercle may change (Yotov et al., 2011). In the latter study, 6 percent of fetuses had unidentified sex in buffaloes; these false diagnoses could be attributed to the absence or unclear visualisation of the sex indicators and their locations. In the present study, diagnosis of the sex of some fetuses was altered as the appearance of this structure changed after their sex was established initially. Yotov et al. (2011) reported that the sex can be determined best in sagittal or cross-sectional position, whereas Azevedo et al. (2009) considered the longitudinal plane best in ovine or caprine fetuses.

In this study, there were more undetermined cases of fetal sex at weeks 11, 13 and 14 due to the growth of the fetuses. Gonzalez de Bulnes et al. (1998) reported that in Manchega dairy ewes the estimation of fetal development using a transrectal ultrasound device can be efficient to detect pregnancy until day 90 to 91, after which the fetus cannot be detected properly with this technique. Similarly, Yotov et al. (2011) observed that after the fetus was grown, accuracy of sex determination could be reduced in buffaloes. Pedreira et al. (2001) reported a variation in the orientation of the genital tubercle during their study period in human fetuses. In the present study, comparison of the transrectal and transabdominal approaches during the 10th through 14th weeks of gestation indicated that the transabdominal technique was more difficult and required more time to evaluate and establish the sex and the BPD.

Biparietal diameter has been used to predict fetal sex in human beings (Mazza et al., 1999). These authors found that, for accurate detection of fetal sex in humans, the threshold above 23 mm of BPD provided the best results. Reichle and Haibel (1991) reported that the fetal BPD measurement was most accurate in mid-gestation due to the larger size of the fetus; this study's results agree with theirs. Abdelghafar et al. (2011) measured weekly BPD of the fetuses starting from 6th week until the end of gestation in Saanen goats. In this study, ultrasonographic examinations were not continued beyond week 14, due to the difficulties of imaging the fetuses as fetal size increased.

In one animal, a mummified fetus was evaluated after birth. The animal's pregnancy was normal during our ultrasonographic examinations, so the fetus apparently died after the completion of ultrasonographic examinations, i.e., after the 14th week of gestation.

Conclusions

In conclusion, one cannot establish the sex of the fetuses by using only ultrasonographic measurements of BPD in either twin or single pregnancies in Saanen does. Birth type did not affect BPD of Saanen goat fetuses, and sexes did not differ until the 14th week of gestation. Even then, the difference was too small to be useful to predict the sex of the offspring. Direct observation of the genital tubercle appears to be necessary to evaluate fetal sex, and that is difficult in multiple pregnancies.

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Appendix Table 1. Measurements of biparietal diameter (mm) and predicted sex of twin fetuses from 6th week to 14 week of gestation and after birth in Saanen goats.

		Gestation periods									
Groups	Doe id.	6. week BPD (mm)	7. week BPD (mm)	8. week BPD (mm)	9. week BPD (mm)	10. week BPD (mm)	11. week BPD (mm)	12. week BPD (mm)	13. week BPD (mm)	14. week BPD (mm)	After birth BPD (mm)
Female/Male	1	10.1 (M) 11.2 (M)	21.5 (F) 22.2 (M)	26.6 (M) 28.0 (M)	30.6 (F) 31.0 (M)	31.2 (F) 32.4 (M)	33.1 (F) 34.0 (M)	35.3 (F) 36.0 (M)	38.0 (F) 38.6 (M)	43.0 (F) 44.3 (M)	80.0 (F) 80.4 (M)
	2	8.94 (M) 8.92 (F)	11.7 (M) 10.8 (F)	17.3 (F) 16.8 (F)	21.2 (M) 20.5 (F)	28.7 (M) 27.6 (F)	33.0 (M) 32.1 (F)	35.4 (M) 34.2 (F)	39.2 (M) 38.5 (F)	47.0 (M) 46.3 (F)	85.0 (M) 82.0 (F)
	3	13.0 (M) 12.8 (F)	13.9 (M) 13.4 (F)	23.3 (M) 23.0 (F)	27.8 (M) 26.0 (F)	34.0 (M) 32.8 (F)	36.0 (M) 35.2 (F)	38.4 (M) 36.7 (F)	42.0 (M) 39.2 (F)	46.3 (M) 43.2 (F)	86.0 (M) 80.0 (F)
	5	11.2 (M) 12.2 (M)	16.2 (M) 17.3 (M)	18.3 (M) 19.0 (M)	22.8 (F) 24.2 (M)	25.4 (F) 27.0 (M)	31.3 (F) 33.0 (M)	33.0 (F) 34.2 (M)	36.0 (NI) 38.2 (M)	44.5 (F) 45.6 (M)	85.0 (F) 87.0 (M)
	6	13.0 (F) 12.3 (M)	16.2 (F) 15.3 (M)	18.3 (F) 17.6 (M)	22.0 (F) 21.3 (M)	NI 26.0 (M)	27.3 (F) 29.0 (NI)	33.3 (F) 32.0 (M)	42.4 (F) 43.0 (M)	47.3 (F) 46.0 (M)	85.0 (F) 80.0 (M)
	7	12.8 (M) 13.2 (M)	17 (F) 16 (M)	21.4 (F) 22.3 (M)	28.2 (F) 29.1 (M)	34.6 (F) 35 (M)	38 (M) 38.2 (M)	38.7 (F) 38.9 (M)	41.2 (F) 42 (M)	43.0 (F) 44.0 (M)	80.0 (F) Mummified
	13	11.2 (F) 13.4 (M)	15.8 (F) 16.7 (M)	18.0 (F) 21.0 (M)	22.0 (F) 23.4 (M)	24.4 (F) 25.6 (M)	32.8 (F) 33.2 (M)	37.6 (F) 38.0 (M)	44.6 (F) 44.7 (M)	45.6 (F) 46.2 (M)	78.0 (F) 80.0 (M)
Only Female	9	13.1 (F) 14.0 (F)	16.0 (F) 15.8 (F)	19.0 (F) 18.8 (F)	22.0 (F) 21.2 (F)	28.2 (F) 26.0 (F)	33.5 (F) 32.0 (F)	37.8 (F) 36.7 (F)	41.2 (F) 40.9 (F)	44.0 (F) 42.7 (F)	82.0 (F) 80.0 (F)
	10	14.5 (F) 13.2 (F)	16.3 (F) 15.3 (F)	18.3 (F) 17.6 (F)	23.0 (F) 23.2 (F)	29.9 (F) 29.8 (F)	37.5 (F) 36.5 (F)	38.3 (F) 38.4 (F)	39.4 (F) 39.6 (F)	43.3 (F) 44.0 (F)	82.0 (F) 83.0 (F)
	12	13.1 (F) 13.6 (M)	16.3 (F) 17.2 (M)	22.6 (M) 18.4 (F)	26.0 (F) 24.3 (F)	30.8 (F) 31.2 (F)	33.2 (F) 34.1 (F)	36.5 (F) 37.0 (F)	42.7 (F) 42.7 (F)	45.2 (F) 45.3 (F)	84.0 (F) 85.0 (F)
Only Male	4	10.4 (F) 9.0 (M)	14.5 (F) 13.0 (M)	17.3 (F) 16.7 (M)	22.0 (M) 19.0 (M)	NI 25.3 (M)	NI (M) 29.0 (M)	29.6 (M) 31.0 (M)	38.3 (M) 36.0 (M)	46.2 (M) 43.0 (M)	80.0 (M) 75.0 (M)
	8	12.8 (M) 11.7 (M)	17 (M) 16.7 (M)	18.3 (M) 18.2 (M)	21.4 (M) 20.9 (M)	28.7 (M) 27.4 (M)	32.4 (M) 31.8 (M)	38.3 (M) 37.9 (M)	42.8 (M) 41.7 (M)	46.0 (M) 45.7 (M)	83.0 (M) 80.0 (M)
	11	12.7 (M) 13.2 (M)	16.6 (M) 17.0 (M)	18.0 (M) 18.3 (M)	22.8 (M) 23.0 (M)	26.5 (M) 26.8 (M)	33.3 (M) 34.0 (M)	37.4 (M) 38.2 (M)	42.3 (M) 43.5 (M)	44.8 (M) 45.4 (M)	80.0 (M) 82.0 (M)

NI: Not Identified

Appendix Table 2. Measurements of biparietal diameter (mm) and predicted sex of single fetuses from 6th week to 14 week of gestation and after birth in Saanen goats.

	Gestation periods									
No	6. week BPD (mm)	7. week BPD (mm)	8. week BPD (mm)	9. week BPD (mm)	10. week BPD (mm)	11. week BPD (mm)	12. week BPD (mm)	13. week BPD (mm)	14. week BPD (mm)	After birth BPD (mm)
14	12.8 (F)	17.7 (F)	28.2 (M)	31.0 (M)	32.8 (M)	35.2 (M)	38.2 (M)	42.0 (M)	47.0 (M)	90.0 (M)
15	9.6 (F)	17.1 (F)	20.4 (F)	23.2 (F)	26.3 (F)	28.0 (F)	33.0 (F)	41.6 (F)	44.0 (F)	80.0 (F)
16	10.8 (M)	14.6 (M)	18.4 (M)	23.0 (M)	33.0 (M)	35.5 (M)	39.0 (M)	41.8 (M)	45.0 (M)	80.0 (F)*
17	10.2 (F)	14.4 (F)	15.8 (F)	23.1 (F)	28.4 (F)	31.0 (F)	36.6 (F)	38.0 (F)	46.3 (F)	80.0 (F)
18	11.1 (F)	15.4 (F)	18.0 (F)	24.2 (M)	NI	33.6 (M)	36.0 (M)	38.2 (M)	46.8 (M)	90.0 (F)*
19	11.1 (NI)	14.3 (M)	16.4 (M)	21.0 (F)	27.6 (F)	33.0 (F)	35.6 (F)	37.0 (F)	41.3 (F)	72.0 (F)
20	8.3 (M)	12.2 (F)	14.7 (M)	21.8 (F)	33.2 (M)	36.0 (M)	38.4 (M)	43.1 (M)	46.2 (M)	85.0 (M)
21	11.4 (M)	16.3 (M)	18.2 (M)	23.0 (M)	28.0 (M)	NI (F)	32.6 (F)	34.0 (F)	36.0 (F)	69.0 (F)
22	16.1 (F)	18.2 (F)	24.3 (F)	30.4 (F)	38.7 (M)	40.2 (M)	43.9 (M)	44.2 (M)	46.3 (M)	89.0 (M)
23	14.2 (F)	17.0 (F)	19.6 (F)	23.0 (F)	28.5 (F)	36.2 (F)	41.4 (F)	42.8 (F)	44.2 (F)	80.0 (F)
24	12.2 (NI)	16.3 (M)	18.4 (M)	23.0 (M)	26.5 (M)	31.4 (M)	36.2 (M)	38.6 (M)	47.2 (M)	85.0 (M)
25	12.7 (F)	15.7 (M)	19.4 (M)	23.0 (M)	26.7 (M)	34.3 (M)	38.3 (M)	43.0 (M)	47.5 (M)	93.0 (M)
26	11.2 (F)	15.8 (F)	18.0 (F)	22.0 (F)	24.4 (F)	32.8 (F)	37.6 (F)	44.6 (F)	45.6 (F)	78.0 (F)
27	15.4 (M)	18.3 (M)	23.8 (M)	31.0 (M)	35.6 (M)	38.8 (M)	42.3 (M)	44.4 (M)	46.2 (M)	89.0 (M)
28	13.4 (M)	16.7 (M)	21.0 (M)	23.4 (M)	25.6 (M)	33.2 (M)	38.0 (M)	44.7 (M)	46.2 (M)	85.0 (M)

NI: Not Identified
*: Misdiagnosed Cases