

## An application of total serum protein, serum gamma glutamyltransferase and serum glutaraldehyde coagulation test as markers of passive immunity transfer in neonatal Brahman calves.

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### Abstract

Newborn calves acquire passive maternal immunity through the ingestion of colostrums. Inadequate colostrums ingestion will result in failure of passive immunity transfer (FPT). The purpose of this study is applying the use of total serum protein (TSP), serum gamma glutamyl transferase enzyme (GGT) and serum glutaraldehyde coagulation test (GCT) as markers of passive immunity transfer in neonatal Brahman calves. Blood samples from 107 calves aged between 2 – 7 days old were collected from January 2012 to April 2013. Age, gender and the dam's parity were recorded in addition with mortality from birth to weaning. The value of TSP < 5.5 g/dl as determined by refractometer, GGT level <200 U/l by Reflotron and GCT > 10 min were employed as the cutoff points of the FPT. Logistic regression was used to analyze the data. It was found that there was no correlation among successive immunity level, age and gender. In contrary calves borne from cows with parity  $\leq 4$  showed significantly higher immunity than calves from cows with the parity >4 ( $P < 0.05$ ) with odds ratio equivalent to 2.9 and 3.2 times for TSP and GGT, respectively. The results of FPT determined from 107 calves revealed 21.5%, 23.7% and 29.0% FPT by TSP, GGT and GCT respectively. Consequently, the combination of special care, treatment with antibiotics and improvement of farm managements in the separated FPT calves for provided effectively benefit to reduce the calves mortality rate to 0% by the end of the study period. In conclusion TSP, GGT and GCT could be applied for health care and reduce losses in neonatal calves.

**Keywords :** newborne Brahman calves, passive immunity transfer, total serum protein, gamma glutamyltransferase enzyme, glutaraldehyde coagulation test

## Introduction

Calves are born without circulating Immunoglobulin (Ig) because the bovine syndesmochorial type placenta does not allow transferring of macromolecule, including Ig, from the dam to fetus in uterus (Barrington, 2001). The newborn calves, therefore, acquire passive immunity by ingesting maternal immunity from colostrums and absorbing via intestine within 24 to 36 hours after birth (Stott et al., 1979; Matte et al., 1982). This passive immunity will last up to 2 months after birth (Logan, 1994), and protect newborn calves from natural infections such as diarrhea, respiratory diseases, joint ill or navel ill, until they are capable of making their own antibodies (McGuire et al., 1976; Besser and Gay, 1985; Wittum and Perino, 1995). If adequate colostrums cannot be ingested, failure of passive transfer (FPT) occurs and put calves at significant risk of infection before weaning (Caldow et al., 1988; McGuirk and Ruegg, 2011). Negative health effects can continue into the feeding period (Wittum and Perino, 1995). The assessments of passive immune status to identify newborn calves with FPT for providing preventive care is one method for preventing neonatal losses due to FPT (Morrill and Tyler, 2012).

Colostrum contains three types of immunoglobulin: IgG (subtype IgG1 and IgG2) IgM and IgA, where IgG represents 80% of the total (Kehoe et al., 2007). As there is significant correlation between Ig concentration in colostrums and in calves serum (Burton et al., 1989; Ježek et al., 2010), sera IgG concentration in new borne calves is commonly used to estimate passive immunity transfer from their dam (Morrill and Tyler, 2012). Calves with serum IgG concentration  $\geq 1,000$  mg/dl are considered to have adequate passive transfer (NAHMS, 1992; Tyler et al., 1996). From 24 hours up to 7 days is time limited to measure IgG without any change in their level (Basoglu et al., 1999; Walder and Rosengren, 2009). There are many methods, both direct and indirect, to assess serum IgG (Weaver et al., 2000). Radial Immunodiffusion test (RID) and the enzyme-linked immunosorbent assay (ELISA) are the only tests that directly measure serum IgG concentration. However, these tests are costly, take time, equipment and technical expertise (Tyler et al., 1996; Basoglu et al., 1999). Therefore, the indirect methods which cheaper and more rapid are commonly used. These included total serum protein (TSP) determination by refractometry, measurement of serum gamma glutamyltransferase (GGT) activity and serum glutaraldehyde coagulation test (GCT). The study by Güngör et al. (2004) and Ježek et al. (2010) showed a good correlation between TSP and IgG in serum. Calves that have TSP concentration  $\geq 5.5$  g/dl are considered to have adequate passive transfer (Tyler et al., 1996). The enzyme GGT is present in colostrums in high concentration and is absorbed like IgG with passive transfer. Therefore, it can be used for indirect estimation of colostrums supply (Bostedt, 1983). In addition, there was a statistically correlation between serum IgG concentrations and serum GGT activities (Güngör et al., 2004). The concentration of GGT level above 200 U/l is considered to give a good immune status (Perrino et al., 1993; Ježek et al., 2010). Serum glutaraldehyde coagulation test (GCT) is commonly used to measure IgG in foal (Lewis, 1995) as well as in calves (Tennant et al., 1979). Significant correlation between IgG1 and GCT was demonstrated by Baggar and Eriksen (2003).

The objective of this study was to assess passive immune status of neonatal Brahman calves by 3 indirect parameters, namely serum TSP, GGT and GCT. Additional studies were performed to evaluate the factors influencing their passive immune status.

## Materials and methods

### Study Site

This study was conducted in a government livestock breeding station in Khonkaen province where the cattle was left grazing freely on the pasture. From 2010 to 2011, 6.09% out of 200 calves of this station got sick and died. Navel ill (omphalitis) and diarrhea caused by *Streptococcus* spp., *Arcanobacterium pyogenase* and *Escherichia coli*, were common causes.

### Samples Collection

The period of this study took 16 months long from January 2012 to April 2013. Blood samples were collected from 107 newborn Brahman calves, aged between 2–7 days, by jugular vein puncture using monovette syringe. Age (day), gender of these calves, the dam parity and the mortality rate of calves during the study were recorded.

Serum was separated by centrifugation of blood samples at 4,000 rpm for 10 minutes and stored at -20°C until used.

### Test methods

#### Total serum protein concentration by Refractometer

Total serum protein (TSP) concentrations were determined by refractometer (N.O.W., Japan). The cutoff value was 5.5 g/dl (Tyler et al., 1996; Baggar and Eriksen, 2003). Calves with serum GGT concentration <5.5 g/dl were considered to have FPT.

#### Serum GGT activity

Serum GGT activity was determined by reflectance photometry method using commercial Reflotron strip (Roche). The cutoff value was 200 U/l (Perino et al., 1993; Ježek et al., 2010). Calves with serum GGT concentration <200 U/l were considered to have FPT.

#### Serum GCT

A modified GCT, that commonly used to determined IgG in foal (Lewis, 1995), was applied to determine the passive immunity transfer of neonatal Brahman calves. Briefly, 25% of glutaraldehyde was diluted to 10% glutaraldehyde by 0.9% normal saline. Put 50 µL of 10% glutaraldehyde in 1 dram vial with screw caps. After that added 0.5 ml of tested serum and mixed well, then observed “gelification” forming time. The cutoff time for FPT was more than 10 minutes, which meant that IgG level was less than 800 mg/dl.

## Data analysis

Descriptive analysis was done by SPSS statistical software 17.0. Furthermore, Logistic regression was performed by the same program to find out the association among age, gender of calves and parity of their dams with their passive immunity level. McNemar Chi-square analysis and Kappa value were employed to find out the significance and strength of agreement among those 3 methods.

## Results

Descriptive analysis from 107 calves used in this study showed more or less the same average in age and parity with no statistic significant by gender. The number of female and male calves were 54 and 53, of which the average age were 3.3 and 3.0 days respectively. The number of calves from parity 1-6 were 7, 24, 10, 34, 24 and 8, respectively.

The percentage of FPT calves determined by those 3 methods namely serum TSP, GGT and GCT were 21.5, 23.7, and 29.0 % respectively (Table. 1).

Table 1. Classification of passive immunity transfer in calves by 3 assessments

| Method  | No. of tested calves | % of Successive Passive Transfer<br>(no. of SPT/total) | % of Failure of Passive Transfer<br>(no. of FPT/total) |
|---------|----------------------|--------------------------------------------------------|--------------------------------------------------------|
| TSP     | 107                  | 78.5(84/107)                                           | 21.5(23/107)                                           |
| GGT     | 97                   | 76.3(74/97)                                            | 23.7(23/97)                                            |
| GCT     | 107                  | 71.0(76/107)                                           | 29.0(31/107)                                           |
| Average |                      | 75.2                                                   | 24.8                                                   |

There were no association among immunity level, sex and age. Furthermore, calves born from cows with parity 4 or less were 2.9 and 3.2 times more likely to have sufficient immunity level compared to the calves borne from cow with higher parities when measured by TSP concentration by refractometer ( $P<0.05$ ) and serum GGT activity ( $P<0.05$ ), respectively (Table 2, 3 ).

Table 2. Association between immunity level (TSP>5.5) and parity, age and sex.

| Factor           | P     | Odds ratio |
|------------------|-------|------------|
| Parity 4 or less | <0.05 | 2.89       |
| Age              | >0.05 | 1.30       |
| Sex              | >0.05 | 0.55       |

Table 3. Association between immunity level (GGT&gt;200) and parity, age and sex.

| Factor           | P     | Odds ratio |
|------------------|-------|------------|
| Parity 4 or less | <0.05 | 3.20       |
| Age              | >0.05 | 0.92       |
| Sex              | >0.05 | 0.43       |

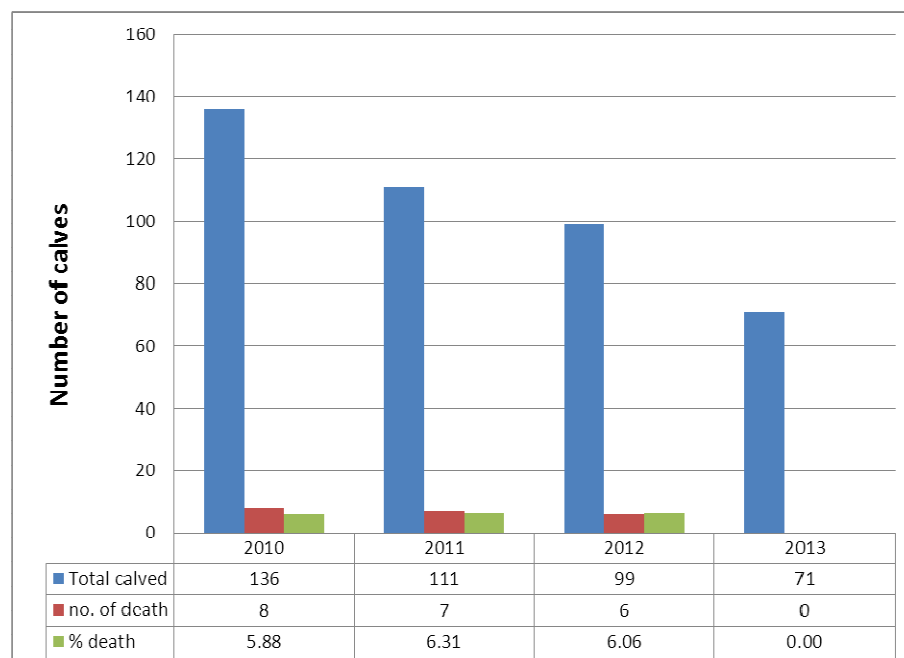
There were slightly agreements of those three tests. The Kappa value for diagnostic of successive passive immunity transfer, using activities of GGT vs TSP, GGT vs modified GCT and TSP vs modified GCT, were 0.392, 0.281 and 0.508, respectively (Table 4).

Table 4. Measurement of agreement of GGT, TSP and GCT

| Test method         | McNemar test | Kappa |
|---------------------|--------------|-------|
| GGT vs TSP          | P>0.05       | 0.392 |
| GGT vs modified GCT | P>0.05       | 0.281 |
| TSP vs modified GCT | P>0.05       | 0.508 |

After providing special care for calves with FPT parallel with improvement of farm management, the calves' mortality rate was decrease to 0% at the end of the study (Figure 1).

Figure 1. Calves mortality rate between the years 2010 – April 2013



## Discussion

In this study, there were no significant difference between age, and parity by gender among those 107 neonatal Brahman calves. The assessment of passive immune status of these calves by TSP concentration by refractometer, serum GGT activity and serum GCT revealed that 21.5, 23.7 and 29.0 % of the calves had FPT respectively (Table 1). In Thailand so far there was no other report on prevalence of FPT in neither beef calves nor dairy calves to compare with these figures. However, the study by Walder and Rosengren (2009) showed that 6% of beef calves in Alberta and Saskatchewan had FPT ( $\text{IgG} \leq 8 \text{ g/L}$ ) and 10% had marginal passive transfer ( $\text{IgG} \leq 16 \text{ g/L}$ ). The National Animal Health Monitoring Systems reported that 41% of dairy calves in USA had FPT (NAHMS, 1992) while the study of prevalence of FPT in US newborn heifer calves by Beam et al. (2009) revealed that 19.2% of US dairy heifer calves had FPT. Therefore, the percentage of neonatal Brahman calves that had FPT in this study showed more or less within the same level as in those studies. According to Larson and Tyler (2005), if the FPT proportion exceeds 15%, FPT may be the important contributing factor to a calf health problem. As a result, from 2010 to 2013 around 6% of the neonatal calves in this study got sick and died of infections (Figure 1). The only factor that may influence the passive immunity transfer in this study was parity. The calves born from older dams that their parity greater than 4 had 2.2 to 3.2 times less immunity than calves born from younger dams with lower parity (Table 2, 3). During the study we noticed that teats of younger Brahman dams were smaller than the older one with higher parity especially after calving period. For a beef calf, to consume adequate amounts of colostrums, the dam must have teat that can be grasped by the calf. Oversized teat was the obstacle of calves borne from the older dams to suckle the cow's milk and lead to inadequate colostrums intake and low calf serum IgG concentration (Larson and Tyler, 2005).

The test agreement among those 3 methods employed in this study was shown in Table 4. Although McNemar test indicated significant agreement of these tests, the Kappa value was too low to select individually and should be assessed in combination. However, the measurement of TSP by refractometer is the most reliable for herd screening, based on a reviewed by Weaver et al. (2000). The sensitivity and specificity of the test (5.5 g/dL cut off) were 0.94 and 0.74 respectively (Tyler *et al.*, 1996). The advantages of this method are simple, rapid and inexpensive that can be easily performed by practitioner on farm as a part of calf health investigation (Morrill and Tyler, 2012). Furthermore, serum GGT activity can be used as a reliable criterion for the determination of passive transfer status in newborn calves (Güngör et al., 2004; Pekcan et al., 2013). The sensitivity and specificity of the test (200 unit of GGT/L cut off) were 0.80 and 0.97 respectively (Perino et al., 1993). However, the assay method of GGT activity requires laboratory equipments which is a limiting factor of on-farm application.

The retrospective study indicated that calve mortality rate from the year 2009 to 2012 was between 5.9-6.0 %. This rate decreased to 0 % in the year 2013 (Figure1). During the

study period, started in January 2012, improvement of farm management was performed. These included providing calving stall for the high pregnant cows in state of calving in pasture, cleaned up and disinfected calving stalls, regularly disinfected newborn calves' umbilicus and bottle fed dams' colostrums to her calves in addition to udder feeding. Besides that, calves identified as low passive immunity and FPT were separated for providing special care and treatment with antibiotics. This combination showed effectively benefit to reduce the calves mortality rate by the end of the study period.

## Conclusion

The objective of this study was to assess passive immune status of neonatal Brahman calves by 3 indirect tests namely total serum protein (TSP) concentration by refractometer, serum GGT activity and serum glutaraldehyde coagulation test (GCT). Brahman calves showed failure of passive transfer between 21.5 – 29.0% (in average=24.8%). Age (1-7days old) and gender showed no significantly associated with the immune level. The calves borne from cows with higher parity (> 5) showed lower successive passive immunity. However, all 3 methods so far should be done together to confirm the successive immunity status. Otherwise TSP>5.5 g/dl by refractometer is one of the screening choice to define the calves passive immunity status.

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## การประยุกต์ใช้ค่าซีรัมโปรตีนรวม ค่าซีรัมเอนไซม์แกมมากลูตามิลทรานสเฟอเรส และ การทดสอบการตกตะกอนซีรัมด้วยสารกลูตาไรลดีไฮด์ เป็นค่าสังเกตของระดับ ซีรัมอิมมูโนโกลอบบูลินในลูกโคברהมันแรกเกิด

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### บทคัดย่อ

ลูกโคแรกเกิดจำเป็นต้องได้รับนม น้ำเหลืองซึ่งอุดมด้วยภูมิคุ้มโรคอย่างเพียงพอ การที่ลูกสัตว์ได้รับนม น้ำเหลืองน้อย ส่งผลให้ลูกสัตว์มีภูมิต้านทานโรคต่ำ อ่อนแอ ติดโรคน่าย การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อ ประยุกต์ใช้ค่าซีรัมโปรตีนรวม (TSP) ค่าซีรัมเอนไซม์แกมมากลูตามิลทรานสเฟอเรส (GGT) และระดับ IgG ด้วย วิธีการตกตะกอนซีรัมด้วยสารกลูตาไรลดีไฮด์ (GCT) มาเป็นค่าสังเกตเพื่อประเมินการได้รับนม น้ำเหลืองจากแม่ซึ่ง จะสะท้อนระดับภูมิคุ้มกันโรคของลูกโคברהมันแรกเกิด โดยการเก็บตัวอย่างเลือดลูกโคเมื่อברהมันพันธุ์แท้ ช่วงอายุ 2-7 วันหลังคลอด จำนวนทั้งสิ้น 107 ตัว ระหว่างเดือนมกราคม 2555 ถึงเมษายน 2556 บันทึกประวัติ ลูกโคได้แก่ อายุ เพศ ลำดับลูกที่คลอดของแม่โค และอัตราการตายของลูกโคอายุตั้งแต่แรกคลอดจนถึงหย่านม ทำการวัดระดับ TSP ด้วยเครื่อง Refractometer วัดระดับ GGT ด้วยเครื่อง Reflotron และวัดระดับ IgG ด้วย วิธี GCT โดยระยะเวลาในการฟอร์มเจลหลังเติมน้ำยาทดสอบ ประเมินระดับภูมิคุ้มกันโรคของลูกโคที่ได้รับจาก แม่ โดยถือเกณฑ์การวัดที่ต่ำกว่ามาตรฐาน 3 ชนิด ได้แก่ ระดับ TSP ที่น้อยกว่า 5.5 g/dl ระดับ GGT ที่น้อยกว่า 200 U/L และระยะเวลาในการฟอร์มเจลหลังเติมน้ำยาทดสอบ GCT มากกว่า 10 นาที ในการตัดสินใจว่าลูก โคได้รับภูมิคุ้มกันโรคจากแม่ไม่เพียงพอ ทำการวิเคราะห์ข้อมูลทางสถิติโดยใช้ Logistic regression เพื่อหา ความสัมพันธ์ระหว่างปัจจัยได้แก่ อายุ เพศ และลำดับลูกที่คลอดของแม่โค กับระดับภูมิคุ้มกันโรคของลูกโคที่ ได้รับจากแม่ ผลการศึกษาพบว่าระดับภูมิคุ้มกันโรคไม่มีความสัมพันธ์กับอายุ และเพศ แต่มีความสัมพันธ์กับลำดับ ลูกที่คลอดของแม่โคอย่างมีนัยสำคัญทางสถิติ เมื่อประเมินจากค่าซีรัมโปรตีนรวม และค่าซีรัม GGT ( $P < 0.05$ ) โดยลูกโคที่เกิดเป็นลำดับที่ 4 หรือน้อยกว่ามีระดับภูมิคุ้มกันโรคที่ได้รับจากแม่ในระดับเพียงพอจำนวนสูงกว่าใน ลูกโคที่เกิดในลำดับที่สูงกว่า 4 ถึง 2.9 และ 3.2 เท่าตามลำดับ (odds ratio=2.9 และ 3.2 ตามลำดับ) ผลการ ประเมินจากลูกโคทั้งหมด 107 ตัว พบลูกโคที่มีระดับภูมิคุ้มกันโรคอยู่ในเกณฑ์ต่ำกว่ามาตรฐาน เมื่อวัดจากค่า TSP, GGT และ GCT ร้อยละ 21.5, 23.7 และ 29.0 ตามลำดับ เมื่อทำการแก้ไขโดยการแยกลูกโคที่มีระดับ ภูมิคุ้มกันโรคไม่เพียงพอออกมาดูแลเป็นพิเศษร่วมกับการให้ยาปฏิชีวนะ และการปรับปรุงการจัดการฟาร์ม ช่วย ให้อัตราการตายของลูกโคลดลงเหลือเป็นศูนย์เมื่อสิ้นสุดการศึกษาครั้งนี้ ผลจากการศึกษาครั้งนี้สรุปได้ว่า ค่า TSP, GGT และ GCT สามารถนำมาประยุกต์ใช้เป็นค่าสังเกตสำหรับดูแลสุขภาพ และลดการสูญเสียลูกโคแรก เกิดได้

**คำสำคัญ :** ลูกโคברהมันแรกเกิด ภูมิคุ้มกันโรคที่ได้รับจากแม่ ซีรัมโปรตีนรวม ซีรัมเอนไซม์แกมมากลูตา มิลทรานสเฟอเรส การตกตะกอนซีรัมด้วยสารกลูตาไรลดีไฮด์