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## **Prevalence of failure of passive transfer of immunoglobulins in Holstein calves in Northern Greece and association with management practices**

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## **Συχνότητα εμφάνισης της ελλιπούς μεταφοράς ανοσοσφαιρινών σε μόσχους φυλής Holstein στη Βόρεια Ελλάδα και συσχέτισή της με διαχειριστικά πρότυπα**

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**ABSTRACT.** Objectives of the present study were to estimate prevalence of failure of passive transfer of immunoglobulins in dairy calves in Northern Greece and to investigate factors potentially associated with it. Four hundred and thirty seven clinically healthy calves in 30 farms were included in the study. Age of calves was 18 h to 7 d. Animals were blood sampled and serum total protein concentrations were measured by a refractometer. Two thresholds of total protein concentration were used: 5.2 or 5.5 g dL<sup>-1</sup>. At calf level, an animal was considered to have failure of passive transfer of immunoglobulins when total protein concentrations were lower than the above thresholds. At herd level, a herd was considered to have failure of passive transfer of immunoglobulins when >20% of sampled calves had total protein concentration was <5.2 or <5.5 g dL<sup>-1</sup>. Moreover, data on health management on the farm were collected in a purpose-built questionnaire. At 5.2 g dL<sup>-1</sup>, 20% of the calves and 40% of the herds were considered to have failure of passive transfer of immunoglobulins; when the 5.5 g dL<sup>-1</sup> threshold was used, respective prevalences were 26% and 53%. At herd level, mean blood serum total protein concentration tended to be positively affected by a short interval between birth and first colostrum meal, by maintenance of a stock of frozen colostrum and by establishment of a close-up group of dry cows. At calf level, the same factors had a statistically

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significant positive effect on blood serum total protein concentration. Moreover, quantity of colostrum received by calves and colostrum condition were also positively related with blood serum total protein concentration. In conclusion, failure of passive transfer of immunoglobulins is a common problem in Holstein calves in Northern Greece. Increased prevalence of the problem implies that increased efforts and management practices need to be applied to ensure the adequate transfer of maternal immunoglobulins to newborn calves. Also, it becomes obvious from all the above findings that many farmers are not well informed for management practices that have to implement to ensure adequate amounts of immunoglobulins to newborn calves. Hence, dissemination of knowledge concerning best management practices for achieving adequate passive immunity is considered to be of significant importance.

**Keywords:** colostrum, dairy calves, Greece, immunoglobulins, management practices, passive immunity, total proteins.

**ΠΕΡΙΛΗΨΗ.** Η παρούσα έρευνα αποσκοπούσε στη διερεύνηση της συχνότητας εμφάνισης ελλιπούς παθητικής μεταφοράς ανοσοσφαιρινών σε μόσχους στη Βόρεια Ελλάδα και στη συσχέτισή της με πιθανούς παράγοντες κινδύνου. Συλλέχθηκαν δείγματα αίματος από 437 κλινικά υγιείς μόσχους, ηλικίας >18 ωρών μέχρι 7 ημερών, σε 30 εκτροφές, στα οποία προσδιορίστηκε με διαθλασίμετρο η συγκέντρωση ολικών πρωτεϊνών στο ορό του αίματος. Στη μελέτη χρησιμοποιήθηκαν δύο διαφορετικά όρια: 5,2 και 5,5 g dL<sup>-1</sup>. Ένας μόσχος θεωρήθηκε ότι είχε έλλειψη επαρκούς παθητικής ανοσίας όταν η συγκέντρωση ολικών πρωτεϊνών ήταν μικρότερη από τα παραπάνω όρια. Μία εκτροφή θεωρήθηκε πως αντιμετώπιζε πρόβλημα ελλιπούς παθητικής μεταφοράς ανοσοσφαιρινών όταν >20% από τους μόσχους στους οποίους είχε γίνει δειγματοληψία, είχε συγκεντρώσεις ολικών πρωτεϊνών <5,2 ή <5,5 g dL<sup>-1</sup>. Επιπλέον, δεδομένα για τα ακολουθούμενα πρότυπα διαχείρισης της υγείας στην εκτροφή συγκεντρώθηκαν σε σχετικό ερωτηματολόγιο, μέσω συνέντευξης με τους κτηνοτρόφους. Με όριο το 5,2 g dL<sup>-1</sup>, 20% των μόσχων και 40% των εκτροφών παρουσίασαν ελλιπή παθητική μεταφορά ανοσοσφαιρινών, ενώ με όριο το 5,5 g dL<sup>-1</sup> τα αντίστοιχα ευρήματα ήταν 26% και 53%. Σε επίπεδο εκτροφής, η μέση συγκέντρωση ολικών πρωτεϊνών έτεινε να επηρεαστεί θετικά από την έγκαιρη χορήγηση πρωτογάλακτος, από τη διατήρηση κατεψυγμένων αποθεμάτων πρωτογάλακτος και από την ύπαρξη ξεχωριστής ομάδας αγελάδων στις τελευταίες 3 εβδομάδες της ξηράς περιόδου. Σε ατομικό επίπεδο, οι παραπάνω παράγοντες είχαν σημαντική θετική επίδραση στη συγκέντρωση των ολικών πρωτεϊνών. Ακόμη, η ποσότητα του χορηγούμενου πρωτογάλακτος και η υγιεινή του κατάσταση παρουσίασαν θετική συσχέτιση με τη συγκέντρωση των ολικών πρωτεϊνών. Συμπερασματικά, η ελλιπής παθητική μεταφορά ανοσοσφαιρινών σε μόσχους φυλής Holstein είναι συχνή στις εκτροφές της Β. Ελλάδας. Είναι εμφανές από τα αποτελέσματα της έρευνας πως πολλοί κτηνοτρόφοι δεν είναι σωστά ενημερωμένοι για τα διαχειριστικά πρότυπα που πρέπει να εφαρμοστούν ώστε να διασφαλιστεί η επαρκής παθητική μεταφορά ανοσοσφαιρινών στους μόσχους. Ως εκ τούτου, κρίνεται ιδιαίτερα σημαντική η μεταφορά γνώσης προς τους κτηνοτρόφους σχετικά με την εφαρμογή των ορθών διαχειριστικών προτύπων και ενεργειών που θα οδηγήσουν στην ελάττωση ή εξάλειψη του προβλήματος.

**Λέξεις ευρετηρίασης:** ανοσοσφαιρίνες, διαχειριστικά πρότυπα, Ελλάδα, έλλειψη παθητικής ανοσίας, μόσχοι, ολικές πρωτεΐνες, πρωτόγαλα.

## INTRODUCTION

In ruminants, failure of passive transfer of immunoglobulins from the dam to the newborn animal predisposes neonates to various diseases (Weaver et al., 2000). There is a recognized association between calf morbidity/mortality and reduced maternal immunoglobulin transfer to neonatal calves (Paré et al., 1993; Donovan et al., 1998; Virtala et al., 1999). Poor performance, as well as increased morbidity/mortality in calves result in higher production costs and reduced profitability for the dairy industry (Trotz-Williams et

al., 2008). Therefore, it is important to ensure that dairy calves receive an adequate quantity of good-quality (high IgG1) colostrum within the first hours of life, in order to facilitate optimal passive transfer of maternal Ig from dam to calf (Morin et al., 1997; Jaster, 2005).

Passive transfer of immunoglobulins in calves may be assessed by several methods, among these direct measurement of serum IgG by ELISA or radial immunodiffusion (Filteau et al., 2003) and estimation of serum IgG concentration by measuring blood serum total protein concentration using refractometry (Wallace et al., 2006). The last method has been

reported to be a reliable screening test for diagnosis of failure of passive transfer in calves (Calloway et al., 2002, McGuirk and Collins, 2004). Blood serum total protein concentration  $\geq 5.2$  g dL<sup>-1</sup> in healthy, well-hydrated calves or  $\geq 5.5$  g dL<sup>-1</sup> in clinically ill calves is considered a measure of adequate passive transfer of immunoglobulins (McBeath et al., 1971; Tyler et al., 1996; Tyler et al., 1999; Weaver et al., 2000).

Although importance of colostrum has been appreciated for almost 100 years (Crowther and Raistrick, 1916), failure of transfer of immunoglobulins is a common problem amongst dairy calves (Davidson et al., 1981; Hancock, 1985; Besser et al., 1991; Tyler et al., 1998). In the USA, ~35% of dairy calves are estimated with failure of passive transfer of immunoglobulins (Stott et al., 1979; Brignole and Stott, 1980), a fact that makes the problem of great significance for cattle farmers. To date, several reports have been published on factors associated with the acquisition of passive immunity in newborn calves in the United States (Besser et al., 1991; Perino et al., 1995; Quigley et al., 2001) or Canada (Filteau et al., 2003).

Information regarding failure of passive transfer of immunoglobulins in Greece is limited. Objectives of the present study were to estimate prevalence of failure of passive transfer of immunoglobulins in Holstein dairy calves in Northern Greece and to investigate factors potentially associated with it.

## MATERIALS AND METHODS

### Farms, samplings and techniques

A cross-sectional study was conducted in 30 dairy farms in Northern Greece. Four hundred and thirty seven (437) Holstein calves, varied from 12 to 22 calves from each farm, were overall enrolled. Each herd was visited once by the same author (EK) between January and May, 2009.

On each visit, clinical examination was performed on each calf and animals found unhealthy were not included in the study. A blood sample was collected from all calves aged 18 hours (which had received colostrum >6 hours earlier) to 7 days at the time of the visit. Samples were collected within 30 min to 2 h after a colostrum or milk meal.

Blood samples were collected from the jugular vein, by using 21¼ G disposable needles, into 10 mL plain glass vacuum tubes (BD Vacutainer®; Plymouth, United Kingdom) with no anticoagulant. Samples were placed in a cooler immediately after collection and refrigerated at 4 °C. Serum was separated by low speed centrifugation (1600 g for 15 min) within 24 hours after collection. Total protein concentration was determined with a desktop refractometer (ATAGO T2-NE CLINICAL, Atago Ltd, Tokyo, Japan), in accordance with manufacturers' instructions.

### Data management

During the study, two different serum total protein thresholds were employed to evaluate potential failure of passive transfer of immunoglobulins: 5.2 or 5.5 g dL<sup>-1</sup>. At calf level, an animal was considered to have failure of passive transfer of immunoglobulins, when blood serum total protein concentration was <5.2 or <5.5 g dL<sup>-1</sup>. At herd level, a herd was considered to have problems with failure of passive transfer of immunoglobulins, when >20% of calves sampled had serum total proteins <5.2 or <5.5 g dL<sup>-1</sup>, as used for individual calves.

Data on colostrum and dry cow management were collected completing a purpose-built questionnaire, by means of interviews and observations by the above author (EK). The following data were included: mode of colostrum administration, quantity of colostrum received by calves, interval between birth and first colostrum meal, administration of colostrum from heifers, maintenance of a stock of frozen colostrum, administration of a colostrum supplement, colostrum administrator, colostrum conditions, equipment condition, interval between birth and second colostrum meal, establishment of a close-up group of dry cows and duration of dry period. Based on above information, farms were allocated in a cluster as per model description below and all calves on that farm were included in the farm cluster.

To estimate the effect of each of the above factors on mean total protein concentration (both at herd and calf level) and on classifying calves in a failure of passive transfer of immunoglobulins or not by using the above two thresholds (5.2 or 5.5 g dL<sup>-1</sup>).

A general linear model (univariate analysis

of variance) was used in SPSS 21.0. Factors were included in the model as categorical variables, as follows: (a) mode of colostrum administration (binary, hand-feeding or sucking), (b) quantity of colostrum received by calves (binary,  $\leq 1.5$  L or  $>1.5$  L), (c) interval between birth and first colostrum meal (1:  $<2.5$  h, 2: 2.5-5 h or 3:  $>5$  h), (d) administration of colostrum from heifers (binary, yes or no), (e) maintenance of a stock of frozen colostrum (binary, yes or no), (f) administration of a colostrum supplement (binary, yes or no), (g) colostrum administrator (binary, farmer or worker), (h) colostrum condition (1: good, 2: moderate or 3: bad), (i) equipment condition (1: good, 2: moderate or 3: bad), (j) interval between birth and second colostrum meal (binary,  $\leq 12$  h or  $>12$  h), (k) establishment of a close-up group of dry cows (binary, yes or no) and (l) duration of dry period (1:  $<45$  d, 2: 45-60 d or 3:  $>60$  d).

Significance level was set at  $P < 0.05$ .

## RESULTS

Using blood serum total protein concentration of  $5.2 \text{ g dL}^{-1}$  as threshold, 87 of 437 calves (20%) and 12 of 30 (40%) herds were found to have failure of passive transfer of immunoglobulins. When the  $5.5 \text{ g dL}^{-1}$  threshold was used, prevalence of failure of passive transfer of immunoglobulins increased to 26% and 53%, respectively.

Total protein concentrations of calves ranged from  $4.0$  to  $8.4 \text{ g dL}^{-1}$  (mean:  $6.21 \text{ g dL}^{-1}$ ; median:  $6.18 \text{ g dL}^{-1}$ ). In each farm, number of calves with total concentration  $<5.2 \text{ g dL}^{-1}$  ranged from 0 (4 farms) to 8 (1 farm) calves; when the threshold of  $5.5 \text{ g dL}^{-1}$  was used, the respective number was 0 (2 farms) to 10 (1 farm) calves (Table 1).

Associations of management practices and prevalence of failure of passive transfer of immunoglobulins were as follows. At farm level, mean blood serum total protein concentration tended to be positively affected by a short interval between birth and first colostrum meal, by maintenance of a stock of frozen colostrum and by establishment of a close-up group of dry cows ( $P = 0.082$ ,  $P = 0.054$ ,  $P = 0.086$ , respectively;  $R^2 = 0.878$ ). At calf level, the same factors had a statistically significant positive effect on blood serum total protein concentra-

tion ( $P = 0.001$ ,  $P = 0.001$ ,  $P = 0.006$ , respectively;  $R^2 = 0.980$ ). Moreover, quantity of colostrum received by calves and colostrum condition were also positively related with blood serum total protein concentration ( $P = 0.072$ ,  $P = 0.068$ , respectively). Short interval between birth and first colostrum meal and maintenance of a stock of frozen colostrum also had a significant positive effect on calves with no failure of passive transfer of immunoglobulins when either threshold ( $5.2 \text{ g dL}^{-1}$  or  $5.5 \text{ g dL}^{-1}$ ) was employed ( $P = 0.027$ ,  $P = 0.049$ , respectively;  $R^2 = 0.960$  or  $P = 0.024$ ,  $P = 0.026$ , respectively;  $R^2 = 0.949$ ).

## DISCUSSION

Failure of passive transfer of immunoglobulins in dairy herds puts calves at increased risk for development of various diseases. Even clinically healthy animals, in which failure of passive transfer of immunoglobulins was noted, can shed increased numbers of pathogens (McGuirk, 2010). Calves with severe failure (blood serum concentration of total proteins  $<4.5 \text{ g dL}^{-1}$ ) were found to be around 10 times more likely to die in the first 100 days of life than were calves with blood serum concentration of total proteins  $>5.5 \text{ g dL}^{-1}$  or  $\leq 6 \text{ g dL}^{-1}$  (Tyler et al., 1999). Moreover, the potential long-term effects should not be overlooked. Average daily gain was lower and post-weaning mortality rate was higher in dairy heifers with failure of passive transfer of immunoglobulins at calthood (Robison et al., 1988). Also, such heifers had lower mature equivalent milk production during the 1st lactation (DeNise et al., 1989).

Herd-based assessment of passive immunity is different than testing individual calves and focuses on the proportion of calves with reduced immunoglobulin blood concentration. Accurate conclusions require a discriminating test, an appropriate sample size and a suitable population of calves to test (McGuirk and Collins, 2004).

Measurement of blood total protein concentration is practical and well-priced and has been validated for estimating passive immunity at herd level (McBeath et al., 1971; Tyler et al., 1996; 1999; Weaver et al., 2000). Immediacy of results permits practitioners to provide feedback to farmers and

**Table 1.** Detailed results of calves sampled for failure of passive transfer of immunoglobulins in 30 dairy farms in Northern Greece; two thresholds were employed for blood serum total proteins concentrations to indicate the problem:  $<5.2 \text{ g dL}^{-1}$  or  $<5.5 \text{ g dL}^{-1}$ .

arm	No of calves sampled	No of calves with blood serum total proteins concentration $<5.2 \text{ g dL}^{-1}$	No of calves with blood serum total proteins concentration $<5.5 \text{ g dL}^{-1}$
1	13	2	2
2	13	4	5
3	12	7	8
4	15	2	3
5	15	2	3
6	14	1	3
7	14	3	3
8	13	2	2
9	16	4	6
10	15	4	6
11	14	1	1
12	13	2	2
13	17	6	9
14	18	8	10
15	14	0	1
16	14	7	8
17	15	1	2
18	12	2	2
19	13	2	4
20	11	0	0
21	12	4	4
22	13	1	2
23	13	0	0
24	22	0	1
25	16	2	3
26	15	2	4
27	13	3	3
28	21	6	7
29	16	6	6
30	15	3	5
total	437	87	115



make clinical decisions regarding necessity of targeted intervention in individual calves, as well as in management practices leading to the problem at herd-level (Tyler et al., 1996).

Measurement of serum total protein by a refractometer for indirect serum immunoglobulin concentration estimation was first proposed by McBeath and co-workers in 1971. They demonstrated that blood serum protein measurement by a refractometer had good correlation with blood serum immunoglobulin concentration measured by radial immunodiffusion. In another study, it was shown that a blood serum protein concentration of  $5.2 \text{ g dL}^{-1}$  was equivalent to IgG concentration of  $1,000 \text{ mg dL}^{-1}$ ; a cut-off point of  $5.5 \text{ g dL}^{-1}$  may be preferable in clinically ill calves (Tyler et al., 1999); on healthy, adequately hydrated calves, blood serum total protein of  $\geq 5.2 \text{ g dL}^{-1}$  is associated with adequate passive transfer (Tyler et al., 1996).

A sample size of 12 calves provides adequate confidence level in the results. In a small herd, test results can be delayed until 12 calves would have been tested. Using an established cut-point of  $5.5 \text{ g dL}^{-1}$  blood serum protein concentration, the proportion of calves below the cut-point determines the adequacy of passive transfer of immunoglobulins. An alarm level is set at 20%; however, if the result is around that, more calves should be tested, in order to increase power of the procedure. Herd-based testing for failure of passive transfer of immunoglobulins serves as the basis for troubleshooting calf health problems (McGuirk and Collins, 2004).

Prevalence of failure of passive transfer of immunoglobulins in the USA was found to be >40% (USDA, 1993), although later it declined. In a large-scale study conducted in 2007, 1816 healthy dairy calves from 394 dairy operations in 17 states were sampled; the results showed that 19% of the calves had failure of passive transfer of immunoglobulins. Moreover, 41% of those farms had at least one calf with failure of passive transfer of immunoglobulins (Beam et al., 2009). In general, similar findings have also been reported from Canada (Filteau et al., 2003; Trotz-Williams et al., 2008). Results of the present study indicate that situation in Greece is similar to that reported elsewhere.

This high prevalence of FPT encountered in

farms in Northern Greece appears to be due to managerial deficiencies concerning colostrum composition, collection, handling, storage and feeding. Nevertheless, there were farms with good management practices, as shown by detection of no calves with failure of passive transfer of immunoglobulins in some of them.

The results of the questionnaire indicate that mean total protein concentrations were positively affected by short interval between birth and first colostrum meal, by maintenance of a stock of frozen colostrum and by establishment of a close-up group of dry cows, as well as, to a lesser degree, by quantity of colostrum received by calves and good colostrum condition and are in accord with results of previous studies reported in the literature (Weaver et al., 2000; Morin et al., 2001; McGuirk and Collins, 2004). In dairy cattle, the variability in colostrum immunoglobulin concentration and volume requires that 3 to 4 L (depending on the breed) of colostrum be given to assure that an adequate immunoglobulin mass is received by the newborns (Besser et al., 1991; Tyler et al., 1999).

Calves, which had received >1.5 L of colostrum per meal, had higher total protein concentrations and better status. Also, calves which received two meals within 12 h after birth, had higher total protein concentrations. Hence, in order to decrease prevalence of failure of passive transfer of immunoglobulins and to improve calf immunity, it was suggested to farmers to adjust colostrum feeding plan accordingly, i.e. to administer a larger quantity at an earlier stage.

Mean total protein concentrations were also positively affected by formation of a close-up group of dry cows. In farms where dry cows were divided into two groups (for earlier or later expected calving), higher total protein concentrations, and thus better passive immunity status in calves, were recorded. That way cows would be fed in an improved way, which is better suited to their needs, hence produce a higher quality colostrum. Moreover, one can suggest that application of such practices indicates improved overall management in the farm, which again is associated with improved colostrum quality.

Finally, good conditions of colostrum and equipment also has been found to have a positive effect on reducing prevalence of failure of passive

transfer of immunoglobulins to calves. Taking into account the present results, farmers were advised to improve general hygiene conditions in the farms and were also informed regarding the beneficial effects of maintaining a stock of frozen or refrigerated colostrum available for administration to calves in need.

## CONCLUDING REMARKS

In conclusion, failure of passive transfer of immunoglobulins is a common problem in Holstein calves in Northern Greece. Increased prevalence of the problem implies that increased efforts and management practices need to be applied to ensure the

adequate transfer of maternal immunoglobulins to newborn calves. Also, it becomes obvious from all the above findings that many farmers are not well informed for management practices that have to implement to ensure adequate amounts of immunoglobulins to newborn calves. Hence, dissemination of knowledge concerning best management practices for achieving adequate passive immunity is considered to be of significant importance.

## CONFLICT OF INTEREST STATEMENT

There is no conflict of interest. ■

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