ARTICLES

A case-control study of the congenital hypothyroidism and dysmaturity syndrome of foals

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Abstract

A case-control study was conducted to identify risk factors for the congenital hypothyroidism and dysmaturity syndrome of foals. A questionnaire was used during personal interviews of foal owners and farm managers to collect information on animal signalment, farm environment, and mare management. Information on 39 foals with the congenital hypothyroidism and dysmaturity syndrome were compared with 39 control foals. Foals with the syndrome had a significantly ($P < 0.0001$) longer gestation (357.6 d) than control foals (338.9 d). Pregnant mares that were fed greenfeed, did not receive any supplemental mineral, left their "home farm" during gestation, or grazed irrigated pasture had 13.1 ($P = 0.0068$), 5.6 ($P = 0.0472$), 4.3 ($P = 0.0076$), and approximately 15.3 ($P = 0.0245$), respectively, greater odds of producing an affected foal than mares not experiencing these events.

Greenfeed often contains high levels of nitrate (NO_3^-), which is known to impair thyroid gland function. In light of this, forage samples from participating farms were analyzed for nitrate levels. The odds of one or more congenitally hypothyroid and dysmature foals being born on a farm feeding forage with at least a trace of nitrate was 8.0 times greater ($P = 0.0873$) than the odds of the disease occurring on a farm that fed forage free of nitrate. Further, the odds of a mare producing an affected foal when fed forage containing at least a trace of nitrate were 5.9 times greater ($P = 0.0007$) than those of a mare fed nitrate free forage. This study suggests that congenital hypothyroidism and dysmaturity syndrome in foals may be the result of diets that contain nitrate or that are low in iodine being fed to pregnant mares.

Résumé

Étude de cas-témoins de poulains présentant le syndrome d'hypothyroïdisme et de dysmaturité congénitale

Une étude des cas a été menée pour identifier les facteurs de risques associés au syndrome d'hypothyroïdisme et de dysmaturité du poulain. Un questionnaire a été utilisé au cours d'entrevues personnelles avec des propriétaires de poulains et des gérants de ferme afin d'accumuler des renseignements sur la description des animaux, l'environnement des fermes et la gestion des juments. Les renseignements obtenus sur 39 poulains présentant le syndrome d'hypothyroïdisme et de dysmaturité congénitale ont été comparés avec 39 poulains témoins. Les poulains souffrant du syndrome avaient eu une durée de gestation significativement plus longue ($P < 0,0001$) que celle des témoins (357,6 j contre 338,9 j). Les juments en gestation qui ont été nourries avec des fourrages verts, qui n'ont reçu aucun supplément minéral, qui ont quitté leur ferme d'origine pendant la gestation ou qui ont brouté dans un pâturage irrigué ont eut respectivement 13,1 ($P = 0,0068$), 5,6 ($P = 0,0472$), 4,3 ($P = 0,0076$) et approximativement 15,3 ($P = 0,0245$) fois plus de risques de produire un poulain atteint que les juments n'ayant pas été exposées à ces événements. Le fourrage vert contient de hauts niveaux de nitrate (NO_3), reconnus pour affecter la fonction de la glande thyroïde. Ces faits étant connus, les niveaux de nitrates des échantillons de fourrage provenant des fermes participantes ont été analysés. Les risques qu'un ou plusieurs poulains souffrant d'hypothyroïdie et de dysmaturité naissent dans une ferme servant des fourrages avec au moins des traces de nitrates étaient 8,0 fois plus élevés ($P = 0,0873$) que les risques encourus dans une ferme servant des fourrages exempts de nitrates. De plus, les risques qu'une jument nourrie avec un fourrage contenant au moins des traces de nitrates donne naissance à un poulain affecté étaient 5,9 fois plus élevées ($P = 0,0007$) que ceux d'une jument nourrie avec un fourrage exempt de nitrates. Cette étude suggère que le syndrome d'hypothyroïdisme et la dysmaturité congénitale du poulain

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puisse provenir du fait que les juments aient reçu des diètes qui contenaient des nitrates ou qui étaient carencées en iode.

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Introduction

In 1981, McLaughlin and Doige (1) described a syndrome of neonatal foals that was characterized by hyperplasia of the thyroid gland and multiple congenital musculoskeletal anomalies. This syndrome has continued to be an important cause of reproductive loss and foal mortality in western Canada (2–6). Affected foals represented 4.5% of all full-term foals submitted to veterinary diagnostic laboratories in western Canada between 1980 and 1989 (5,6). The syndrome may also have been associated with additional reproductive losses in the form of abortions that went undetected (6). We have investigated farm outbreaks of this syndrome over the past 5 y, during which 30% to 100% of the foals had the disease. About 6 affected foals have also been examined at the Large Animal Clinic or Diagnostic Laboratory of the Western College of Veterinary Medicine during each foaling season since 1990. Almost all of these foals died or were euthanized (4).

The most common musculoskeletal lesions of affected foals were mandibular prognathia, osteochondrosis in the form of inappropriately ossified carpal and tarsal bones, flexural deformities of the forelimbs, and ruptured common digital extensor tendons. This syndrome was initially recognized in full-term foals and was associated with normal or prolonged gestation (1–5). Despite the length of gestation, foals with this syndrome often had signs of immaturity (7), including a short, soft (silky) coat; pliable ears; lax tendons and joints; incomplete closure of the abdominal wall; and immature carpal and tarsal bones. These foals also had marked hyperplasia of their thyroid glands and were hypothyroid (2,4). Therefore, the syndrome has been referred to as either thyroid hyperplasia and musculoskeletal deformities (TH-MSD)(5) or congenital hypothyroidism and dysmaturity (CHD)(4).

The cause of the CHD syndrome remains unknown. However, a recent study (5) suggested that an investigation into various environmental exposures, particularly feed, of the dams producing CHD foals was warranted. The purpose of this study was to identify risk factors for the development of the CHD syndrome of foals.

Materials and methods

Subjects

A case-control study was conducted using privately owned foals born in Alberta in 1993. Members of the Alberta Standardbred Horse Association, the Alberta Division of the Canadian Thoroughbred Horse Society, the Western Canadian Association of Equine Practitioners, and the Alberta Veterinary Medical Association were informed of the study. They were requested to contact the 1st author if a foal believed to be affected with the CHD syndrome was born. Attempts were then made to identify a nearby farm expecting a similar number of foals in 1993, as a source of control foals.

A foal was classified as affected if it was examined by the 1st author and found to have any 2 of the following musculoskeletal anomalies: 1) any degree of mandibular prognathism; 2) flexural deformities of the legs; 3) rupture of one or both common digital extensor tendons; or 4) incomplete ossification of the carpal or tarsal bones. Ossification of the carpal or tarsal bones was assessed using radiographs and a skeletal ossification index (8,9). The radiographs were evaluated independently of the 1st author by a specialist and a resident in veterinary radiology. The radiologists were not given any information, except for the foal's gestational age and its age at the time the radiographs were made. In addition, any thyroid gland that was available from stillborn or dead CHD foals was examined histologically for

Table 1. Summary of the independent variables investigated for association with the CHD syndrome

Abridged description of variables; possible responses
Animal signalment
Breed of dam; Arab/mixed/other/quarter horse/standardbred/Thoroughbred
Age of dam; years
Number of pregnancies dam has experienced, including 1992–93; number > 0
Dam has competed or dam was a standardbred or Thoroughbred and has raced; no/yes
Breed of foal; Arab/mixed/other/quarter horse/standardbred/Thoroughbred
Sex of foal; female/male
Length of gestation; days
Pasture
Dam kept on native grass/improved (reseeded) pasture during summer; no/yes
Pastures were irrigated/fertilized/treated with pesticide/treated with herbicide in 1992; no/yes
Diet
Dam was regularly fed hay/greenfeed/silage/other forage — during summer/during winter; no/yes
Dam was regularly fed grain (oats, barley, wheat, other)/complete horse feed — during summer/during winter; no/yes
Dam was regularly fed protein/protein-vitamin-mineral supplement — during summer/during winter; no/yes
Dam regularly had access to salt or mineral blocks/had access to loose salt or mineral/had salt or mineral added to concentrate — during summer/during winter; no/yes
Dam regularly had access to water supplied to local city/from local well/from dug-out/from stream or river/from other source — during summer/during winter; no/yes
Farm
Number of years owners/operators have been producing foals at current location; number > 0
Dam regularly had access to trees and/or bushes; no/yes
Dam regularly exposed to fences and/or buildings treated with paint or stain/creosote/diesel fuel/used motor oil; no/yes
Number of horses that came onto farm during 1992; 0/1 or 2/between 3 and 10/more than 10
Cattle/other livestock (pigs, sheep, poultry) were kept on the farm in 1992; no/yes
Dam management
Dam received ivermectin in paste for horses/ivermectin injectable for cattle given orally/other anthelmintic prior to foaling; no/yes
Dam was vaccinated against rabies/equine encephalitis/equine influenza/equine rhinopneumonitis/equine viral arteritis/tetanus/strangles/Potomac horse fever prior to foaling; no/yes
Dam was artificially inseminated/bred naturally during her last estrous period in 1992; no/yes
Dam received a drug or medication prior to foaling; no/yes
Dam was off or new to the farm during gestation; no/yes

Table 2. Characteristics and comparisons of mares and foals from affected and control farms

	Affected Farms	Control Farms	*P*-value
Total number of farms	26	23	
Total number of mares/foals	97	89	
Number of foaling mares per farm			
1	7 (26.9%)	7 (30.4%)	
2 to 5	14 (53.8%)	10 (43.5%)	
6 to 10	4 (15.4%)	4 (17.4%)	
11 to 15	1 (3.8%)	2 (8.7%)	
mean (*s*)	3.7 (2.8)	3.9 (3.7)	0.8822[a]
Age of foaling mares			
number (missing)	91 (6)	73 (13)	
range	4 to 25	3 to 23	
mean (*s*)	10.0 (4.6)	10.6 (5.0)	0.3687[a]
Parity of foaling mares			
number (missing)	90 (7)	82 (4)	
range	1 to 12	1 to 15	
mean (*s*)	3.8 (2.8)	4.3 (3.6)	0.3437[a]
Breed of foaling mares			
Arab	11 (11.3%)	6 (6.7%)	
other (includes mixed and Thoroughbred)	3 (3.1%)	13 (14.5%)	
Quarter horse	34 (35.1%)	36 (40.5%)	
Standardbred	49 (50.5%)	34 (38.2%)	0.0255[b]
Breed of foal			
Arab	10 (10.3%)	6 (6.7%)	
mixed	33 (34.0%)	35 (39.3%)	
other (includes Thoroughbred)	6 (6.2%)	14 (15.7%)	
Standardbred	48 (49.5%)	34 (38.2%)	0.0972[c]

[a]*P*-value associated with Student's *t*-test
[b]*P*-value associated with chi-square test after mixed, other and Thoroughbred were combined
[c]*P*-value associated with chi-square test after other and Thoroughbred were combined

evidence of hyperplasia (1,5,10). Foals that did not have the CHD syndrome and were born to a mare that spent the majority of her gestation on an affected farm were referred to as exposed foals. An affected farm was any farm where a mare producing an affected foal had spent most of her gestation.

A foal was classified as a control if it was thought to be normal by the foal's owner or farm manager, found to be free of anomalies when examined by a local veterinary practitioner or the 1st author, and born to a mare that had spent most of her gestation on a farm that did not produce an affected foal. Farms that did not produce an affected foal were control farms.

Data collection

Information was collected to investigate the potential transmission of infectious agents, the likelihood of exposure to a toxic substance, and the possibility of a dietary deficiency. A questionnaire was used to assist with the collection of this information and was administered by the 1st author during a personal interview with the owner or farm manager. Consultation with the local veterinary practitioner was sometimes required to complete the questionnaire.

For the purposes of this study, summer referred to the period from about June 1 to about September 30, 1992, and winter referred to the period from about October 1, 1992, until the time when mares foaled in 1993.

Whenever possible, samples of forage fed to pregnant mares were collected at the time of the personal interview, and stored. Following preliminary statistical analyses, these samples were analyzed for nitrate levels using previously established methods (11).

Variables

The outcome or dependent variable was whether or not a foal used in the study was an affected (CHD) or control foal. The independent variables investigated for possible association with the CHD syndrome are summarized in Table 1.

The term greenfeed was used to refer to a cereal crop, almost always oats, harvested prior to maturity, that is, "green", and baled for use as a livestock feed. Green oats, green oat hay, oat hay, green oat forage and oat straw are other terms that have been used to describe similar types of forage.

A variable representing the presence or absence of mineral supplementation of any kind was constructed from the variables concerned with feeding complete horse feed, feeding a protein-vitamin-mineral supplement, adding granular salt or mineral to grain, having free access to granular salt or mineral, and having free access to salt or mineral blocks.

Statistical analysis

The data set was checked for completeness and accuracy. Descriptive statistics were calculated for each of the continuous variables, and frequency tables were constructed for all categorical variables. Differences among farms and among mares producing affected and control foals were examined using Student's *t*-test, the chi-square test, and Fisher's exact test. Stratified analyses (Mantel-Haenszel)

Table 3. Length of gestation of congenitally hypothyroid dysmature foals and control foals

	Affected Foals	Control Foals	*P*-value
number (missing)	28 (11)	28 (11)	
mean (*s*)	357.6 (11.7)	338.9 (10.8)	<0.0001[a]
range[b]	330 to 378	322 to 357	

[a]Two-sided *P*-value associated with Student's *t*-test
[b]Range = the minimum and the maximum values greater than 320 d

Table 4. Categorical independent variables[a] unconditionally associated ($P \leq 0.15$) with congenital hypothyroidism and dysmaturity of foals

Variable	Affected	Control	Odds ratio	95% CI[b]	*P*-value[c]
greenfeed					
yes	10	1	13.1	1.6 to 108.3	0.0068
no	29	38			
left farm					
yes	19	7	4.3	1.5 to 12.2	0.0076
no	20	32			
irrigated pasture[d]					
yes	6	0	15.3	0.8 to 282.2	0.0254
no	33	39			
cattle on farm					
yes	19	9	3.2	1.2 to 8.4	0.0327
no	20	30			
no mineral					
yes	9	2	5.6	1.1 to 27.7	0.0472
no	30	37			
mineral block					
yes	22	31	0.3	0.1 to 0.9	0.0512
no	17	8			
other forage					
yes	7	15	0.4	0.1 to 1.0	0.0769
no	32	24			
creosote					
yes	11	19	0.4	0.2 to 1.1	0.1026
no	28	20			
ivermectin[e]					
yes	14	22	0.4	0.2 to 1.1	0.1113
no	25	17			

[a]Variables as described in Table 1
[b]Precision-based 95% confidence interval
[c]Two-sided *P*-value associated with Fisher's exact test
[d]To make calculations possible 0.5 was added to each value in the table
[e]Injectable ivermectin for cattle (Ivomec, Merck AgVet, Kirkland, Quebec) given orally

were performed to examine the association of various combinations of risk factors with the occurrence of disease (12). The analyses were performed using the Statistical Analysis System (SAS, Version 6.08, SAS Institute, Cary, North Carolina, USA) and InStat2 (GraphPad Software, San Diego, California, USA).

The association of all risk factors, except the presence of nitrate in forage samples, with the occurrence of disease was examined using all affected foals and a subset of control foals. The subset of control foals was selected from a list of all control foals using a random numbers table. The probability of selection was proportional to the number of foals on each control farm, with all control farms being represented by at least 1 foal. The association between the presence of nitrate in forage samples and CHD in foals was examined at the level of the farm and the individual animal. The later analysis included all foals examined on farms from which forage was collected.

Results

Fifty-four foals from 38 different farms were identified as affected foals. However, 15 foals from 12 farms could not be used in the study, as the foal owners or farm managers were either unable or unwilling to provide the information of interest. As a result, 186 foals comprising 39 affected foals and 58 exposed foals from 26 affected farms, and 89 control foals from 23 control farms were available for analysis. The 39 affected foals included 2 aborted foals with lesions and farm histories consistent with CHD syndrome (6). One hundred and twenty-four of the 186 foals received a detailed physical examination by the 1st author, and 69 foals, including 36 of the 39 CHD foals, were subjected to a radiographic examination of the carpal and tarsal regions.

The thyroid glands from 25 of the 39 affected foals were examined histologically. All of them were found to be hyperplastic and lacking normal amounts of colloid. Only 10 of the 54 foals with CHD were alive at the

Table 5. Association of selected independent variables with congenitally hypothyroid and dysmature foals after controlling for the effect of the absence of mineral supplementation in winter

Variable[a]	cOR[b]	sOR+[c]	sOR−[d]	chi-square[e]	*P*-value[f]	Summary ORmh[g]	95% CI[h]
greenfeed	13.1	0.3[i]	18	6.43[j]	<0.025	NA[k]	NA[k]
left farm	4.3	0.4[i]	4.9	2.95	>0.05	NA[k]	NA[k]
irrigated pasture	15.3	0.9[i]	16.2[i]	2.04[j]	>0.1	NA[k]	NA[k]
other forage	0.4	0.1[i]	0.5	2.53	>0.1	0.4	0.2 to 1.2
creosote	0.4	0.3[i]	0.6	0.12[j]	>0.7	0.6	0.2 to 1.5
ivermectin[l]	0.4	0.1	0.6	0.76	>0.3	0.5	0.2 to 1.3

[a]Variables as described in Table 1
[b]Crude odds ratio used in Table 3
[c]Stratum specific odds ratio for dams that did not have regular access to supplemental minerals during the winter
[d]Stratum specific odds ratio for dams that had regular access to supplemental minerals during the winter
[e]Breslow-Day test for homogeneity of the odds ratio
[f]*P*-value associated with the Breslow-Day test for homogeneity with 1 df
[g]Mantel-Haenszel summary odds ratio
[h]Test-based 95% confidence interval for the Mantel-Haenszel summary odds ratio
[i]Odds ratio calculated by adding 0.5 to all values in the stratum
[j]Breslow-Day test for homogeneity calculated after adding 0.5 to all values of those strata that contain a zero
[k]Use of a summary odds ratio (and 95% CI) is not appropriate when interaction is thought to be present
[l]Injectable ivermectin for cattle (Ivomec, Merck AgVet, Kirkland, Quebec) given orally

end of 1993. The remaining 44 foals were delivered dead, died, or were killed within a few days of birth.

A statistical comparison of the affected and control farms found that the 2 groups were similar in terms of size and animal signalment, except that standardbred mares and foals were moderately overrepresented in the affected group, relative to the control group (Table 2). The mean number of years of producing foals at the current location was considered equal ($P = 0.2349$). In both groups, there were 5 farms that were in their 1st foaling season on their present premises.

Of the 77 independent variables generated from the questionnaire, 10 were unconditionally associated with CHD at a $P \leq 0.15$ level of significance. The other 67 independent variables were not pursued further.

Only gestation periods over 320 d were used in analyses and, as expected (4,5), length of gestation was found to be significantly ($P < 0.0001$) longer in the CHD-affected foals compared to the subset of control foals (Table 3). Interestingly, the mean length of gestation (348.3 d) of exposed foals ($n = 46$) was significantly ($P < 0.0154$) longer than the mean gestation for all control foals (341.9 d, $n = 63$). Overall, the mean length of gestation (352.4 d) of all foals from affected farms ($n = 75$) was significantly ($P < 0.0001$) longer than that of control foals.

The association between each of the other 9 variables and CHD are summarized in Table 4. Further analyses were stratified on the variables concerning the absence of any supplemental mineral during the winter and the feeding of greenfeed during the winter and are presented in Tables 5 and 6.

The association between CHD and variables reflecting the feeding of forage other than hay, greenfeed, or silage; the use of creosote on fences and buildings; and the use of injectable ivermectin for cattle (Ivomec, Merck AgVet, Kirkland, Quebec) administered orally to horses, lacked statistical significance and did not reveal interaction or confounding after controlling for the effects of no mineral supplementation (Table 5). The same is true for the presence of cattle on the farms used in this study after controlling for the use of greenfeed in the winter (Table 6).

The association between mares grazing irrigated pasture and CHD could not be interpreted, and further evaluation could not be undertaken as the data were too sparse. Specifically, only 6 of the 78 dams grazed irrigated pasture and all 6 produced foals having CHD syndrome. Only 1 of these 6 dams failed to receive mineral supplementation, and none of the 6 dams were exposed to greenfeed.

An examination of "left farm" in Tables 5 and 6 reveals that its association with CHD varies with the presence or absence of supplemental mineral and with the feeding of greenfeed.

Samples of 20 different forages were collected from 14 of the 26 affected farms and samples of 10 different forages were collected from 7 of the 23 control farms. Nitrate was present more often (8 of 14 farms) and at higher concentrations in those samples collected from affected farms compared with those from control farms (1 of 7). The odds of at least 1 case of the CHD syndrome occurring on farms feeding forage with at least a trace of nitrate was 8.0 times greater ($P = 0.0873$) than the odds of disease occurring on farms that fed forage free of nitrate. On an individual animal basis, the odds of a mare producing a CHD foal when exposed to forage containing at least a trace of nitrate was 5.9 times greater ($P = 0.0007$) than those of mares exposed to nitrate-free forage.

Discussion

The case-control method of investigation has been widely accepted as the research strategy of choice when initiating an exploratory study of disease etiology (13–16). Thomas *et al* (17) have recommended that all associations under study should be reported, so that they are open to scrutiny by the reader. For this reason, all variables examined in this study are presented in abridged form in Table 1, with those deemed appropriate for additional study presented in Table 4.

The major concern associated with case-control studies is the potential for systematic errors or bias (13–16). The most likely source of potential bias in this study would have been differential misclassification of either disease or exposure status. Given the obvious

Table 6. Association of selected independent variables with congenitally hypothyroid and dysmature foals after controlling for the effect of feeding greenfeed in winter

Variable[a]	cOR[b]	sOR+[c]	sOR−[d]	chi-square[e]	*P*-value[f]	Summary ORmh[g]	95% CI[h]
left farm	4.3	0.1[i]	7.6	12.14	<0.005	NA[j]	NA[j]
irrigated pasture	15.3	0.1[i]	21.3[i]	8.53[k]	<0.005	NA[j]	NA[j]
cattle on farm	3.2	1.1[i]	2.3	0.52	>0.4	2.1	0.7 to 6.1
no mineral	5.6	0.1[i]	8.1	6.27[k]	<0.025	NA[j]	NA[j]

[a]Variables as described in Table 1
[b]Crude odds ratio used in Table 3
[c]Stratum specific odds ratio for dams fed greenfeed during the winter
[d]Stratum specific odds ratio for dams not fed greenfeed during the winter
[e]Breslow-Day test for homogeneity of the odds ratio
[f]*P*-value associated with the Breslow-Day test for homogeneity with 1 df
[g]Mantel-Haenszel summary odds ratio
[h]Test-based 95% confidence interval for the Mantel-Haenszel summary odds ratio
[i]Odds ratio calculated by adding 0.5 to all values in the stratum
[j]Use of a summary odds ratio (and 95% CI) is not appropriate when interaction is thought to be present
[k]Breslow-Day test for homogeneity calculated after adding 0.5 to all values of those strata that contain a zero

nature of the specific and uncommon combination of lesions required to classify a foal as affected, misclassification of disease status seemed unlikely. In addition, the histologic appearance of the thyroid gland was used to support the diagnosis of a foal with the CHD syndrome.

The potential for misclassification of exposure status was a greater concern. However, if misclassification had occurred with regard to the feeding of greenfeed or the failure to supplement mares with mineral in winter, it would have acted to decrease the strength of association of these variables with the disease, and the true odds ratios would have been even greater than those reported here. We believed that most horse producers would have viewed the feeding of greenfeed as a nontraditional and questionable practice. We also believed that most horse producers would have felt that supplementing the diet of pregnant mares with minerals was beneficial and should have been done. For these reasons, we felt that participants in this study would, if anything, have been inclined to underreport the feeding of greenfeed and the failure to supplement mineral. In this situation, the study would have produced an underestimate of the true exposure-disease association.

The feeding of greenfeed in winter had a strong (OR = 13.1) and highly significant ($P = 0.0068$) measure of association with CHD. The potential for greenfeed to accumulate high levels of nitrate (NO_3^-) and nitrite (NO_2^-) has been well recognized (18–27). In fact, prior to identifying nitrate as the causative agent of methemoglobinemia or nitrate poisoning of cattle, the condition was referred to as oat hay poisoning (18–22). Smith and Suleiman (27) have reported that Alberta producers often provide high-nitrate feeds, including oat greenfeed, to livestock. Pertinent to this discussion is the association between nitrate exposure and alterations in iodine metabolism, thyroid activity or thyroid gland morphology reported in a variety of animals, including fish (28), rats (29–34), growing pigs (35–38), goats (39), lambs and sheep (31,40–44), and cattle (43). Further, there is correlational evidence that suggests that high levels of nitrate present in drinking water is associated with an elevated rate of goitre in people of specific regions of Germany (45) and Nigeria (46). It has also been shown that nitrate is able to cross the placenta of rats (47,48), guinea pigs (49), and cattle (50–52). Interestingly, ingestion of nitrate by pregnant cows has been implicated as a cause of congenital arthrogryposis (53), as well as prolonged gestation, depressed thyroxine levels, and enlarged thyroid glands (54) in their calves.

If nitrate present in the diet of pregnant mares can cross the placenta and interfere with fetal thyroid function, it will be important to consider all sources of environmental nitrate to fully understand and prevent disease. There can be considerable variation in the nitrate levels found in plants due to the plant species, stage of maturity, nitrogen content of the soil and water, and other growing conditions. Many of the plants commonly made available to horses in western Canada can accumulate high levels of nitrate; these include alfalfa, timothy, ryegrass, sweet clover, and a wide variety of weeds. Many cereal crops, such as oats, wheat, barley, rye, corn, and flax, are also able to concentrate nitrate. Fortunately, different parts of the plant contain different levels of nitrate, and very little tends to appear in the seed or grain. Nitrate is water soluble and water can also be an important source of nitrate for livestock, particularly near areas of heavy fertilization, feedlots, dairies, landfills, and some types of industry. The potential for problems are likely to be increased following periods of high surface runoff that would be created by such things as spring snow melts, heavy rainfall, or irrigation. It is worth emphasizing that feed, water, and other potential sources of nitrate are additive in their effect, and all will have to be considered when investigating a suspected nitrate problem (25,52,53,55,56).

An absence of supplemental salt or mineral during the winter had a moderately strong measure of association (OR = 5.6) with CHD, and this association was probably underestimated, since no attempt was made to estimate the quantity or the quality of the mineral consumed on a farm or individual animal basis. Despite this, the relationship was statistically significant ($P = 0.0472$) and a biologically plausible association among a deficiency of minerals, thyroid function, and the CHD syndrome has been discussed previously (5). Iodine is essential for normal thyroid function and failure to supplement pregnant mares with mineral may have been associated with an iodine deficiency, since soils in western Canada and the plants grown on these soils are very low in iodine.

Information concerning a mare's movement off and onto her "home farm" was included as one of several variables to evaluate the possibility of an infectious agent being the cause of the CHD syndrome. However, the failure of other related variables to be associated with CHD invites a different interpretation of the results that were obtained. An examination of "left farm" in Tables 5 and 6 demonstrates that the association between movement off and onto the "home farm" and CHD varies with the presence or absence of supplemental mineral and the feeding of greenfeed. This effect, referred to as interaction, supports the conclusion that feeding greenfeed and failing to provide supplemental minerals during the winter were risk factors for disease. It would appear that dams that left a high risk environment, that is, one that failed to provide supplemental mineral or that fed greenfeed, had a reduced risk of producing a CHD foal; while dams that left a low risk environment had an increased risk of producing a CHD foal.

Unfortunately, the combined effects of the lack of supplemental mineral and the feeding of greenfeed on the occurrence of CHD syndrome could not be pursued with this data set, as none of the farms included in this study fed greenfeed and failed to provide supplemental mineral during the winter. There is evidence in rats (32) and pigs (37) that increased levels of iodine in the diet can counteract, to some degree, the effects of nitrate on thyroid activity.

This study has determined that the lack of mineral supplementation and presence of greenfeed in the diet of pregnant mares significantly increases the risk of producing a foal with CHD syndrome. It has been argued that the ingestion of nitrate and a deficiency of iodine are 2 underlying factors that produce disease by interfering with fetal thyroid function.

While this study has been successful in generating new hypotheses about the cause or causes of CHD syndrome, it will be important to test these hypotheses through additional epidemiologic investigations (17) and controlled experiments.

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