Samen werken aan diergezondheid

A rickets-like bone disease in young dairy goat kids

C. Honingh
Msc Student Veterinary Medicine, Utrecht University
Student number: 3744094

February – May 2016

Dr. K. Lievaart-Peterson
Animal Health Service GD Deventer
Department of Small Ruminant Health

Dr. G. Koop
Faculty of Veterinary Medicine, Utrecht University
Department of Farm Animal Health

Abstract: On a yearly base approximately 10-15% of the goat kids on a commercial dairy goat farm in the Netherlands was affected with a rickets-like bone disease. Clinical features, which consisted of bended (front)legs and a stiff posture and gate, were seen from the age of 14 to 21 days onwards. The objectives of this study were to quantify clinical symptoms and determine whether or not the incidence of disease was associated with external risk factors, derived from does, paternity and diet. A total of 36 yearlings and 112 second parity does lamb between 5 and 19 March 2016. In the last trimester of pregnancy, 61 of 112 second parity does were treated with a vitamin D preparation. In a four-weeks longitudinal study, clinical features of this rickets-like disease were measured in a total of 235 goat kids. On the basis of appendix I and II, each kid was classified into a category. Calcium, inorganic phosphate and 25-hydroxyvitamin D_3 levels were determined in blood samples. Furthermore, three goat kids were selected for pathological examination of kidney, liver and bones. The number of clinically diseased goat kids was not significantly associated with the vitamin D treatment of does (p=0,744), parity of mothers (p=0,559) and bucks (p=0,264). The external risk factor diet, was associated with the number of goat kids, which exposed mild clinical symptoms (p=0,002). The mean concentrations of calcium, inorganic phosphate and 25-hydroxyvitamin D_3 in the clinically diseased offspring of vitamin D treated does were 2,45 mmol/L (σ=0,11), 2,90 mmol/L (σ=0,14) and 92 nmol/L (σ=35), respectively. The results show that neither the existence of clinical signs nor the blood concentrations of calcium, inorganic phosphate and 25-hydroxyvitamin D_3 were associated with the vitamin D treatment of does. Pathological examination showed several bone deformations, which were most probably related to a rickets-like bone disease.

Introduction

In recent years, goat kids with curved legs were observed on a commercial dairy goat farm in the southern part of the Netherlands. Changes in posture and gate were observed from 14-21 days of age onwards. Especially the front legs grew curved and seemed as a result of the bending, too short in relation to the hind legs. In gait, the scapula and the elbow joint made lateral movements, in addition to physiologically cranio-caudal movements. The bending of the legs and the affected gait appeared irreversible in most animals, although some goat kids seemed to recover over time. On a yearly base 10-15% of the approximately 300 female goat kids, were permanently affected with curved legs. This number of diseased goat kids was observed since the pregnant does were moved to a new stable in 2006. In contrast to the females, the male slaughter kids, which were directed to the fattening location at a mean age of 20 days, seemed to be clinically unaffected. The most appropriate diagnosis, corresponding to the clinically affected goat kids, was a rickets-like bone disease. Information about this disease was collected by previous diagnostics in 2013. Mean calcium concentrations of affected kids were reported within reference, although the average phosphorus concentration was too high in the young animals.[1,2] On the basis of dietary calcium, phosphorus and vitamin D_3 requirements for pregnant goats and growing kids, several diet changes for pregnant does and rearing kids were implemented on this commercial dairy goat farm. In 2015, pathological examination of four rickets-like diseased goat kids showed severe bone abnormalities and moreover a chronic nephritis was found in two of them.

In literature, rickets was described as a metabolic bone disease, characterized by a defective mineralization of cartilage in growing bone.[3,4] This metabolic bone disease was described in children and young growing domestic animals, including cattle, pigs, llama's, alpaca's, sheep and goats.[5] In 1992, another case of rickets-like bone changes was described in Dutch dairy goat kids, which were suckled with a synthetic milk replacer for calves.[6] Bone length growth is normally a balance between

periosteal bone formation by osteoblasts and endosteal bone resorption by osteoclasts. In case of rickets, this balance has been disturbed, resulting in enlarged trabeculae and an increased percentage of osteoid.[7,8] Culminating characteristics of disease were enlarged costochondral junctions, a stiff gait, an arched back, lameness and bending of long bones.[5] Vitamin D deficiencies, caused by low dietary vitamin D_3 concentrations, malabsorption of vitamin D_3 in the intestines, low sunlight exposure or an abnormal synthesis of 1α,25-dihyroxyvitamin D_3 by liver and kidney, have been shown to be related to rickets.[5] Literature in humans and ewes showed also that vitamin D concentrations between mothers and their neonates were closely related.[9,10] Congenital rickets in man was associated with maternal vitamin D deficiency during pregnancy.[9,11,12]

The vitamin D metabolism in the kidney was influenced by calcium and phosphorus.[5] On the other hand were concentrations of calcium and phosphorus influenced by 1α,25-dihyroxyvitamin D_3 and its effects on intestines, kidney and bone, which were induced by parathyroid hormone (PTH).[5,8] According to those interactions, calcium and inorganic phosphate concentrations could also be affected in case of rickets.

This study on the ethology and underlying risk factors was initiated because the cause and solution corresponding to this disease were not found yet. It was questioned whether or not the young goat kids were born with a possible hypovitaminosis D and a misbalanced calcium phosphorus ratio as a result of low vitamin D in the does. In response to the hypothesis that the symptoms were related to piglet grid housing systems, it was questioned whether kids, housed on layer of crushed straw remained unaffected. The objectives of this research were to quantify clinical symptoms, identify the beginning and development of clinical signs and calculate the incidence of rickets-like diseased goat kids. Another purpose of this research was to determine whether or not clinical symptoms were associated with external risk factors, such as vitamin D treatment and parity of does, heritability and diet. Other aims of this study were to measure blood concentrations of calcium, inorganic phosphate, and 25-hydroxyvitamin D_3 in newborn and affected kids and determine any pathological abnormalities in two clinically diseased kids. A longitudinal study design was used to identify clinical signs of disease.

Materials and methods

Animals

This longitudinal study was designed to fit on a commercial dairy goat farm. On the farm, around 1000 dairy goats were year-round housed in six separated pens with straw bedding. Yearlings were housed in the nearby rearing stable. All dairy goats had free access to tap water, which was offered by a nose paddled drinking bowls, and were fed with pelleted feed and a mixture of grass silage, corn silage and pressed beet pulp. Yearly, approximately 250 does lambed. In 2015, 100 yearlings were mated naturally and 150 older does were synchronized and artificial inseminated. In the remaining 750 goats, new pregnancies were not initiated to maintain prolonged milking.

All future breeding stock, excluding slaughter kids, which were born between 5 and 19 March 2016, were selected for further research. The total of 235 goat kids, were the offspring of 36 naturally mated yearlings (45 kids) and 112 second parity does, which were artificial inseminated with semen of five different bucks (190 kids). In the last trimester of pregnancy, 61 second parity does (107 kids) were subcutaneously injected with 2,5 mL of a vitamin preparation (VITAMINE AD3 80/40 PRO INJ.®, Alfasan, Woerden, The Netherlands). Each doe was injected with 200.000 IE vitamin A, 100.000 IE cholecalciferol and 25 mg alfa-tocoferolacetaat. The remaining 51 second parity does (83 kids) were not injected with the vitamin preparation.

The goat kids were separated from their mothers directly after birth, and were reared in the breeding pen. In the first hours of life, the kids were fed individually with two doses of 100 mL colostrum replacer (Capracol®, Arts Food Products B.V. 's-Hertogenbosch, The

Netherlands). Subsequently, goat kids were suckled ad lib with a milk replacer by a drinking automat. Kids, which were born up to and including 10 march 2016 were suckled during the first 10 days with Chivalac® milk powder (Nutrifeed, Veghel, The Netherlands) and then transferred to Chivalac Rendement® milk powder (Nutrifeed, Veghel, The Netherlands). The remaining kids, which were born since 11 march 2016, were suckled with the Chivalac Rendement® milk replacer till weaning. To create those milk replacers, each liter of water was mixed with 180 grams of skimmed milk powder (21% protein and 28% vegetable fat) in the machine. The kids also had free access to tap water, offered by a nose paddled drinking bowls during the entire rearing period. At the age of twenty days, crushed rapeseed straw and pellets for pre-ruminated kids (Vita CAPRI Pensstart Z20®, ForFarmers Hendrix, Lochem, The Netherlands), were offered. Animals, which were weighing more than 12 kilograms in the fourth week of life, were weaned.

The weaned kids were housed in groups of 25 to 50 animals, in each pen three to four animals are housed per square meter. The grids in the slatted flour, were covered with cardboard and the ground was covered with a layer of crushed straw. The climate in the rearing stable was mechanically maintained by the outfall of air, and was stored at a minimum of 16°C, a relative air humidity of 72 percent was analyzed and mean concentrations of CO_2 and NH_3 in the rearing stable were 1350 and 5 ppm, respectively.

During the rearing period, the kids were dehorned and vaccinated with Gudair® (CZ Veterinaria, Porriño, Spanje) against Johne's Disease, around the age of ten days.

Scoring clinical features

During the first twenty-six days of life, all 235 selected goat kids were examined on clinical features two times a week. The kids were three to six days old, when they were inspected for the first time. Abnormalities were classified on the basis of the scoring table, which is contained in appendix I. According to the eight awarded scores per animal, each individual kid was assigned into one of four categories. Appendix II summarized the four different categories, in which individual kids were classified.

Analyzing risk factors

It was analyzed whether external risk factors were associated with the four granted categories of disease. In the offspring of second parity does (190 kids), it was studied whether vitamin D treatment of does, heritability and diet were associated with the incidence of disease in each category. Finally, parity was analyzed as external risk factor for disease in the offspring of vitamin D untreated does (128 kids). The flow chart, which is enclosed in appendix III, showed all relationships between the 235 kids and their mothers, fathers and diets.

Blood measurements

Blood samples were collected to determine the concentrations of calcium, inorganic phosphorus and 25-hydroxyvitamin D_3 in unaffected newborn and clinically diseased goat kids of 35 days old. Blood samples of 4 mL were collected from the jugular vein by the use of a single syringe and needle, 2 mL of each sample was stored in serum tubes and 2 mL was stored in heparin. Then the serum samples were centrifuged for six minutes at 5000 RPM by the EBA 20 Hettich Zentrifugen® (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Calcium and inorganic phosphate concentrations were determined for nine unaffected newborn kids and ten clinically diseased kids by the Spotchem EZ Anaysator®, which used the Spotchem II Calcium stick and the Spotchem II Inorganic Phosphate stick respectively. The blood plasma samples for the determination of the 25-hydroxyvitamin D_3 concentration were centrifuged during fifteen minutes at 3000 RPM. Plasma samples of sixteen affected animals and eight newborn kids were stored at -20°C before determination of 25-hydroxyvitamin D_3 concentrations. The 25-hydroxyvitamin D_3 concentration was determined in plasma samples of 0,2 mL by the use of high performance liquid chromatography, as described in earlier studies.[13,14]

Pathological examination

Two kids, which were category 3 classified on the basis of Appendix II, were selected for pathological examination. One clinically unaffected kid, which died for other reasons, was also pathologically examined. The carcasses were evaluated macroscopically, and in addition, samples for histology were taken from liver, kidney and bone. From the long bones in the front legs, longitudinal sections were sawed. Sections through the growth retardation lattice of the distal condyle of the humerus and the proximal side of the radius, were cut and stained in hematoxylin and eosin (HE) for further histological examination.

Statistical analysis

The data were analyzed with the Chi-Square test and logistic regression (SPSS version 22). The independent T-test was used to analyze mean serum calcium and inorganic phosphate levels and mean plasma 25-hydroxyvitamin D_3 concentrations between the newborn offspring of vitamin D treated and untreated mothers and the affected offspring from vitamin D treated and untreated does (SPSS version 22).

Results

Clinical features and risk factors

At the first scoring moment, when goat kids were 3-6 days of age, 90% were awarded as normal and 9% were score 1 assigned. From the second to the final scoring-moment, approximately 73% of the kids were classified as normal. Score 1 was assigned to 22% of the kids at the second time of examination. Subsequently, the percentage of score 1 awarded kids was reduced to almost zero at the eight scoring moment. Scores 2 and 3 were perceived from the fourth scoring moment, and then gradually increased. At the final scoring moment, 15% of the kids were awarded in score 2 and 6% were classified in score 3. The time-dependent pattern of those existing clinical features, and corresponding scores, was demonstrated in figure 1. Afterwards, 33% of the individual kids were classified into category 0, 28% of the kids were category 1 assigned, 11% were category 2 assigned and 21% were classified in category 3.

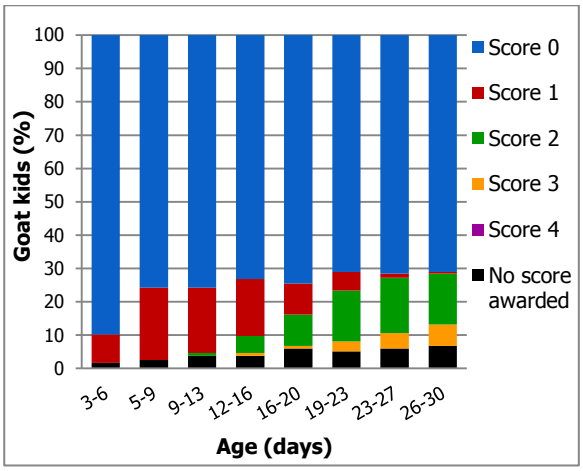

Figure 1| Scored clinical features of a rickets-like bone disease, assigned at eight scoring moments during at least the first 26 days of life in dairy goat kids on a commercial dairy goat farm in the Netherlands (n=235; score 0, normal; score 1, mild clinical features; score 2, moderate clinical features; score 3, considerable clinical features; score 4, severe clinical symptoms).

Figure 2a focused on the categories, which were assigned to the offspring of vitamin D treated and untreated second parity does. A p-value of 0,744 showed that the number of unaffected kids (category 0) differed not significantly between the offspring of vitamin D treated and untreated second parity does. According to p-values of 0,534, 0,660 and 0,869, no significant differences were found for categories 1, 2 and 3 Furthermore, the number of category 0 awarded kids, differed not significantly between the offspring of vitamin D untreated first and second parity mothers (p=0,559). Differences between categories 1, 2 and 3 were also not significant for the offspring of first and second parity does. Corresponding p-values were 0,840, 0,299 and 0,184 for categories 1, 2 and 3, respectively. Figure 2b presented the awarded categories for the offspring of vitamin D untreated first and second parity does. In figure 2c were the granted categories summarized for the natives of five different fathers. P-values of 0,264, 0,202, 0,158 and 0,161 demonstrated that the number of kids, which were assigned to categories 0,1,2 and 3, differed not significantly per father. The categories, which were awarded

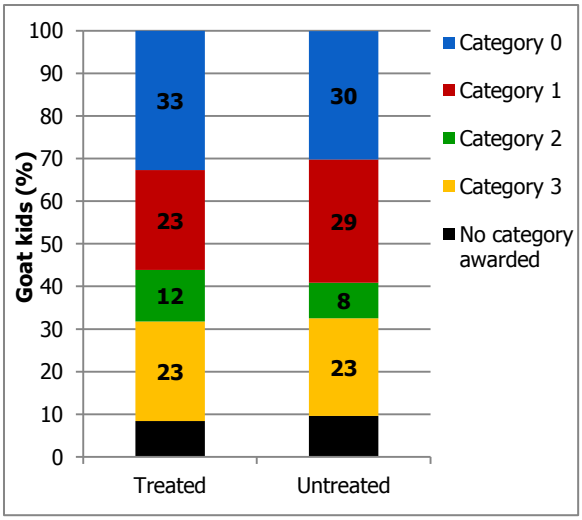

Figure 2a| Clinical features of a rickets-like bone disease were classified into categories after the first 26 days of life in dairy goat kids on a commercial dairy goat farm in the Netherlands; (n=107 in the offspring of vitamin D treated second parity mothers and in the offspring of vitamin D untreated second parity mothers n=83; category 0, scored normal (score 0) over the entire scoring period; category 1, mild clinical features were scored one or more times; category 2, animals were one of eight times classified in score 2 or 3; category 3, animals were scored two or more times as score 2 or 3.

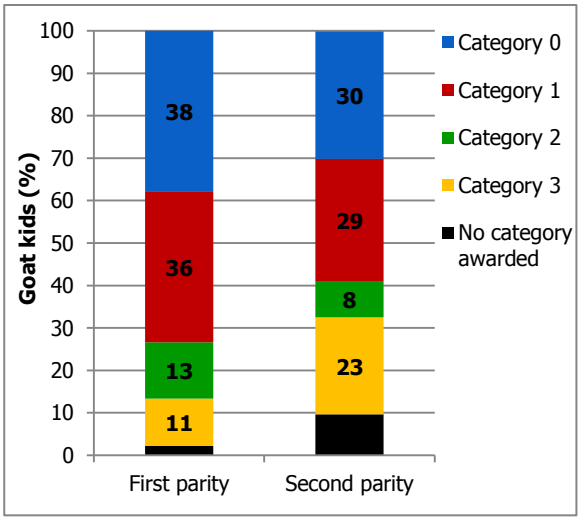

Figure 2b| Clinical features of a rickets-like bone disease were classified into categories after the first 26 days of life in dairy goat kids on a commercial dairy goat farm in the Netherlands; (n=45 in the offspring of vitamin D untreated, first parity mothers and in the offspring of vitamin D untreated, second parity mothers n=83; category 0, scored normal (score 0) over the entire scoring period; category 1, mild clinical features (score 1) were scored one or more times; category 2, animals were one of eight times classified in score 2 or 3; category 3, animals were scored two or more times as score 2 or 3).

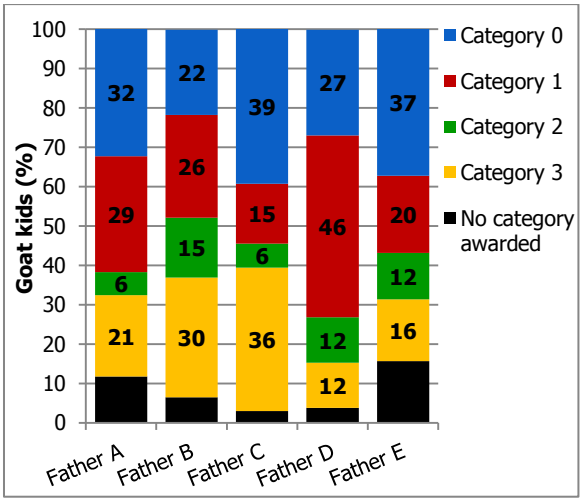

Figure 2c| Clinical features of a rickets-like bone disease were classified into categories after the first 26 days of life in dairy goat kids on a commercial dairy goat farm in the Netherlands; (n=34 father A, n=46 father B, n=33 father C, n=26 father D, n=51 father E; category 0, scored normal (score 0) over the entire scoring period; category 1, mild clinical features (score 1) were scored one or more times; category 2, animals were one of eight times classified in score 2 or 3; category 3, animals were scored two or more times as score 2 or 3).

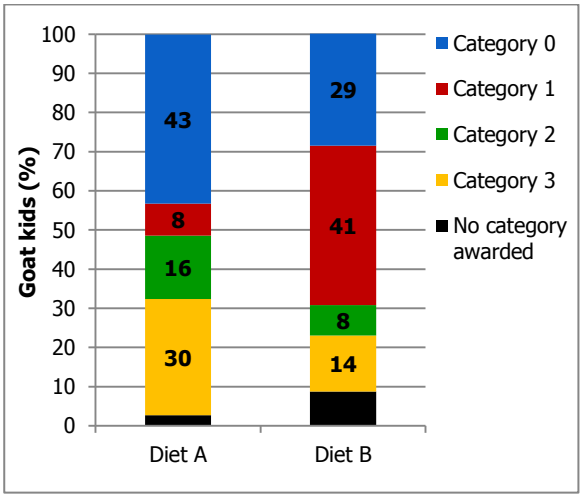

Figure 2d| Clinical features of a rickets-like bone disease were classified into categories after the first 26 days of life in dairy goat kids on a commercial dairy goat farm in the Netherlands; (n=49 suckled with diet A and n=141 contained diet B; category 0, scored normal (score 0) over the entire scoring period; category 1, mild clinical features (score 1) were scored one or more times; category 2, animals were one of eight times classified in score 2 or 3; category 3, animals were scored two or more times as score 2 or 3).

to diet A and diet B suckled kids, were summarized in figure 2d. The number of category 1 awarded kids was significantly different between the group of diet A and B suckled kids (p=0,002). The different occurrence in category 2 awarded kids was, considering to a p-value of 0,08, almost significant. Odds ratios were 3,2 and 0,5, when diet A was compared to diet B for categories 1 and 2. P-values of 0,401 and 0,221 showed that no significant differences were found for categories 0 and 3, respectively. A slightly increased difference between diet A and B was demonstrated in category 1, when p-values were corrected for the adjusted effects of the fathers. The odds ratio became 3,7 when diet A was compared to diet B (p= 0,001). The adjusted effect of the fathers did not affect the other categories.

Blood measurements

The mean calcium concentrations between the newborn offspring of vitamin D treated does (2,28 mmol/L; σ=0,20) and vitamin D untreated does (2,45 mmol/L; σ=0,07) were according to a t-value of -1,798 and a p-value of 0,115 not significantly different. The mean calcium concentration in both groups of clinically diseased kids (2,41 mmol/L; σ=0,09 and 2,45 mmol/L; σ=0,11) differed also not significantly (t=-0,688; p=0,511). Mean inorganic phosphate concentrations in both groups of newborn kids (1,71 mmol/L; σ=0,22 and 1,85 mmol/L; σ=0,19) did not significantly vary (t=1,069; p=0,320). Mean inorganic phosphate concentrations in both groups of affected kids were 2,90 mmol/L (σ=0,14) and 2,82 mmol/L (σ=0,09). Considering to the t-value of 1,036 and the p-value of 0,331, no significant difference was charged. Mean concentrations of calcium and inorganic phosphate were presented in figure 3a.

Mean 25-hydroxyvitamin D_3 concentrations of 50 nmol/L (σ=8) and 47 nmol/L (σ=12) were found in the newborn offspring of vitamin D treated and untreated does. According to a t-value of 0,379 and a p-value of 0,384, the average 25-hydroxyvitamin D_3 values differed not significantly. Mean 25-hydroxyvitamin D_3 concentrations of 92 nmol/L (σ=35) and 85 nmol/L (σ=25) were found for the clinically diseased kids, whose mothers were treated and untreated with vitamin D. Between the two groups of affected goat kids, mean 25-hydroxyvitamin D_3 concentrations were not significantly different (t=0,424; p=0,164).

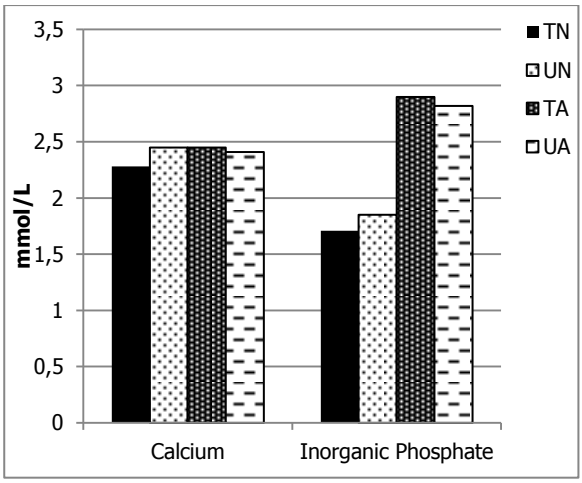

Figure 3a| Mean Calcium and Inorganic Phosphate concentrations in blood serum samples of nine newborn goat kids and ten clinically diseased kids, which were 35 days of age. (NT, Newborn goat kids, which was the offspring of vitamin D treated mothers (n=4); NU, Newborn goat kids, which was the offspring of vitamin D untreated mothers (n=5); AT, Affected goat kids, whose mothers were treated with vitamin D (n=5); AU, Affected goat kids, which was the offspring of vitamin D untreated mothers (n=5)).

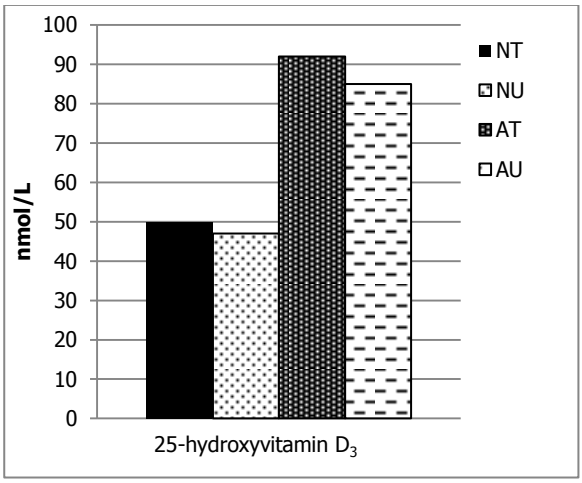

Figure 3b| Mean 25-hydroxyvitamin D_3 concentrations in blood plasma samples of eight newborn goat kids and sixteen clinically diseased kids, which were 35 days of age. (NT, Newborn goat kids, which was the offspring of vitamin D treated mothers (n=4); NU, Newborn goat kids, which is the offspring of vitamin D untreated mothers (n=4); AT, Affected goat kids, whose mothers were treated with vitamin D (n=8); AU, Affected goat kids, which was the offspring of vitamin D untreated mothers (n=8)).

Figure 3b summarized the mean concentrations of 25-hydroxyvitamin D_3.

Pathological examination

Macroscopically, no abnormalities were seen in liver, kidney and longitudinal dissections of long bones from all three kids. Histological were the three kids affected with a little aspecific hepatitis and in the two kids, which were clinically affected with the rickets-like bone disease, also degeneracy of hepatic cells was perceived. Microscopically, in both clinically affected and unaffected kids, the longitudinal sections of the distal condyle of the humerus and the proximal shown in figures 4a and 4b, the coupes were characterized by wide trabeculae and also a lot condyle of the radius showed abnormalities in the formation of bone. As of hypertrophic osteoblasts were found. However, osteoclasts were almost not presented in the longitudinal sections through the growth retardation lattice of the humerus and radius. Figure 4c showed signs of inflammation and degeneration around a blood vessel in the cartilage of a clinically diseased kid. An irretrievable fissure through the growth retardation lattice was also observed in the radius of this kid (figure 4d).

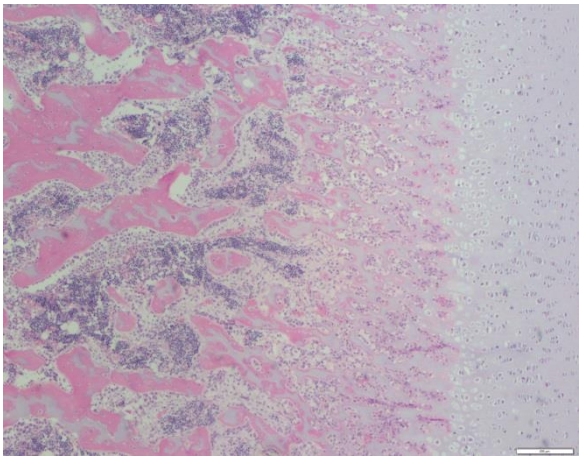

Figure 4a| Proximal radius of a 21-days old goat kid which was clinically not affected with the rickets like-disease, showed wider metaphyseal trabeculae (Bar = 200 µm).

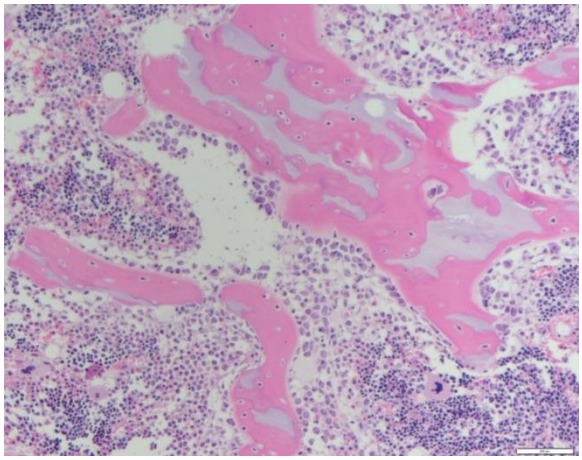

Figure 4b| Proximal radius of a 21-days old goat kid which was clinically not affected with the rickets like-disease, showed wider metaphyseal trabeculae and the absence of osteoclasts (Bar = 100 µm).

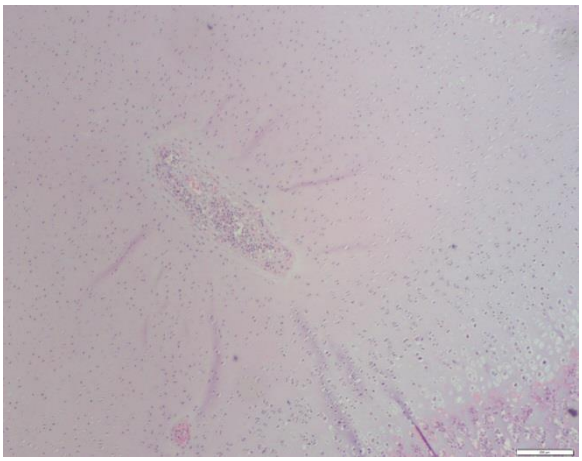

Figure 4c| Blood vessel in the radius cartilage of a 9-days old goat kid, which was clinically diseased with the rickets-like bone disease. Inflammation and degeneration were presented around the blood vessel (Bar = 200 µm).

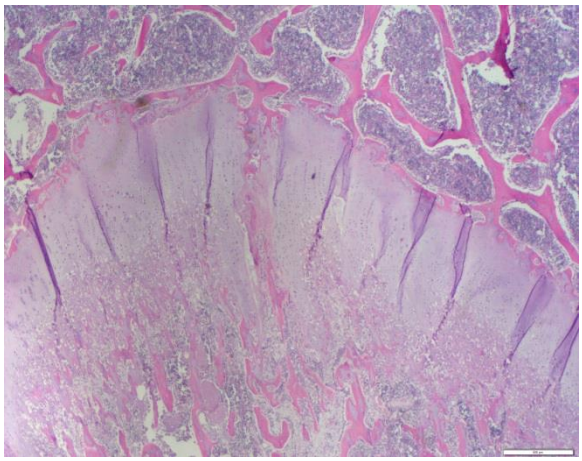

Figure 4d| Growth retardation lattice of the proximal radius of a 9-days old goat lamb, which was clinically affected with the rickets like-disease, was injured with an irrecoverable fissure (Bar = 500 µm).

Discussion and conclusion

Approximately 75% of rearing kids were awarded as normal (score 0) on each scoring moment. However, only 33% of the kids were awarded as normal over the entire period (category 0) and at least 60% of the kids were one or more times clinically affected with the rickets-like bone disease (categories 1-3). This difference between score 0 and category 0 awarded kids was probably caused by time-dependent changes in the total number of affected kids. On the one hand, kids with an increasing number of clinical abnormalities were observed, but on the other hand kids with a reduced amount of clinical features were detected in time. These findings consist with literature on spontaneously recovered experimental valgus deformations in kids.[15] Another theory was that category 2 awarded kids, which presented at only one of the scoring moments clinical abnormalities, were related to the vaccination with Gudair®. This vaccine, on the basis of a mineral oil, was subcutaneously injected at the cranioventral side of the rib wall. It was mentioned that skin thickness and pain at the injection site were caused by the vaccination with Gudair®.[16,17]

In the 235 kids, which were born between 5 and 19 March 2016, clinical signs of disease were well quantified by the use of the scoring tables out of Appendix I and II. The present study primarily focused on the replacement breading stock, and therefore the incidence of disease amongst the slaughter kids was not recorded. Because of the high number of diseased rearing kids, it was suspected that an unspecified number of slaughter kids was also affected with (sub-)clinical abnormalities.

The percentages of affected kids in each category were almost identical in both the offspring of vitamin D treated and untreated mothers. It was concluded that the treatment of does with 2,5 mL VITAMINE AD3 80/40 PRO INJ.® (Alfasan, Woerden, The Netherlands), did not reduce the number of clinically diseased goat kids. Although the association between congenital rickets and low maternal vitamin D levels was described in human literature, blood samples of pregnant does were not analyzed in the present study. [9,11,12] It was still possible that vitamin D levels of pregnant does were not deficient.

In addition, it was analyzed whether or not the parity of does influenced the number of clinically affected kids. Especially during cold weather, when windows were screened in the rearing stable and the milking doe stable, the exposure to ultraviolet light was reduced in the rearing stable. It was hypothesized that this reduced exposure to ultraviolet light, caused a vitamin D deficiency in especially pregnant first parity does.[5] This hypothesis was not supported by the current study, because no significant differences were found between the offspring of first and second parity does.

A hereditary form of rickets was investigated in Corriedale Sheep, but to our knowledge no inherited forms of rickets were reported for goat.[18,19] In previous years, it seemed that clinically diseased kids were not related to one specific father. In the present study, it was analyzed whether the number of clinically diseased goat kids, was influenced by the fathers. In the offspring of five dissimilar fathers, no significant differences were found for the four different categories, in which clinical features were classified. However, figure 2c showed for father D an increased tendency of category 1 assigned kids. Furthermore the percentage of category 3 awarded kids seemed increased in the offspring of fathers B and C. Although no significant differences were found between the offspring of different bucks, the incidence of clinically diseased kids was probably influenced by hereditary factors of father.

Recently, several diet changes for growing kids were implemented on this dairy goat farm. Because of the seemingly unaffected male slaughter kids, which were suckled with diet B, the current study analyzed diet as risk factors for rickets-like bone disease. The odds ratio of 3,2 (p= 0,002) showed that the relative risk of category 1 classified disease was higher for diet B suckled kids, compared to diet A suckled ones. The calculated odds ratios between diet A and B were not significant for categories 2 and 3, however the number of goat

kids which was awarded with considerable clinical features seemed reduced in the diet B suckled group. The ratio between calcium and phosphorus should be maintained between 1:1 to 2:1 in the diet of rearing goat kids.[4,20] A ratio of 0,8:1 was found between calcium and phosphorus in the diet of clinically diseased kids, which were suckled with a synthetic milk replacer for calves.[6] However, ratios of 1,1:1 and 1,2:1, which were calculated for diet A and B respectively, were both within the reference range and furthermore comparable to caprine milk.[4,20,21] The composition of analytical components were almost similar for diet A and B, however different concentrations were found for zinc and manganese. Earlier research assumed that a manganese deficiency caused bone deformations in goat.[22] A manganese deficiency was regarded as a potential cause for the occurring rickets-like bone disease in kids.

Mean serum calcium concentrations of 2,7 mmol/L were mentioned as normal in previous years. However, the average phosphate concentrations of 4,0 mmol/L was interpreted as too high, considered to reference values of 1,4-3,1 mmol/L for inorganic phosphate.[4,20] In the present study, the mean calcium concentrations of 2,28 mmol/L (σ=0,20) and 2,45 mmol/L (σ=0,07), which were found in the newborn offspring of vitamin D treated and untreated does, remained within the reference range of 2,2-2,9 mmol/L.[4,20] The mean calcium concentration of 2,41 mmol/L (σ=0,09) in the clinically affected offspring of vitamin D treated mothers and 2,45 mmol/L (σ=0,11) in the clinically affected offspring of vitamin D untreated mothers, were also interpreted as normal. Concentrations of inorganic phosphate were 1,71 mmol/L (σ=0,22) and 1,85 mmol/L (σ=0,19) for the newborn ones with a vitamin D treated or untreated mother, respectively. Higher amounts of phosphorus were found for affected kids, mean concentrations were 2,90 mmol/L (σ=0,14) for the offspring whose mothers were treated with vitamin D and 2,82 mmol/L (σ=0,09) for the offspring of the untreated mothers. It can be concluded that the vitamin D treatment of mothers, in both newborn offspring and clinically affected goat kids of 35 days old, had no effect on mean concentrations of calcium and inorganic phosphate. However, between newborn kids and affected kids of 35 days, a different phosphate concentration was deduced out of figure 2a. Whether this difference was caused by disease, age or dietary effects cannot be stated for sure. A recent study on the biochemical profile of goat kids, showed that calcium and phosphorus were age-dependent parameters.[23] Mean calcium and phosphorus concentrations out of the present study, were closely related to concentrations, which were reported for Saanen goat kids.[23] Changed concentrations of phosphorus, may also be related to dietary influences and the hormonal regulation of the phosphate homeostasis.[24] Higher concentrations of phosphorus in young animals were caused by an increased reabsorption in the kidney, which was induced by growth hormone.[24] Consequently, it seemed most probable, that concentrations of inorganic phosphate were affected by dietary influences and age.

In recent literature, which was on the influence of exposure to ultraviolet radiation, 25-hydroxyvitamin D_3 concentrations around 100 nmol/L were reported in growing lambs and in growing goat kids of 18 weeks old, concentrations of 90 nmol/L were described.[25] In another study, an average concentration of 100 nmol/L was determined in six to seven months old control goat.[26] In the present study, mean 25-hydroxyvitamin D_3 concentrations of 92 nmol/L (σ=35) and 85 nmol/L (σ=25), were calculated for the clinically diseased offspring of vitamin D treated and untreated does, respectively. This mean 25-hydroxyvitamin D_3 concentrations corresponded to earlier investigations.[25,26] Concentrations of 1α,25-dihyroxyvitamin D_3 were not determined in the present study, and consequently a deficiency of active vitamin D, which was produced in the kidney, cannot be completely excluded. Mean 25-hydroxyvitamin D_3 concentrations of 50 nmol/L (σ=8) and 47 nmol/L (σ=12) were calculated for the newborn offspring of vitamin D treated and untreated does, respectively. Considered to previous studies in humans, 25-

hydroxyvitamin D_3 concentrations between neonates and their mothers were closely associated.[9,10] The somewhat lowered average concentrations of 25-hydroxyvitamin D_3 in the newborn kids, supported the hypothesis that pregnant does were possibly affected with a hypovitaminosis D.

Post mortem investigations were performed in three kids, one of them seemed clinically unaffected and the remaining two kids were category 3 classified. Considering to the total number of kids, which was clinically affected, this section of the population seemed correctly. Abnormalities in liver, kidney and bone, which were found at pathological examination of the three goat kids, were corresponding to previous post mortem investigations. In contrast to pathological examination of four affected goat kids in 2015, which showed severe deformations and several bone cysts at macroscopically examination of humerus and radius, these three carcasses seemed macroscopically unaffected. Microscopically examination showed no abnormalities of the kidney's. However, in a previous histological evaluation, a chronic inflammation of the kidney's was observed in two out of four animals. The wider trabeculae, hypertrophic osteoblasts and the reduced number of osteoclast, which were found in the longitudinal sections of the proximal condyle of the radius, were most probably related to a rickets-like bone disease.[27] The histopathological examination of Corriedale sheep affected with inherited rickets, showed also an irregular thickening of trabeculae in the hypertrophic zone and trabeculae which crossed the metaphyseal zone, parallel to the growth retardation lattice.[18,19] In addition, in one of the clinically diseased goat kids an irretrievable fissure through the growth retardation lattice of the radius was observed, and also signs of inflammation and degeneration around blood vessels in the cartilage were perceived. These histological findings were probably related to osteochondritis.[27] A study in fattening pigs, showed that the prevalence of osteochondritis was related to the floor type. The prevalence of disease was higher in pigs, which were housed on a conventional floor type, compared to pigs housed on a deep litter floor.[28] These results, supported the hypothesis that piglet grid housing systems for goat kids, were related to number of clinically diseased kids. Besides the reduced osteoclastic activity and the enlarged number of trabeculae under the growth retardation zone in the radius, more severe abnormalities were presented in earlier pathological examination. The severe bone deviations, including bone cysts, bone collaboration, supplemental connective tissue, plugs and fissures, were probably related to the more chronic stage of disease, which was observed in 2015.

The underlying reasons for the rickets-like bone disease in rearing goat kids were still not completely understood, however the present study clearly described the emerging clinical features. The results of this study, showed that the vitamin D treatment and the parity of the does, were not related to the number of clinical affected kids and furthermore the concentrations of calcium, inorganic phosphate and 25-hydroxyvitamin D_3 in blood samples were not influenced. The present study, analyzed the effect of four risk factors for disease at the same time in the 235 kids. Because the effects of diet and heritability were doubtful, it was preferred to repeat the study and investigate the effects of both risk factors individually. To collect more information about the synthesis of 1α,25-dihyroxyvitamin D_3 in the kidney, it was also recommended to analyze the levels of the active vitamin D in kids. Considering to the abnormalities of bone, which were observed at post mortem examination, it was required to collect more information about the activity of bone. To investigate the activity of osteoclasts and osteoblasts, it was suggested to determine serum osteocalcin and C-telopeptide (CTx) levels and specify the activity of bone by the osteocalcin/CTx ratio.[29]

Acknowledgements

I would like to say a word of thanks to my supervisors dr. K. Lievaart-Peterson (Animal Health Service GD, Department of Small Ruminant Health Management) and dr. G. Koop

(Utrecht University, Department of Farm Animal Health) for their guidance during this research. I would also like to acknowledge dr. K. Peperkamp and dr. R. Dijkman (Animal Health Service GD, Department of Pathological Research), who performed pathological examination of the goat kids. Last but not least I would like to thank dr. A.B. Vaandrager and drs. M. Molenaar (Utrecht University, Faculty of Veterinary Medicine, Department of Biochemistry and Cell Biology) for the provision of the High Performance Liquid Chromatography and their laboratory assistance, drs. P. van den Oord (Veterinary practioner) for the collection of blood samples and Dr. G. Counotte (Animal Health Service GD, Department of Toxicology), who helped with the interpretation of the blood results.

References

1. Pugh DG. Appendix III. In: *Sheep and Goat Medicine.* 1st ed. ; 2002:451-544.

2. Kramer JW. Normal hematology of cattle, sheep and goats. . 2000;5.

3. Smith MC, Sherman DM. Chapter 4: Musculoskeletal system. In: *Goat Medicine.* ; 2009:131-148.

4. Reilly LK, Baird AN, Pugh DG. Chapter 9: Diseases of the musculoskeletal system. In: *Sheep and Goat Medicine.* 1st ed. ; 2002:223-254.

5. Dittmer KE, Thompson KG. Vitamin D metabolism and rickets in domestic animals: a review. *Vet Pathol*. 2011;48:389-407.

6. Dercksen DP, Berger JM. [Rickets-like bone changes in goat kids fed with artificial milk intended for calves]. *Tijdschr Diergeneeskd*. 1992;117:629-31.

7. Sykes AR. Chapter 53: Deficiency of mineral macro-elements. In: Aitken ID, ed. *Diseases of Sheep.* ; 2007:363-377.

8. Revell PA. Chapter 5: Metabolic bone disease. In: *Pathology of Bone.* ; 1986:113-146.

9. Anatoliotaki M, Tsilimigaki A, Tsekoura T, Schinaki A, Stefanaki S, Nikolaidou P. Congenital rickets due to maternal vitamin D deficiency in a sunny island of Greece. *Acta Paediatrica*. 2003;92:389-391.

10. Smith BS, Wright H, Brown KG. Effect of vitamin D supplementation during pregnancy on the vitamin D status of ewes and their lambs. *Vet Rec*. 1987;120:199-201.

11. Mølgaard C, Michaelsen KF. Vitamin D and bone health in early life. *Proc Nutr Soc*. 2003;62:823-828.

12. Paterson CR, Ayoub D. Congenital rickets due to vitamin D deficiency in the mothers. *Clinical Nutrition*. 2015;34:793-798.

13. Corbee R, Tryfonidou M, Grinwis G, et al. Skeletal and hepatic changes induced by chronic vitamin A supplementation in cats. *The Veterinary Journal*. 2014;202:503-509.

14. Testerink N, Ajat M, Houweling M, et al. Replacement of retinyl esters by polyunsaturated triacylglycerol species in lipid droplets of hepatic stellate cells during activation. *PloS one*. 2012;7:e34945.

15. Meynaud-Collard P, Asimus E, Mathon D, et al. Spontaneous recovery of experimental valgus deformity in lambs. *Veterinary and Comparative Orthopaedics and Traumatology (VCOT)*. 2009;22:356-362.

16. Ministerie van Economische Zaken. GUDAIR emulsie voor injectie bij schapen en geiten. Available at: http://db.cbg-meb.nl/MarketedAuth/v113936-90sr-05102014.pdf.

17. Stau A, Ganter M. Immune reactions to and side effects of a vaccination against paratuberculosis in milk goats. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2012;40:14-20.

18. Thompson K, Dittmer K, Blair H, Fairley R, Sim D. An outbreak of rickets in Corriedale sheep: evidence for a genetic aetiology. *N Z Vet J*. 2007;55:137-142.

19. Dittmer K, Thompson K, Blair H. Pathology of inherited rickets in Corriedale sheep. *J Comp Pathol*. 2009;141:147-155.

20. Smith MC, Sherman DM. Chapter 19: Nutrition and metabolic diseases. In: *Goat Medicine.* ; 2009:733-785.

21. Park YW, Haenlein GFW. Chapter 2: Goat milk. In: *Handbook of Milk of Non-Bovine Mammals.* John Wiley & Sons; 2008:11-135.

22. Hidiroglou M. Zinc, copper and manganese deficiencies and the ruminant skeleton: A review. *Canadian Journal of Animal Science*. 1980;60:579-590.

23. Zaeemi M, Mohri M, Naserian AA. Age related changes in serum biochemical profile of Saanen goat kids during the first three months of life. *Rev Med Vet*. 2016;167.

24. Muscher A, Hattendorf J, Pfeffer E, Breves G, Huber K. Hormonal regulation of phosphate homeostasis in goats during transition to rumination. *Journal of Comparative Physiology B*. 2008;178:585-596.

25. Kovács S, Wilkens M, Liesegang A. Influence of UVB exposure on the vitamin D status and calcium homoeostasis of growing sheep and goats. *J Anim Physiol Anim Nutr*. 2015;99:1-12.

26. Herm G, Muscher-Banse A, Breves G, Schröder B, Wilkens M. Renal mechanisms of calcium homeostasis in sheep and goats. *J Anim Sci*. 2015;93:1608-1621.

27. Zachary JF, McGavin MD. *Pathologic Basis of Veterinary Disease.* Elsevier Health Sciences; 2013.

28. van Grevenhof EM, Ott S, Hazeleger W, van Weeren PR, Bijma P, Kemp B. The effects of housing system and feeding level on the joint-specific prevalence of osteochondrosis in fattening pigs. *Livestock Science*. 2011;135:53-61.

29. Pastoureau P, Meunier P, Delmas P. Serum osteocalcin (bone Gla-protein), an index of bone growth in lambs. Comparison with age-related histomorphometric changes. *Bone*. 1991;12:143-149.

Appendix I: Scoring clinical features

Scoring clinical features of a rickets-like bone disease	
Score 0 Normal	**Posture and gait** • Regular gait; horizontal back **Joints (shoulder, elbow, costochondral)** • Scapula is narrowly connected with the body • Surface of costochondral joints feels regular and flat **Bones (humerus, ulna, radius, femur, tibia and fibula)** • Straight bones • Front- and hind legs are correctly in proportion
Score 1 Mild clinical symptoms	**Posture and gait** • Regular gait; horizontal back **Joints (shoulder, elbow, costochondral)** • Scapula is narrowly connected with the body • Surface of costochondral joints feels a little enlarged **Bones (humerus, ulna, radius, femur, tibia and fibula)** • Straight bones • Front- and hind legs are correctly in proportion
Score 2 Moderate clinical symptoms	**Posture and gait** • Regular gait; mild arched back **Joints (shoulder, elbow, costochondral)** • Scapula is narrowly connected with the body • Surface of costochondral joints feels enlarged **Bones (humerus, ulna, radius, femur, tibia and fibula)** • Mild bending of humerus. Ulna, radius, femur, tibia and fibula are straight. • Front- and hind legs are correctly in proportion
Score 3 Considerable clinical symptoms	**Posture and gait** • Mild stiff gait; arched back **Joints (shoulder, elbow, costochondral)** • Scapula is not narrowly connected with the body • Surface of costochondral junctions feels enlarged **Bones (humerus, ulna, radius, femur, tibia and fibula)** • Bending of humerus, ulna and radius. Femur, tibia and fibula are straight. • Front legs seems a little too short in relation to the hind legs
Score 4 Severe clinical symptoms	**Posture and gait** • Stiff gait, arched back and unwillingness to stand **Joints (shoulder, elbow, costochondral)** • Scapula is not narrowly connected with the body (exorotation) • Surface of costochondral joint feels enlarged **Bones (humerus, ulna, radius, femur, tibia and fibula)** • Widespread bending of humerus, ulna and radius. Mild bending of femur, tibia and fibula. • Front legs seems to short in relation to the hind legs

* Scores were awarded when three out of five scoring points matched the clinical condition of the goat lamb.

Appendix II: Classification of clinical features

Clinical features of a rickets-like bone disease classified into categories	
Category 0	Goat kids were awarded as normal (score 0) eight of eight times considering to the scoring table out of Appendix I.
Category 1	Goat kids were one or more of eight scoring moments classified as score 1 out of the scoring table for clinical features out of Appendix I.
Category 2	Goat kids were one of the eight scoring moments classified as score 2 or 3 out of the scoring table for clinical features out of Appendix I.
Category 3	Goat kids were two or more of the eight scoring moments classified as score 2 or 3 out of the scoring table for clinical features out of Appendix I.

** Only the highest category, in which a goat kid was classified, was registered.

Appendix III: Specification of kids