AN INVESTIGATION OF SPONTANEOUS HUMERI FRACTURES IN NEW ZEALAND DAIRY CATTLE

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Abstract

This dissertation reports the result from an investigation of spontaneous humeri fractures that happened in New Zealand dairy cattle population between 2007 and 2012. As the syndrome was relatively new in New Zealand and elsewhere, the case definition was derived from a case series report in 2008.

Questionnaires were mailed to potential respondents whose farm suspected to have the outcome of interest as recommended by various parties (veterinarians, researchers, farmers) who had seen or knew farms, which had recorded the spontaneous fractures' syndrome.

A total of 149 cases was reported in the five-year observation period from 2007 to 2012 with an increasing trend (r^2=0.71) in 22 farms that responded to the study. Out of the 149 reported incident of spontaneous fracture, 115 case details managed to be gathered and analysed. The result showed that the spontaneous fracture syndrome exhibit a spatial clustering, which was utilised to compare the persistence of identified risk factors in the different geographical cluster. The spontaneous fracture syndrome also displayed an observable temporal pattern whereby the occurrences were recorded in early spring, peaked in late spring and ended in early summer in every observation year. All case animals were female, relatively young with a noticeable biphasic age profile (24-31 months and 36-40 months), good body condition and reproductively active.

As the fracture occurrences coincided with the period of high calcium demand, transient osteoporosis to pregnancy and lactation was hypothesized to cause the spontaneous humeri fractures in New Zealand dairy cattle population between 2007 and 2012. Other factors which could be the risk factors based on the persistency in the presence prior, and at the time of fractures were: lacked of dietary calcium in the growing stage, the breed of the dairy cows, high-quality index (breeding and production worth), increased walk speed of the lactating cows, and the involvement of a truck for heifer transfer from grazier to the case farm.

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Chapter 1

1 A general introduction to the bone biology and literature review

1.1 Introduction

Bone is the hardest specialised connective tissue of the vertebrates which contributes between 15-22% of total body weight of ruminants depending on the age group; the lower age group consistently shows the higher bone to weight percentage (Martinsson & Olsson, 1993; Piedrafita et al., 2003; Refsgaard Andersen, 1975). The bone strength is chiefly contributed by the mineral salt that constitutes 45% of adult mammals' wet bone weight, which predominantly calcium (37%) and phosphorus (18.5%) (Carter & Spengler, 1978). Based on the strength per unit weight, bone (femur) is apparently stronger than aluminium alloy (Fung, 1993; Kaplan, Lee, & Keaveny, 1994) hence spontaneous fracture on a healthy bone is next to impossible. While bones appear to be rigid, it is inherently dynamic and endures active process of model and remodel throughout life (Delahay 2010) as a response to physiological need, trauma, disease, use or disuse (McGeady, 2006), which differentiate bones from any inert hard materials such as aluminium alloy.

This general introduction will give an overview of bone biology and describe the potential differential diagnosis of spontaneous/low force fracture that serves as a guide in investigating the potential aetiology of the spontaneous humeri fracture. The literature cited may come from different species besides dairy cattle, especially in introducing the bone biology.

1.2 Bone composition

As any other connective tissues, bone tissue that is originated from the embryonic mesoderm, contains cells, fibre and amorphous ground substance(McGeady, 2006). The cellular components of bone that actively involved in model and remodeling process are osteoblast, osteocytes and osteoclast(Jee, 1989). The fibrous component of bone tissue is primarily type one collagen. The amorphous substances of the bone are mainly organic materials that excreted by the cellular component: sulphated glycosaminoglycan and glycoprotein; as well as inorganic materials that contribute to the tensile strength known as calcium-phosphate-carbonate complex (Gentili & Cancedda, 2009).

1.2.1 Bone cells

1.2.1.1 Osteoblasts

The osteoblasts are responsible for the construction of the bone tissue. Osteoblasts derived from the lineage-restricted potential cells in the mesenchyme that are able to differentiate into either fat cells or osteoblasts (McGeady, 2006). The presence of Cbfa1 promotes the osteoblasts' formation (Karsenty, 2000). Histologically, osteoblasts are cuboidal and columnar in shape with large, round, centrally located nuclei and could be found on the bone lining(Jee, 1989). The osteoblasts have receptors for systemic hormones such as oestrogen and PTH (Ernst, Parker, & Rodan, 1991; Ono et al., 2007)indicating that the endocrine system may dictates the osteoblast activities; and secrete other local regulators that could influence other cellular processes such as activation (via RANK-Ligand) or neutralisation (via OPG) of osteoclastogenesis (F. Gori et al., 2000). The activated osteoblasts synthesize organic matrix (osteoid), which will be calcified and entrapped the cells in it. In the matrix, osteoblasts differentiate into the osteocytes. The remaining osteoblasts on the bone lining are either differentiates and becoming the lining cells that serve as a protective cover of the new bone or undergo apoptosis and disintegrate (Xing & Boyce, 2005).

1.2.1.2 Osteocytes

The osteocytes are the entrapped, mature osteoblasts that become less active in the secretions of the organic matrix (McGeady, 2006). The site where osteocytes are located is referred to lacuna. As the osteocytes embedded deeper into the mineralised matrix, the cytoplasmic content becomes less but the cytoplasmic processes that lie within the calcified matrix establish contact with other osteoclasts (Eurell & Van Sickle, 1998). The cytoplasmic canals, known as the canaliculi plays an important role in low molecular weight substance transfer between osteoclasts and the stimulation of the cell linings or the osteoblast precursor for a targeted remodelling process (Bonewald, 2007).

1.2.1.3 Osteoclasts

The osteoclasts are the bone cells that responsible for bone matrix resorption. The osteoclasts act to dissolve the inorganic component of the bone matrix under through the reduction of environment pH and the degradation of organic component via the proteolytic enzymes (McGeady, 2006). The attachment site of the osteocytes to the bone in which active bone resorption occurs is called ruffle borders. The matrix-resorption activities produce erosion lacuna that remains even after the cells are no longer present. Histologically, the active osteoclasts are large (up to 150um in diameter), multinucleated (up to 50 nuclei), acidophilic cytoplasm and located at the surface of the surface of bone (Eurell & Van Sickle, 1998; Nicolin et al., 2006). Osteoclasts derived from the linage-restricted pluripotential cells that also produce macrophages and monocytes, which are circulating in the blood and bone marrow (Eurell & Van Sickle, 1998). The osteoclast precursors that are activated by the osteoblastic RANK-L merge to become multinucleated, large cells. While resorping the bone matrix, BMPs will be released, which regulates the osteoclastic activities (Dieudonné, Foo, Van Zoelen, & Burger, 1991). When the bone resorption is over, the osteoclasts undergo apoptosis and disintegrate (Xing & Boyce, 2005).

1.2.2 Bone matrix

The bone matrix is consisting of the other two components (beside cells) that classified bone as a type of connective tissue: fibre and amorphous compounds (McGeady, 2006). The fibres that are produced by osteoblasts are mainly the collagen type-I that exist in triple helices form. The collagen provides strength to the bone by their spiral orientation in the osteon lamella and the right angle alternation to the adjacent lamella (Eurell & Van Sickle, 1998). The inorganic substances of the amorphous compound of the bone matrix are chiefly calcium and phosphate. The minerals are deposited within the collagen fibre network in the form of crystal thus further augment the tensile strength of the bone. The inorganic substance is accounted for approximately 60% of the dry bone weight (Carter & Spengler, 1978). The bone matrix also contains several proteins that possess important bone homeostasis function. The expression of the matrix protein is controlled and coordinated by the encoding genes through complex mechanisms that are directly or indirectly modulated by systemic hormones (Zaidi, 2007). The function of the matrix proteins may overlap, but certain matrix proteins have specific deficiency effect on bone. The function and regulation of the bone matrix have been reviewed elaborately by Gunberg (2003) and Young (2003), and the summary of bone matrix function is shown in table 1-1.

Table 1-1: Summary of the function of Bone Matrix Protein and the effect of deficiency as demonstrated in gene deleted mice

Group	Name	Functions in bone	Effect of deficiency (Knock-out gene in mice)
Proteoglycans	Biglycan	Collagen fibril formation	Reduction in osteoblast precursor and less responsive to TGF-β hence causing a lower peak bone mass
	Decorin	Regulate TGF-β activities	Amplify the effect of biglycan deficiency
Glycoprotein	Fibronectin	Binds to integrin receptors; proliferation and differentiation of osteoblast;;	Death at the early stage
	Osteonectin	Initiate mineralization of collagen ; matrix metalloprotein production	Osteopenia in older animals
	Osteopontin (OPN)	Promotes osteoclast attachment to mineralised surface; anti-apoptosis	Resistant to PTH, reduced mechanical stress- induced bone resorbtion; increase in trabecular bone volume
	Sialoprotein	Bone primary mineralisation	Higher trabecular bone volume, associated with lower bone remodelling
	Matrix extracellular protein (MEPE)	Inhibitory role in bone formation and mineralization; involved in renal phosphate regulation	Increased bone formation and bone mass
Vit K dependent proteins	Osteocalcin (OCN)	Regulation of bone mineralisation	Increase in functional and quality of bone mass
	Matrix-gla-protein (MGP)	Regulation of bone mineralisation – promote mineralisation	Disproportionate mineralisation of cartilage and arteries
References: (Gundberg, 2003; Young, 2003)			

1.3 Bone function

With the variation in the shape, density, tensile strength and location, bone serves many important functions including support, protection, movement, blood cells' production, mineral storage (Clarke, 2008; Eurell & Van Sickle, 1998; Jee, 1989; McGeady, 2006).

1.3.1 Support function

The common function of any connective tissue is to provide cohesion and internal support. The bone assists the function of other soft tissues by providing attachment sites. Ligaments, muscle and tendons are the examples of tissue that benefits directly the generic function of bone. The bone also supports another function such as hearing through the ability to vibrate as a response to audio stimuli (Monsell, 2004); reproduction in dogs (Kent & Carr, 2001)-the presence of Os penis enables coitus to happen.

1.3.2 Protection function

The bone provides significant protective properties on many vital organs via its shape and tensile strength (Clarke, 2008; Kent & Carr, 2001). The body systems that enjoy the protective properties of bones;

- The central nervous system enjoys an extensive protection by the bones by the skull and vertebrae that act as a tough case which provides shelters that withstand the external force.
- The circulatory and respiratory system in the thoracic cavity is protected by rib cage and sternum.
- Visual system receives a substantial protection by the virtue of ocular sockets.

1.3.3 Movement function

Animal movement is made possible by the synchronised contraction and relaxation of muscles, which attached to the bone. Bones act as levers that assist the body movement on different planes. The type of movement is related to the structure in which two bones are attached. The socket and ball attachment (hip and shoulder) allows all planes movements; hinge on attachment (elbow) allows one plane movement; gliding attachment (vertebrae) allows the flat surface of one slip over the other (MacConaill, 1948; Vogler & Bojsen-Moller, 2000).

1.3.4 Blood cells production function

Blood cells' production (hematopoiesis), prenatally, take place happens in many organs such as liver, spleen, lymph node and bone marrow (Tavian & Peault, 2005). After birth, the production is taken over mainly by bone marrow and partly by lymph node. Nevertheless, maturation or activation of many blood cells produced by bone marrow happens in the other organs. The production began with the differentiation of hematopoietic stem cell under the influence of many sets of cytokines (Taichman, 2005) to produce different cell progenitor that will give rise to specific blood cells (Pittenger et al., 1999).

1.3.5 Mineral storage function

The bone is accounted for 15-22% of body weight (Piedrafita et al., 2003). Approximately, 45% of wet bone weight is mineral salts; of which 37% is calcium (Carter & Spengler, 1978). Hence, via a simple calculation, a 350kg cow may have roughly 19kg of calcium, which is stored in the bone. The above example illustrates the storage capacity of bones. When it is necessary, the mineral reserve in the bone can be used to maintain the narrow range of plasma calcium of 8-10mg/dl (Goff, Reinhardt, & Horst, 1991). The conditions which normally necessitate the withdrawal of bone mineral reserve are pregnancy and lactation (Braithwaite, 1983; Oliveri, Parisi, Zeni, & Mautalen, 2004). When the mineral deficiency has been lifted, the mineral deposition takes place again to the optimal level. If the bone mineral withdrawal was prolonged, for example, in extended lactation, the bone density could be significantly reduced (Sowers M & et al., 1993). The bone's strength, at the same time could be compromised as the mineral crystal function is to strengthen the bone structure (Carter & Spengler, 1978).

1.4 Long bone growth

The long bone growth starts in the embryonic stage. The mesodermic layer that aggregates under the influence of BMP, differentiate into perichondrium and become the template of future bone (McGeady, 2006; Sykaras & Opperman, 2003). The interstitial growth at the both end in the perichondrium is regulated by PTHrp and Indian Hedge Hog (ihh). PTHrp stimulates the proliferation of chondroblast, which elongates the cartilaginous template while ihh stimulates the differentiation of chondroblast into chondrocytes, which increases the width (Karp et al., 2000; McGeady, 2006). TGF-β, however, regulates the hypertrophy of the chondrocyte; therefore, indirectly regulates ihh secretion by the chondrocyte (Vortkamp et al., 1996). The chondrocytes excrete substance such as amorphous ground substance, collagen and fibronectin to fill in the matrix. Alkaline phosphatase that secreted by the hypertropic chondroblasts promotes mineral deposition of the matrix hence trapped the chondrocytes in it (Orimo, 2010). As the chondrocytes entrapped in the calcified matrix degenerates and disintegrates, the primitive medullary space is formed in the diaphysis. The medullary space will be invaded by the periosteal bud form the developing periosteum that consists of blood vessels and mesenchymal cells; that later differentiates into pre-osteoblast and pre-osteoclast (McGeady, 2006). The invasion of the periosteal bud initiates the remodelling of the calcified chondrocytes matrix, which is known as the primary centre of ossification. The secondary centres of ossification occur in the similar manner of the primary ossification, but it occurs at the end of the long bone template (epiphysis) (Eurell & Van Sickle, 1998).

The growth in the long bones is performed by the cartilaginous layer that remains after the invasion of the primary and secondary periosteal bud. The cartilaginous layer, which is known as physes, grows inwards into the diaphyseal region owing to the columnar arrangement of the chondrocytes(McGeady, 2006). The reserve zone of the growth plate, which differentiates into chondroblast, move towards the diaphysis to enter the phases of proliferation, hypertrophy, resorption and ossification (Eurell & Van Sickle, 1998). The rates of chondrocytes' productions in every column are highly regulated by the local hormones; ihh controls the production of PTHrp, and PTHrp promotes the chondroblast differentiation. The local hormones' regulation is important to ensure the simultaneous growth of the growth plate; hence uniform elongation of long bone can be seen (Karp et al., 2000; McGeady, 2006).

The process of width and circumference growth of long bones is the effect of a highly regulated, different rate of osteoblast and osteoclast activities in remodelling the ossified bone matrix (Burr, 2002). The endosteal resorption lags behind the periosteal expansions hence the bone gains the width but maintains the shape (Eurell & Van Sickle, 1998). If the osteoclast fails to resorb the bone, the bone becomes dense from the mineralisation of the cartilage (Van Slyke & Marks, 1987).

1.5 Growth in flat bones

The flat bones such as skull, mandible and clavicle do not undergo the same elongation process as in long bones (McGeady, 2006). This is because of the absence of cartilage stage in the flat bones (Eurell & Van Sickle, 1998). The fibroblast within the well-vascularized connective tissue that entrapped the future flat bones differentiates into osteoblast, produce the osteoid matrix which subsequently calcified to form bone spicules. This isolated bone development within connective tissue is known as the centre of ossification, which intensifies and radiates into several directions to form cancellous bone. Later, the osteoclasts resorb the cancellous bone and replaced by lamellar bone. The outer mesenchyme also differentiates into the periosteum that fuses with both sides of the developing cancellous layer to produce bone plates. The expansion of the centre of ossification is responsible in increasing the thickness of the flat bone (Eurell & Van Sickle, 1998; McGeady, 2006; B.R Olsen, 1999; B. R. Olsen, Reginato, & Wang, 2000; Yang, 2009).

1.6 Bone remodelling

During the inert situation, sclerostin is secreted by osteocytes (Poole et al., 2005); travel through canaliculi to the bone marrow, and inhibits Wnt pathways that would produce the cellular expression which stimulates bone formation (Li et al., 2005). In situation that leads to the death of osteocytes, two major events will take place:

1. Sclerostin that normally be secreted will be reduced hence the Wnt pathways for cell expression on certain bony location will resume (Li et al., 2005; Poole et al., 2005).
2. The adjacent osteoclasts secrete other factors such as prostaglandin, nitric oxide and growth factors that assist in the remodelling process (Bakker, Soejima, Klein-Nulend, & Burger, 2001).

The adjacent stromal cells that are free from the influence of sclerostin generate pre-osteoblasts and secrete M-CSF that assists in preosteoclastic generation; while the lining cells merge with the nearby blood vessels and allowing pre-osteoclastic cells to flood the bony area to be remodelled (Matsuo & Irie, 2008). The pre-osteoblasts proliferate and secrete cytokines while expressing the surface RANK-L which activates the preosteoclast to merge and form the multinucleated osteoclasts (Matsuo & Irie, 2008). The osteoclasts bind and digest the bone matrix using the H ion and Cathepsin-K until receiving signals to cease the activity by the bone growth factor: BMPs, IGF and TGF-B (Dieudonné et al., 1991; Fuller et al., 2008). The osteoclasts then undergo apoptosis under the influence of oestrogen and other factors as to allow for bone formation by osteoblasts (Kameda et al., 1997). The pre-osteoblasts, under the influence of systemic (estrogen, PTH) and local hormones (IL-1,_IL-6) proliferate and differentiate into osteoblasts (Neve, Corrado, & Cantatore, 2011). The osteoblasts stop expressing surface RANK-L and secreting OPG that collectively stops the activation of pre-osteoclasts by the pre-osteoblasts (Kobayashi, Udagawa, & Takahashi, 2009). The mature osteoblasts lined-up the resorption cavity and produce osteoid. Some osteoblasts trapped in the osteoid and while the rest will continue to produce the bone matrix; some differentiate into osteoblasts in the bone matrix, or become flat cells that line the bone (Hill, 1998). The new, mature osteocytes re-establish the link between the bone and the surface cells and continue transmitting sclerostin to surface cells that suppress the cellular expression by the Wnt pathway (Poole et al., 2005).

1.7 Bone regulators

1.7.1 Local regulators

Besides the regular endocrine hormones that modulate the bone dynamics, bone cells also produce local bone regulators, which act on the nearby cells; influencing the proliferation, differentiation, or survival (Hill, 1998). The production of the local regulators such as sclerostin by osteocytes in preserving the quiescence of bone environment may be independent of endocrinal hormones (Poole et al., 2005) but many other local regulators such as IGFs are produced in the presence of PTH (Lombardi et al., 2010).

1.7.1.1 Growth factors

Growth factors are polypeptide that is synthesized by specific tissue, which act by binding to the specific trans-membrane receptors of the target cells and activate the transcription-translation mechanism to produce proteins for intra cellular usage or exported (Trippel, Coutts, Einhorn, Mundy, & Rosenfeld, 1996). The general function of the growth factors as demonstrated in the cell culture experimental studies are influencing proliferation, differentiation and protein synthesis in osteoblastic cultures as well as bone formation in animal models (Bolander, 1992; Boyne, 1996; A. Yamaguchi et al., 1996). There are several families of growth factors identified: bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF-β), insulin-like growth factors I and II (IGF-I and IGF-II), platelet derived growth factors (PDGF) and fibroblast growth factors (FGF); however, only BMPs are known to provoke heterotopical bone formation by osteoinduction of the undifferentiated mesenchymal cells.(Solheim, 1998). The BMPs is important in the regulation of bone induction, maintenance and repair (Sykaras & Opperman, 2003). A simple example that could explain the importance of BMP is the fate of preosteoblast; without the presence of BMP, Cbfa-1 that activates the gene transcription in the stem cells to differentiate into osteoblast may not be produced, hence the stem cells would turn into adipose cells (Akira Yamaguchi, Komori, & Suda, 2000). Most of these growth factors are stored in the bone matrix (Bonewald & Mundy, 1990); therefore, bone resorption by osteoclast may release the growth factors and activates the osteocytes' lineage proliferation and differentiation, which promote bone formation.

1.7.1.2 Cytokines

Cytokines are soluble signaling proteins that produced by haematological cells, which could act as a local regulator of bone cell metabolism (Goldring & Goldring, 1990). Osteoblasts produce a few cytokines: macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factors (GM-CSF), interleukin-6 (IL6) and tumor necrosis factors (TNF-α), which serve as paracrine regulators of osteoclasts as well as autocrine regulators for osteoblast (Metcalf, 1989). The cytokines' production is highly regulated by other regulators such as PTH (Feyen, di Padova, Trechsel, & Elford, 1989) and oestrogen (Masiukiewicz, Mitnick, Grey, & Insogna, 2000). Although the specific function of each cytokine may include the both bone formation and resoption, the main function of cytokines is more related to the latter. IL-6 that influence osteoblast to express RANKL and M-CSF that important in the maturation of osteoclast are the key element for osteoclastogenesis that leads to bone resorption. (Teitelbaum, 2000)

1.7.2 Systemic regulators

1.7.2.1 Hormones

Hormones are organic chemicals that are made by a group of specialised tissues that act as a messenger for other parts of tissue with the specific receptor for the hormone to act. Hormones that are excreted directly into the blood stream for the transportation to the target tissue are called the endocrine hormones, whereas the group of hormones that are secreted through a duct before they reach the target tissue with or without the medium of blood as the transporter is called the exocrine hormones (Griffin & Ojeda, 2004). The process of getting the hormones to produce the intended effect may not be direct. Briefly, in order to have the growth of the long bones under the influence of thyroid hormones, thyrotropin-releasing hormone has to be produced by the hypothalamus to stimulate the anterior pituitary to produce TSH. The TSH will stimulate the thyroid gland to produce thyroid hormones that will act on the specific receptors in the bone tissue to produce the desired effect (Duncan Bassett & Williams, 2003). When the intended effect has been fulfilled, a negative feedback will be produced to signal the tissue to stop producing the hormones (Scanlon & Toft, 2000).

The following paragraph illustrates on how the systemic hormone inter-related to each other in maintaining calcium homeostasis, which affects the bone dynamic. The example given here is relating the dependency on PTH for vitamin D activation and the antagonism between PTH and calcitonin on maintaining the narrow calcium plasma range.

1.7.2.1.1 Parathyroid hormone

Parathyroid Hormone (PTH) is a peptide hormone produced the chief cells of the parathyroid gland. PTH is responsible in increasing the blood calcium level by acting upon the PTH-receptor in the bone and kidney. In the bone tissue, PTH reduces sclerostin (Bellido et al., 2005), and OPG but increases the expression of RANKL (Huang et al., 2004) which collectively promotes bone resorption. In kidneys, PTH acts to increase the blood calcium level by increasing the calcium reabsorption that primarily occurs in the distal tubules (Kennedy, Flanagan, Mills, & Friedman, 1989) and enhancing the phosphorus excretion (Clark, 1991) thus the calcium-phosphorus blood ratio can be increased. Concurrently, PTH involves with the modulation of 25-hydroxy vitamin D activation into 1, 25-dihydroxy vitamin D (Brenza et al., 1998). The activated vitamin D is required to activate calbindin (Hemmingsen, 2000)to intensify the intestinal calcium absorption. The secretion of PTH is highly regulated by the calcium level in the blood; a low blood calcium level will trigger the secretion while a high blood calcium level will serve as a negative feedback to the parathyroid gland (Loupy et al., 2012). Unfortunately, a low blood magnesium level may also compromise the regulation mechanism of the PTH secretion (Rude, Oldham, Sharp, & Singer, 1978). In normal circumstances, magnesium modulates the calcium release from the sarcoplasmic reticulum (Meissner, Darling, & Eveleth, 1986)of the calcium-sensing receptors hence the PTH regulation is triggered primarily by the influx of the extracellular calcium (Kantham et al., 2009). In other words, the state of hypomagnesemia could create a false signal of plasma calcium sufficiency, which incapacitates the PTH secretion which could lead to the state of hypocalcemia.

1.7.2.1.2 Calcitonin

Calcitonin is a peptide hormone produced within the parafollicular cells of the thyroid gland when there is an increase above the normal plasma calcium level (Griffin & Ojeda, 2004). It works antagonistic to PTH by temporarily decreasing the blood calcium level upon stimulating the calcitonin-receptor in the bone, kidney and intestine. Calcitonin receptors that are found abundant in the osteoclast suggesting calcitonin preserve the bone by inhibiting osteoclast activities (Samura, Wada, Suda, Iitaka, & Katayama, 2000). The osteoclasts' inhibition mechanism by calcitonin is important in skeleton preserving effort, especially during the physiologically increased in calcium demand such as pregnancy and lactation (Kovacs & Kronenberg, 1997). In kidney, calcitonin inhibits the tubular calcium reabsorption (de Rouffignac & Elalouf, 1983), while the role of calcitonin on the intestinal calcium absorption is uncertain as the result from experimental studies on animals was not unanimous (Jaeger, Jones, Clemens, & Hayslett, 1986; Matsui, Kuramitsu, Yano, & Kawashima, 1983). Calcitonin production can also be stimulated by other stimulants beside the blood calcium level such as chronic elevation of gastrin (Erdogan, Gursoy, & Kulaksizoglu, 2006). Together with PTH, calcitonin is important in maintaining the calcium-phosphorus homeostasis.

1.7.2.1.3 Vitamin D

Vitamin D is a special lipid hormone that can be synthesized from the cholesterol in the skin by the UVB (290-310 nm wavelength) (Slominski & Wortsman, 2000). The product of the photosynthesis: a pre-vitamin D (7-dehydrocholesterol) (Tian & Holick, 1999), has to be activated before functioning as a regulator of bone density. The pre-vitamin D can be converted into 25-hydroxy vitamin D (calcidiol) in the liver. In the presence of PTH, calcidiol can undergo hydroxylation to form 1, 25-dihydroxy vitamin D (calcitriol) in kidneys (Holick, 1994). This active form vitamin D binds to the vitamin D receptors which are located in the kidney and intestine to regulate the expression of transport protein such as TRVP6 and calbindin. The TRVP6 act as channels that allow selective uptake of calcium ion into the intestinal epithelium (den Dekker, Hoenderop, Nilius, & Bindels, 2003). While the calcium ions are in the enterocyte, Calbindin binds to the ion and facilitates the movement across the enterocytes (Bolt, Cao, Kong, Sitrin, & Li, 2005). The presence of calbindin in bone and cartilage suggest that vitamin D may involve in bone tissue mineralisation (Balmain, 1991).

There are many other systemic hormones that may influence the dynamicity of the bone. Table 1-2 summarises the net effect of selected systemic hormones on the bone density.

Table 1-2: Summary of the net effect of systemic hormon on the bone dynamics

Increase Bone Absorption	Ref	Decrease Bone Absorption	Ref
Parathyroid hormone	(Jilka et al., 1999; Lindsay et al., 1997; Mcsheehy & Chambers, 1986)	Calcitonin	(Ongphiphadhanakul, Piaseu, Chailurkit, & Rajatanavin, 1998; Samura et al., 2000; van der Wiel et al., 1993)
Glucocorticoids	(Ernesto Canalis & Delany, 2002; Gronowicz, McCarthy, & Raisz, 1990; Lukert & Raisz, 1990)	Gonadal steroids (androgen)	(Chiang et al., 2009; Francesca Gori, Hofbauer, Conover, & Khosla, 1999; Vanderschueren et al., 2004)
Thyroid Hormone	(Allain, Chambers, Flanagan, & McGregor, 1992; Mundy, Shapiro, Bandelin, Canalis, & Raisz, 1976; Uzzan et al., 1996)		
Increase Bone Formation	**Ref**	**Decrease Bone Formation**	**Ref**
Growth hormones	(Andreassen, Jørgensen, Oxlund, Flyvbjerg, & Ørskov, 1995; Menagh et al., 2010; Ohlsson, Bengtsson, Isaksson, Andreassen, & Slootweg, 1998)	Glucocorticoids	(Kim et al., 2007; Kim et al., 2006; Rauch et al., 2010)
Vitamin D metabolites	(Baldock et al., 2006; Bordier et al., 1978; Erben, Scutt, Miao, Kollenkirchen, & Haberey, 1997)		
Gonadal steroids (estrogen)	(Chow, Tobias, Colston, & Chambers, 1992; Sjögren et al., 2009; Yilmaz et al., 2005)		

1.8 Biomechanics of bone

Any solid materials inherently have the properties of stiffness and toughness. The stiffness of a solid material, which normally estimated using Young's modulus of elasticity using pascal (Pa) as the measurement unit, is a measure of the force over a reversible displacement (Riley & Zachary, 1989). A stiff material requires a high amount of force to produce a unit of reversible strain. Currey (1999) stated that a solid material can be categorised as stiff if it had a Young's modulus value higher than 5GPa. A quick example to comparatively illustrate the stiffness properties of solid materials using Young's modulus value: the reinforced thermoplastic natural rubber (0.34GPa)(Sahrim, Mou'ad, Yahya, & Rozaidi, 2011), bovine cortical femur bone (14GPa) (J. D. Currey, 1979)and diamond (1050GPa) (Savvides & Bell, 1993).

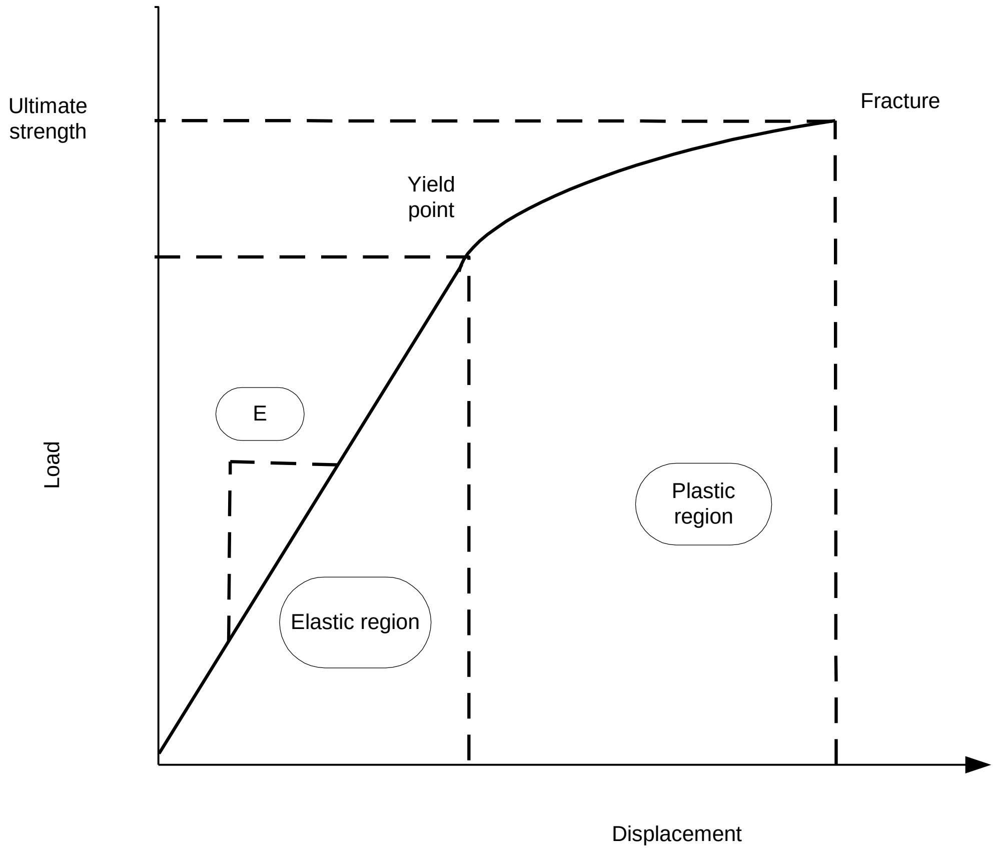


Figure 1-1: A standard load-displacement plot of a solid material. As the load (force) increase, the material displacement takes place. The unit for load may be in the form of pressure (Pascal, N/cm2) but displacement can take length (cm. inch) or percentage of changes

The maximum force on which a material could return to the original shape upon the removal of force correspond to the yield point (Riley & Zachary, 1989). On the load-displacement plot (Fig 1-1), the yield point represents the gradual transition between the linear and the non-linear curve. Any forces placed on a solid material, which exceeded the yield point may permanently misshape the solid material even after the force is removed. When a solid material breaks, it indicates that the ultimate strength of the material was exceeded.

The displacement value that corresponded with the yield point divides the stress-strain plot into two; the lower displacement region that covers the linear plot of the load-displacement curve is called the elastic region, and the higher displacement region that covers the non-linear curve of is called the plastic region. The size of the elastic region can be used to compare the stiffness of two materials. The smaller the elastic region means the lesser work is needed to misshape the material. The size of the plastic region indicates the brittleness of the material. The smaller the plastic region means the lesser work of force is needed to break the materials after the yield point has been reached. Both areas, the elastic and plastic region, indicate the toughness property of a solid material.

The bone tissue is a unique lightweight composite material, which exhibits excellent strength and stiffness to provide structural support (McNamara, 2011) . The mineral and collagen content of bones which contributes to rigidity and flexibility, strongly affect the mechanical properties of bone(Follet, Boivin, Rumelhart, & Meunier, 2004). The composition varies depending on the function of the bone (J. D. Currey, 1979). Tympanic bulla (earbone), which function is to convert the audio wave into the audio signal through vibration, has the mineral content of 86% and Young's modulus of 31.3GPa, while the femur bone, which function is to support weight, has 67% mineral content and Young's modulus of 13.5GPa (J.D. Currey, 1999).

On the age factor, based on Brear (1990), three months old polar bear had a lower femoral bone mineral content (235 mg calcium/g bone) than of a three years-old polar bear (259 mg calcium/g bone). The Young's modulus of the three years-old polar bear was three times higher than the three months-old polar bear. This shows that the young can withstand a large bone displacement better than the old, owing to the lower bone mineral content, which leads to the phenomena of green stick fracture in the young: bone undergoes a large displacement without break. As the elasticity decreasing with age, the strength of the bone increases with the increase of bone mineral content. The advancement of age from three months to three years had the calcium content per gram femur bone of the polar bear increase by 10%; the yield strength increases by 70% (Brear, Currey, & Pond, 1990) to meet the increasing demand in its structural functionality. As the animal grows bigger, the weight needed to be supported efficiently. The increasing demand in tensile strength, especially on the limb's bone is met by increasing the mineral content and cortical diameter of the diaphysis (Follet et al., 2004; C. H. Turner, 2006).

The strength of a long bone is not uniform. As for an example, adult human femur has longitudinal compressive strength of 205MPa and a longitudinal tensile strength of 135MPa (Joon, 1999). That means femur bone is easier to break when the femur is pulled that pushed longitudinally. Conversely, human femur has the shear strength of 52MPa (C. H. Turner, Wang, & Burr, 2001), which means pulling and rotating the bone half the forces needed to pull-breaking the shaft would cause the bone to break. A spiral or oblique fracture of long bone is an indication that shear stress involved in the fracture (C. H. Turner et al., 2001).

1.9 Low force fracture

Bone fracture is also known as the break of bone continuity (McGuigan, 2010). It happened when the loading force exceeded the ultimate strength of the bone causing irreversible permanent damage (Einhorn, 1992). Excessive force is needed to fracture a normal healthy bone due to the presence of mineral and collagen in the bone that strengthen the structure. Conversely, an excessive mineralisation of bone as in osteopetrosis does not contribute to the ultimate bone strength (Stark & Savarirayan, 2009). A standard has been proposed that any deviation from the normal bone mineral density exceed 2 the standard deviation of the normal mean BMD of young healthy population, would put the bone on a risk of fracture (Genant et al., 1999).

Low force or spontaneous fracture is a common term to describe the incident of bone fracture without obvious involvement of excessive external force (Dolinak, 2008; Horiuchi et al., 1988). The spontaneous fracture happened because the inherent strength to withstand fracture failure has been reduced tremendously that normal daily force load exceeded the ultimate strength of bone. Based on the underlying cause of the spontaneous fracture, the fracture can be categorised as pathologic (Torbert & Lackman, 2010) or fatigue(J. M. Morris, 1968). The importance of identifying the underlying causative agent of low force fracture is that the intended treatment options and post –treatment care may not be the same (Hallel, Amit, & Segal, 1976; Torbert & Lackman, 2010).

The following paragraph is to illustrate some of the common conditions that would that would lead to the reduction of bone ultimate strength and posed an increased risk of low force fracture.

1.9.1 Osteoporosis

Osteoporosis is a major health threat to the aging human population worldwide on which a global strategy was indicated by WHO to prevent and control the osteoporosis-related complications (Genant et al., 1999). The WHO task force on osteoporosis predicted that the worldwide incident of hip fracture due to osteoporosis that was recorded 1.7 million in 1990 could increase to more than 6 million in 2050 (Genant et al., 1999). Osteoporosis is generally known as the reduction of bone mass and micro-architectural deterioration of bone tissue, which increases the bone fragility (Rosenzweig & Pignolo, 2010). As bone is a dynamic tissue which undergoes constant remodeling, change in bone density is a relative statement. Therefore, WHO has specifically defined osteoporosis as the reduction of BMD >

2 standard deviation of the BMD of healthy young age group (World Health Organisaton, 1994). During the embryonic stage, the bone started to increase the mineral content and density from the second to third trimester (McGeady, 2006). Approximately, 35 grams of calcium are needed to support human embryonic bone development (Kovacs & Kronenberg, 1997). After birth, bones continue to increase the mineral content but the bone density may not increase as fast due to the rapid elongation of the axial and appendicular bones. The optimum bone density reaches the optimum level only after the puberty and remains within the same range until 50 (female) and 65 (male) for healthy normal human being (Bachrach, 2001). Reduction in bone mass is a result of imbalance in the activities of osteoclast and osteoblast. Obviously, the osteoclast outperformed osteoblast. Reduction of bone mass in osteoporosis is a subclinical condition until the specific function of bone is affected. Human case of osteoporosis is usually presented with tenderness, recurring back and neck pain, which detected at the advance stage of osteoporosis (Glaser & Kaplan, 1997; Orwoll & Klein, 1995). There are many risk factors claimed to cause such imbalance, including aging, physical stress, nutritional deficiency, hormonal and genetic disorder, as well as the lifestyle (Genant et al., 1999).

Osteoporosis can be grouped into two major categories depending on the underlying cause: Primary and secondary osteoporosis.

1.9.1.1 Primary osteoporosis

Primary osteoporosis is a bone disorder of unknown origin or as a result of aging (Glaser & Kaplan, 1997). The peak bone mass is reached within a few years after the closure of the long bone epiphyses, and the BMD remains within its normal range until the sex hormones declined significantly (Bachrach, 2001). Primary osteoporosis is associated with the gradual declination of sex hormone (Riggs, Jowsey, Kelly, Jones, & Maher, 1969). The reduction in bone density is accelerated when the sex hormone producing organ is surgically removed (Russell T. Turner, Wakley, & Hannon, 1990) or the permanent natural cessation of the primary function occurred as in the post menopause period (R. T. Turner, Riggs, & Spelsberg, 1994).

In female, oestrogen is produced primarily by the ovaries but other tissues such as liver and adrenal gland and adipose has the capability of producing estrogen in a smaller quantity through the aromatisation of C19 steroids (Simpson, 2002). During pregnancy, the placenta becomes the primary estrogen producing tissue (Koh et al., 2012). There are three types of estrogen depending on the presence of the hydroxyl group on the carbon ring: Estrone (E1), estradiol (E2) and estriol (E3). E2 is the most potent form of estrogen is mainly produced by the gonads and the major estrogen group during the active reproductive years (Longcope, 1998).

Estrogen is readily diffused across cell membrane and attach with the intercellular estrogen receptors (ER) to control gene expression. Many bone cells except osteoclast and chondrocytes are rich with ER hence estrogen has a direct influence on those cells (Vanderschueren et al., 2004). Estrogen was found to be beneficial in preserving the bone due to its ability to reduce the prevalence of mature osteoblast apoptosis (Almeida et al., 2010). Some researchers have linked estrogen to the suppression of inflammatory cytokines that stimulate osteoclastogenesis and bone resorption, such as IL-1, TNF-α, and IL-6 (Most et al., 1995; Sunyer, Lewis, Collin-Osdoby, & Osdoby, 1999); while other suggest that oestrogen induces Fas-ligand in osteoblasts to regulate the differentiation of preosteoclast (Krum et al., 2008). On the other hand, the failure of the Wnt/β-catenin pathway to adjust the bone cell response to the mechanical strains has been suggested in estrogen deficient individual, which leads to the inability to maintain the appropriate bone mass. (Armstrong et al., 2007).

The androgens, which are the main hormone that induced fetal sexual differentiation and the expression of male characteristic at the puberty, are not only produced by Leydig cells in the testicle but also produced in a small quantity in the non-gender specific tissue such as adrenal glands. Hence androgens are present in female serum as the estrogens are present in male serum. Labrie et al (1997) reported that in serums of human adult between the age of 20-30 years, mean serum testosterone level in female is 7% of the serum testosterone level in male while the estradiol in male is 36% as high as in female. The presence of gender-specific hormones in the opposite sex is due to the capacity of the androgenic steroid, androstenedione, to undergo hydroxylation into testosterone or aromatisation into estrogen (Riggs, Khosla, & Melton, 2002). Androgen can bind to androgen receptors (ARs) which present in nearly all bone cells while androgen-derived oestrogens bind to the ERs. The effect of both estrogen and androgen in preserving the bone may overlap but the distinction of bone responses to the stimulation of ARs and ERs in gene knock-out mice has been reviewed by Vanderschueren et al (2003).

In relating the primary osteoporosis and sex hormones, aging process has been reported to reduce important major sex hormones: mean serum estradiol (E2) in female by 75% while only 20% reduction of mean serum testosterone (Labrie, Belanger, Cusan, Gomez, & Candas, 1997). The protective effect of sex hormones on the bone in aged female may be lifted hence the complications of primary osteoporosis higher in female (Genant et al., 1999).

1.9.1.2 Secondary osteoporosis

Secondary osteoporosis is applied to the reduction of bone masses that are not related to menopause or aging process (Gennari, Martini, & Nuti, 1998). Secondary osteoporosis occurs regardless of the normal aging factor as in the primary osteoporosis hence any age group could be affected. The reduction of bone mass in secondary osteoporosis could be as a result of a single or a combination of various conditions (Templeton, 2005).

The following paragraphs are to illustrate important events that lead to secondary osteoporosis and the clinical manifestations that are may accompany the events. The clinical manifestations may be valuable in the clinical investigation to narrow down the primary causative factor of the reduction of the bones' ultimate strength, which increase the risk of spontaneous fracture.

Medical condition that is associated with secondary osteoporosis, including endocrinal and non-endocrinal diseases. In the endocrinal diseases, the disturbances in the hormonal balance lead to the negative net balance in bone dynamic. An example of endocrinal deficiency that is associated with osteoporosis is hypogonadism (Rochira et al., 2006); the condition whereby the sex hormones are insufficient. As the sex hormones are important in the BMC modulation, the reduction in the hormones causes the disruption in the bone growth and expansion for the pre-pubertal group, while accelerated bone absorption is predictable in the adult group (Vanderschueren et al., 2004). Genetic disorder such as Turner's syndrome that leads to gonadal dysgenesis (K. Rubin, 1998); removal of the sex hormones producing tissue as in surgical gonadectomy or chemical castration (Vanderschueren et al., 2004), would also result-in hypogonadism related osteoporosis. Other clinical symptoms for hypogonadism include the absence of secondary sex characteristics, infertility, muscle wasting, and other abnormalities (Patidar, Thakur, Kumar, & Kumar, 2010).

Excessive in endocrine hormones is also detrimental to the net bone dynamic. In cases such as hyperthyroidism, the bone dynamic is accelerated through the effect of thyroid on both osteoclast and osteoblast. However, the quality of mineralisation is affected hence the net chronic effect will be the reduction in bone density (Vestergaard & Mosekilde, 2003).

Other clinical manifestations of hyperparathyroidism in human are fatigue, nervousness or anxiety, weight loss, palpitations, heat sensitivity, decreased fertility, reduced libido and sometimes gynecomastia in men (Carlson, 1980; Cooper, 2003; Krassas, 2000). Several medication/chemicals which are used to treat other non-bone conditions were found to be detrimental to the bone. The mode of action of those chemicals may not be the same, but the result is a significant reduction in BMD.

Excessive of certain trace elements such as fluoride was demonstrated deteriorative to the bone. While dietary fluoride is known to increase calcium retention in bone, the shear strength of long bone was reduced by 30% (M. Chan, Rucker, Zeman, & Riggins, 1973). The pathological effect of chronic and excessive fluoride exposure on bovine has been reviewed by Shupe (1992) which suggested other three main systems are affected by fluorosis: dental, skeletal and renal system. Clinical features of fluorosis include dental erosion, bony exostosis, lameness debility, poor production with no age preferences (Patra, Dwivedi, Bhardwaj, & Swarup, 2000)

Deficiency of certain trace element such as copper long was associated with osteoporosis as copper is a component of lysyl oxidase that involved in cross-linking of the collagen in the bone (Rucker et al., 1998). Conversely, bone is not the only tissue that can be affected by chronic copper deficiency as there are many enzymes depends on copper for the normal function for examples cytochrome oxidase (erythropoiesis and central nervous system, gastrointestinal system), super oxidase dismutase (immune system, reproduction) (S. Chan, Gerson, & Subramaniam, 1998; Fisher, 1975; Percival, 1998; Xin, Waterman, Hemken, & Harmon, 1991). Therefore, copper deficiency syndromes are usually presented as a multisystem failure. The clinical manifestations of copper deficiency in cattle may include anemia, scour, impaired growth, dull hair coat, unthrifty, poor immunity, and reproduction failure.(Black & French, 2004; Mills, Dalgarno, & Wenham, 1976; Moore, 1991; Smart, Gudmundson, Brockman, Cymbaluk, & Doige, 1980).

Macro minerals that constitute the bone crystal (calcium and phosphorus) would influence the strength of the bone. However, the comparison of the degree of mineralisation of different bony structures to the strength may not be straight forward; Bovine femur (67% mineral content) has 1.4 times higher bending strength than red deer antler (59% mineral content), but the force needed to fracture the antler is 2.2 times the force needed to fracture the femur (J.D. Currey, 1999). Conversely, comparison of the calcium content of the same bone may serve as a relative indicator for the bone strength. An increase of 10% of mineral content of a femur increases the yield strength by 70% (Brear et al., 1990), hence the same proportion of reduction in strength could be assumed when the bone losses the calcium content. A classic work by Bocker et al (1934) identified the association of the calcium supplements in lactating dairy cows with the prevention of various bone fractures (hip, rib and pelvic). The long bone strength of un-supplemented cows was also found less 90% than of the supplemented cows group (Bocker, Neal, & Shealy, 1934).

The endocrine system works efficiently in maintaining the narrow range of plasma calcium level of 8-10mg/dL (Goff et al., 1991) hence the reduction of plasma calcium level in the face of dietary calcium deficiency may be compensated by mobilising the calcium reserve in the bone. A prolonged dietary calcium deficit in the face of an increase in calcium demand such as during the 3^{rd} trimester of pregnancy, continued to the lactation phase may temporarily expose the bone excessive resorption. Therefore, increases the risk of fracture. Transient osteoporosis to pregnancy and lactation has been widely reported to be associated with the incident of low force fracture (Curtiss & Kincaid, 1959; Di Gregorio, Danilowicz, Rubin, & Mautalen, 2000; Samdani, Lachmann, & Nagler, 1998; Sowers M & et al., 1993; Spencer, 1979; Stamp, Mclean, Stewart, & Birdsall, 2001).

While the spontaneous bone fracture is a general term to describe the pathologic fracture, stress fracture is a specific type of non-traumatic fracture, which has no relation with pathological condition of the bone (J. M. Morris, 1968). Stress fractures could occur when there are repetitive forces, constantly applied on the bone (Carter, Caler, Spengler, & Frankel, 1981). Those who were involved in high physical stress activities had been identified as the most likely group to get the stress fracture (Branch, Partin, Chamberland, Emeterio, & Sabetelle, 1992; Freslon et al., 2004; Matheson et al., 1987). In animal kingdom, stress fracture has been reported in horses (Kraus, Ross, & Boswell, 2005). It was revealed by Burr (2007) that bone microdamage, which is an important micro-feature of fatigued bone, could occur without any prominent high-stress activities. The micro damage accumulates over time (which exponentiate after the age of 40) and gender is a risk factor for the accumulation (female accumulate twice as rapid as male).

Osteogenesis imperfect (OI) is another bone disorder that may be presented in animals as spontaneous fracture. OI is a rare inherited bone disorder caused by genetic disorder, which may include the gene that encodes the procollagen molecule or the production matrix protein. The defected gene (COL1A1 and COL1A2) responsible in producing collagen 1 that serves as a template for calcium phosphate deposition that strengthens the bone (Rowe, 2002; Seeliger et al., 2003). Other several non-collagenous matrix proteins that promote mineral deposition on collagen such as osteonectin and proteoglycan were also depleted in OI (Termine et al., 1984). The degree of severity of patients with OI may vary from mild to lethal, but the typical clinical manifestations are the spontaneous fractures of bone and teeth with variety of other signs such as opalescence teeth, blue sclera, joint laxity (Rowe, 2002; Seeliger et al., 2003). OI has been reported in many animal species whereby the young animals were mostly affected (Agerholm et al., 1994; Arthur, Thompson, & Swarbrick, 1992; Cohn & Meuten, 1990).

1.10 Conclusion

Bone is a special type of tissue that involved and support many important functions. As hard as aluminium, bone tissue is very dynamic as it has the capacity to model the original shape in the prepartum period, grow multiple times bigger than the earlier size at birth, or remodel the current bony structure. The bone dynamic is made possible due to the presence of BMU that comprises of osteoclast, osteoblast and osteocytes, supported by local and systemic regulators with the presence of the building block (protein and minerals).

As bones depend on many factors in maintaining its normal functions, it is prone to become defective should any of the supportive factors fall short. Spontaneous fracture of the bone which could prevent the bone from performing its movement function is an indicator that there is a failure of one or a combination of factors to maintain the inherent strength of the bone. There are many causative agents that could result in lower ultimate strength, but the site of bone fracture may not provide a clear indicator of which causative agent involved. The clinical manifestation and the demographic features of cases, as reported by many researchers, may provide some clues that could be used to narrow down the possible underlying problem that leads to the spontaneous fracture.

Chapter 2

2 Spontaneous humeri fractures in New Zealand dairy population 2007-2012; a series of 115 cases

2.1 Introduction

Bone fracture is generally known as a discontinuation of bone integrity as a result of direct or indirect force (McGuigan, 2010). Bone fracture is an important debilitating condition which causes severe pain and mobility restriction (Gangl, Grulke, Serteyn, & Touati, 2006; Nichols, Anderson, Miesner, & Newman, 2010). Even though bone fracture is a treatable condition, the prognosis and the economic aspect of fractures differ according to the underlying causes, location of fracture, treatment of choices and the species involved (Crawford & Fretz, 1985)

Bone fracture, based on the underlying causative agent, can be classified into traumatic or pathologic (Doblaré, García, & Gómez, 2004; Wiesel & Delahay, 2010). In traumatic related fracture, an acute direct force is involved, which deformed the bone immediately if the force exceeded the ultimate strength; or causing micro-damage if the force was between the yield point and ultimate breaking point. The micro-damages could accumulate and have been reported to decrease bone tensile strength up to 40% (Burr et al., 1997). Age and gender were found to be associated with the micro damage accumulation; the crack density exponentiate after the age of 40 years and females accumulate bone micro damage twice as rapidly as males (Schaffler, Choi, & Milgrom, 1995).

In pathologic fracture, bone continuity dissociates without obvious prior traumatic episode suggest that pre-existing pathological bone lesion lower the inherent tensile strength of the bone. The bone strength can be reduced when: there was an excessive bone resorption that affected the BMD (Barrett-Connor et al., 2005); inadequate in bone cell production (Glorieux et al., 2002), pathological remodelling of bone (Dove, 1980); defective in bone mineralisation (Hoikka, Alhava, Savolainen, & Parviainen, 1982); abnormal growth of bone cells (Scully, Ghert, Zurakowski, Thompson, & Gebhardt, 2002).

Spontaneous fracture (also known as low force fracture) is a condition of disrupted bone structure with no apparent involvement of blunt force trauma (Horiuchi et al., 1988).

While the spontaneous fracture is a common outcome of pathologically compromised bone, the condition is not exclusive; other healthy groups of human or animal also recorded the incident. Non-pathogenic spontaneous fractures were also found in baseball players (Branch et al., 1992), cross-country runners (Freslon et al., 2004), horses (Kraus et al., 2005) indicating that repetitious high stress physical activity could lead to this type of fracture.

The decrease in bone strength, which increases the risk of low force fracture, had been previously reported associated with other factors such as:

- Nutrition: Chronic deficiency in copper (Rucker et al., 1998), calcium (H. A. Morris, O'Loughlin, & Anderson, 2010), magnesium (Stending-Lindberg, Tepper, & Leichter, 1993) or excessive in fluoride (M. Chan et al., 1973), phosphate (Huttunen et al., 2007)
- Gender: pregnancy and lactation osteoporosis in human (Di Gregorio et al., 2000), gilt (Spencer, 1979)
- Age: rickets (Chapman et al., 2010), hypogonadism in aging (Ahlborg, Johnell, Turner, Rannevik, & Karlsson, 2003) .
- Medication of other disease such as autoimmune (E. Canalis, Mazziotti, Giustina, & Bilezikian, 2007)

Bone fracture in large ruminant is not a common condition. A four-year retrospective studies in a large animal clinic in Belgium (Gangl et al., 2006) could merely manage to detect 99 cases. Another eight-year retrospective studies in a large animal clinic in Canada (Crawford & Fretz, 1985) only manage to include 213 fracture cases. None of the identified fracture cases were classified as pathologic and spontaneous. Both retrospective studies suggested, at most, 24-26 cases of large ruminant traumatic fracture could be expected to be seen in a year by a veterinary practice. The most frequent fractured bone is not unanimous. Gangl (2006) rank tibia as the most common fractured bone (57% of cases) while Crawford (1985) reported femur was the most common fractured bone in large ruminant (32% of cases). The age group, weight and gender play an insignificant role in determining the frequent fracture site as well as the prognosis (Crawford & Fretz, 1985; Gangl et al., 2006; Nichols et al., 2010). The season in which the fracture would have happened, however, may be significant as many researchers observed that the cases were predominantly in spring (Gangl et al., 2006; Martens et al., 1998; Weston, 2008) which is the busiest season for synchronised spring breeders.

A first case report pertaining to humeri fractures in dairy cattle in New Zealand, which labeled as spontaneous, was reported by Weston (2008). The observed spontaneous fracture, which involved four cows in their first lactation, occurred within a short duration between one another (the shortest duration was within a week). Copper deficiency was suspected to cause the fracture due to the low liver copper level of the case animals. Based on the case report, the first case cow was euthanized, and the remaining three were dried-off. Out of the three remaining, one fractured the other humeri and had to be euthanized. Since then, more reports pertaining to the spontaneous humeri fractures were reported to the veterinarian at Massey University directly or indirectly (Weston, Thompson, Dittmer, & Abdul Rashid, 2012).

We investigate the occurrences of spontaneous humeri fractures' syndrome in New Zealand dairy cattle from 2007 to 2012. Our aims were to describe the demographic, spatial and temporal characteristic of the syndrome as we believe that, at the material time; the syndrome was new and did not occur in random. As reported by Weston (2008), the case animals were either had to be dried-off or put to sleep, therefore, the impact of the cases on the dairy cattle enterprise is non-negligible. The motivation of the study was to identify the most likely causative agent, and the plausible risk factors associated with the spontaneous fracture syndrome; hence appropriate immediate intervention can be advised.

2.2 Objectives

The objectives of the study were;

1. To describe the incident of spontaneous humeri fractures in New Zealand dairy cattle from the aspect of individual case attribute, spatial and temporal related distribution form rearing season 2007/2008 until 2011/2012.
2. To propose the plausible causative agent and the risk factors that may be associated with the spontaneous fracture.

2.3 Materials and method

2.3.1 Study design

The study was set out to describe the occurrences of the spontaneous humeri fracture syndrome in dairy cattle population in New Zealand. The description would include the animals, spatial and temporal factors that exist within the study period. The observation period was set for five years starting from season 2007/08 until 2011/12. A season, such as 2007/08, starts from Jun 2007 until the end of Mei 2008.

2.3.2 The respondent

The study started in December 2011 with the announcement made through the DCV newsletter and group emails regarding the intention to investigate the fracture syndrome. Many veterinarians, who had been introduced to the subject matter via a case report that was presented in the NZVA conference 2008, were contacted to nominate possible case farms to the best of their knowledge. The farmers whose farm had been identified as case farms were also invited to nominate the other farms that they thought might experience the spontaneous fracture syndrome in dairy cattle. The aim was to include as many dairy herds in New Zealand that had the spontaneous fracture syndrome. The intention to conduct the study was also conveyed via email to a list of veterinarians who had been assisting other studies performed by researchers and students in Epicentre, IVABS, of Massey University. The farm owner whose farm had been identified by the veterinarian as the possible case farm, were contacted through email and phone prior to the questionnaire distribution. Those farmers who cannot be contacted, the assistances of the veterinarian were sought to inform them regarding the study.

2.3.3 The case definition

As the spontaneous humeri fracture syndrome was relatively new, there was no standard case definition available. The case definition for spontaneous humeri fractures in this study was derived from the case description in the first case series reported by Watson (2008). In order to include as many possible animals with the syndrome, the definition has been tailored by including those cases which had not been seen by a veterinarian to increase the case inclusion. The working case definition for humeri fracture in New Zealand dairy cattle was:

- Sudden, severe non weight bearing lameness of the front leg, and
- Physical examinations suggest that the bone between the elbow and shoulder is fractured, and
- No sign of external trauma.

2.3.4 The questionnaire

A questionnaire which comprises of open and closed questions was produced (annex 1) to assist in capturing the herd and individual-level information. The herd level information included the farm details, herd history, physical factors and herd's feeding management. Individual level information included case description, reproductive status at the time of fracture and the management of the individual cases. The respondents were required to fill in only one herd-level questionnaire sheets, but the individual-level questionnaire was meant to be filled for each case animal. The questionnaires were beta-tested by a farmer in the Manawatu area to eliminate potential ambiguities, before the distribution to the list of the identified farmers. Distributions of the case questionnaires begun at the end of December 2011 after amendments were made in the questionnaire's structure and words selection. The questionnaire kit contained an open cover letter (annex 2); a set of a standard questionnaire which include the questions on herd and individual level; five extra copies of individual questionnaire sheets. The mode of questionnaire return was a prepaid, self-addressed envelope which was inserted in the questionnaire kit.

The respondents were contacted between one to three times with approximately 30 days apart to remind them about the questionnaire. The first contact was made via email and phone call at the end of January 2012. The veterinarians who suggested the farmer were contacted after the third reminder at the end of March 2012 to assist the follow-up activity.

2.3.5 Data handling and analysis

The returned case questionnaires were recorded in Microsoft Excel spreadsheet. The data were explored to check for missing values and transformation of open answers. The farmers were contacted again through phone to clarify any ambiguities and furnishing the missing values. When the farmers could not recall the information, the information was regarded as a true missing value. All exploratory data analysis was undertaken using SAS version 9.3 using the FREQ and UNIVARIATE procedures (SAS Institute Inc., Cary, NC).

2.4 Results

2.4.1 Descriptive data analysis

The variables used in the study were generally divided into two parts: captured and derivative variables. The captured variables were the usable information recorded on the questionnaire as written by the farmer while the derivative variables were a product of transformation or mathematical operations of two or more captured variables. Out of the captured variables, top three variables with missing values were the wean weight; wean age and the affected leg which recorded the proportion of 56%, 43% and 40 % respectively. Out of the derivative variables, the cattle walk speed was noticed to have the highest proportion of missing value, which was 22%.

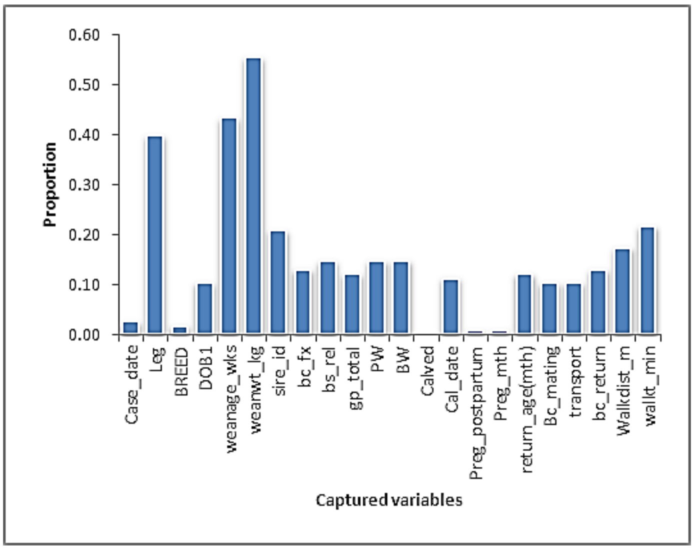


Figure 2-1: Proportion of missing value on captured variables

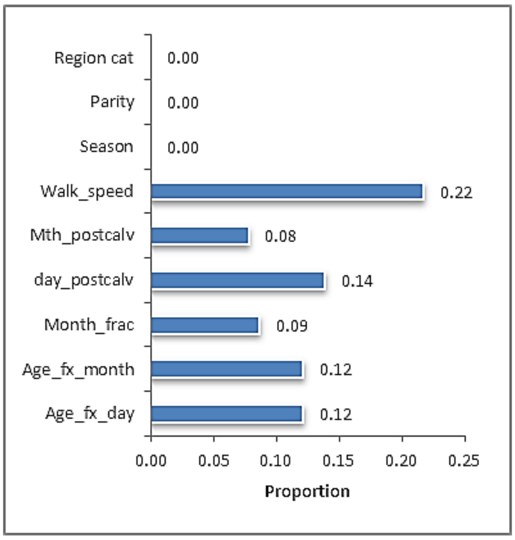


Figure 2-2: Proportion of missing value on the derivative variables

One peculiar aspect of derivative variables was that the missing data proportion depends on the scale of transformation and the original variable from which it derived from. An example of this transformation paradox was the derivative variable used to describe the duration post calving; calving date and case date were needed to produce duration post calving.

Table 2-1: Derivative variables and its description

Derivative variables	Code	Description	Var type	Option
Age of fractured animals (month)	Age_fx_day	Fracture date-birthday in days	Integer	
Age of fractured animals (days)	Age_fx_month	Fracture date-birthday in month	Integer	
Absolute month of fracture	Month_frac	Fracture date converted into absolute calendar month	Nominal	
Day post calving	day_postcalv	Fracture date-calving date in days	Integer	
Month post calving	Mth_postcalv	Fracture date- calving date in months	Integer	
Walking speed	Walk_speed	Walk distance/walk time	Continuous	
Rearing season	Season	The observation year. For example; Rearing season 2011/12= June 2011-Mei 2012	Nominal	
Parity group	Parity	The indicator of the reproductive age. If the animals had calved and in the last trimester of 2nd pregnancy, they are grouped into 1st parity group.	Nominal	0/1/2/3
Region category	Region_cat	Reclassification of region	Nominal	N-North island/S-North island/South island

The missing data percentage in the case date was 3% while the missing data percentage in calving date was 11%. The missing data for duration post calving, which was derived from the calving date and the case date were not a figure in between 3-11% as recorded in the parent variables. If the unit of the derivative variables were higher in the sensitivity, the missing data percentage was higher; day-post calving recorded 14% missing data while month-post calving recorded 8% missing data as many farmers could only recall the month of occurrences, not the exact date.

Questionnaires were mailed to thirty farms, which had been identified by the local veterinarian as the possible farms with spontaneous fracture syndrome. Twenty two farmers (73%) responded by returning the questionnaire; two refused to participate (7%) on the ground of not being able to recall the information as it happened quite sometimes, involving less than two animals and did not recur; the rest potential respondent (20%) did not state the reasons.

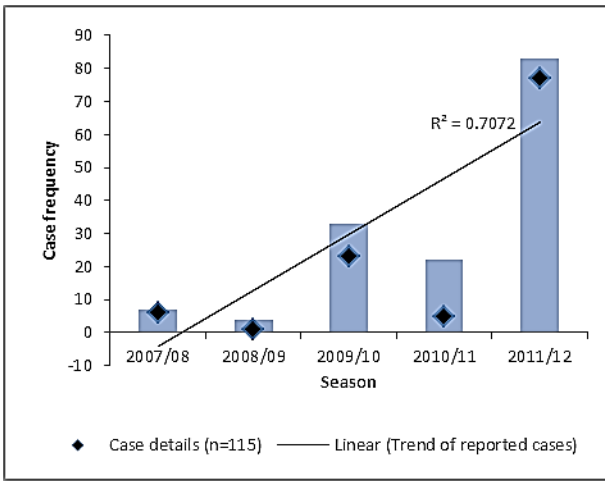


Figure 2-3: Trend of the reported cases over 2007-2012 (n=149)

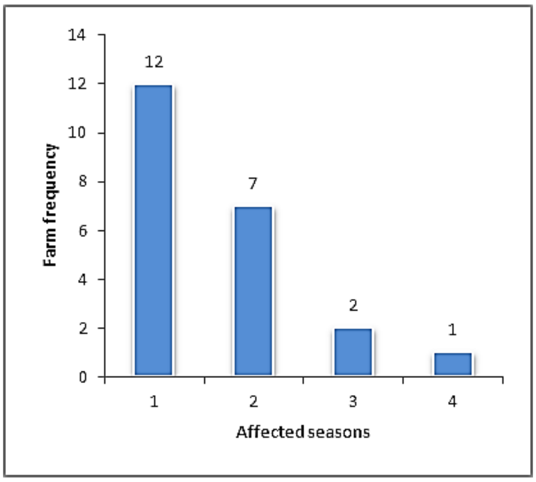
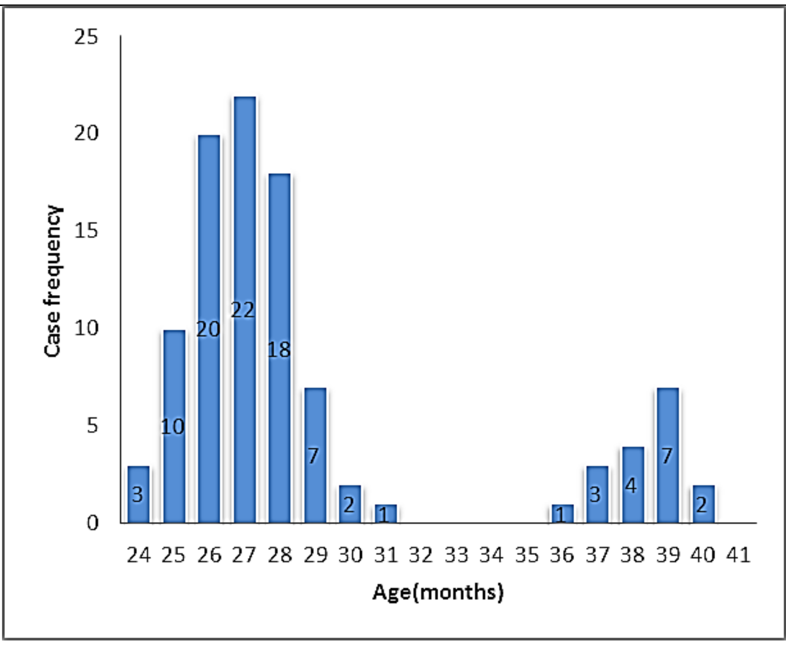


Figure 2-4: The frequency of farm with repeated case season 2007-2012 (n=22)

Overall, 149 cases were recorded based on the working definition suggested, from 22 farms that responded, over five rearing seasons from 2007/2008 until 2011/12. Out of 149 cases, 115 case details (77%) managed to be recorded in the individual cases' sheet. Case details captured in seasons 2008/09 and 2010/11 were less than 50% of the total reported case in respective years, whereas the case details in the other seasons exceeded 70% response. The reported cases were on the increasing trend (R^2=0.7), based on the five-year observation starting from 2007/08. The spontaneous fracture cases were not a one-off experience. One farm experienced the spontaneous fracture in four seasons; two farms had recorded three affected seasons, and seven farms had recorded two affected seasons (fig 2-4).

2.4.2 Animal factors

All 115 animals that fit into the case definition were female, aged between 24 to 40 months. A closer examination on 101 case animals revealed that there were two fairly symmetrical age group's distributions involved. The first age group which consists of 83% (84/101) of the observation were aged between 24 to 31 months with mean age was 26.75 (SD=1.60) months. The second age group seemed to have a smaller density (17%, 17/101) with a range of 36 to 40 months and mean age of 38.35 (SD=1.11) months. No cases were recorded in dairy cattle aged less than 24 months, between 32 and 35 months and more than 40 months.



	1st age group	2nd age group
n	84	17
mean	26.75	38.35
std	1.60	1.11
median	27	39
Q1	26	38
Q3	28	39
min	23	36
max	31	40

Figure 2-5: Case frequency as a function of age and the summary statistics (n=101)

The affected breeds as recorded in 113 cases were Friesian (23/113, 20%), Jersey (24/113, 21%) and crossbreed (66/113, 58%). Regardless the breed, the animal quality index of the case animals as recorded in 'production worth' and 'breeding worth' is shown in table 2-2. The two indices were highly correlated with the calculated correlation coefficients was 0.96. Based on 98 cases, the production worth was fairly symmetrical (Kolmogorov-Smirnov p=0.010) with the mean of 117.29 (SD=54.02), median of 120 (q1=84.25, q3=148.75) and a huge range from -23 to 319. The breeding worth of the case animals was distributed normally (Kolmogorov-Smirnov p=0.086) with a smaller measure of variability compared to the production worth. The breeding worth mean was 115.90 (SD=38.37), median of 112.5 (q1=98.5, q3=142.75), and a range between 16 and 226.

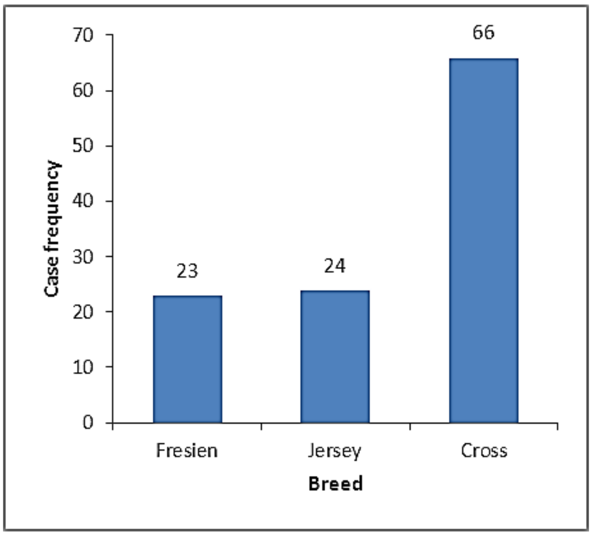


Figure 2-6: Case frequency as a function of cattle breed (n=113)

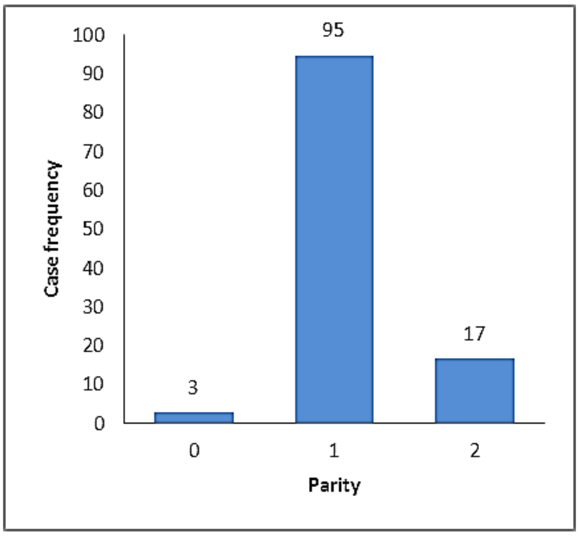


Figure 2-7: Case frequency as a function of parity (n=115)

All 155 cases animal fractures were recorded either in the late pregnancy or had recently calved. They can also be categorised according to the respective parity group. Parity 0, a group which consists of heifers in the final month of the 3rd trimester constitutes of 3% (3/115) of the case details obtained. Parity 1, a group of the recently calved cows to their first calf and possibly pregnant for the second time constitutes the largest portion of the case animals (83%, 95/115). The remaining case animals were in the Parity 2 group, which consist of cows in their second calving.

Stratification of the case animals by the post-partum period is shown in fig 2-16. The calculation of the postpartum period for the first parity was straight forward; the calendar month of the case minus the calving month. If the animals were in the second parity, the calculation of the post-partum period started with the second calving to the month of fracture. Postpartum 0 means, the animal had not calved when the fracture happened. Based on the bar chart of case frequency as a function of the post-partum period, 88% (93/106) of cases occurred in the first-4 month's post-partum.

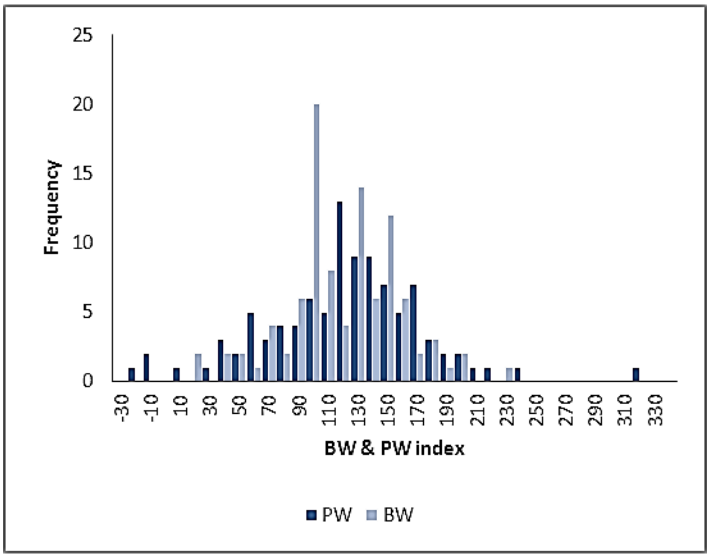


Figure 2-8: The distribution of breeding worth (BW) and production worth (PW) of the case animals (n=98)

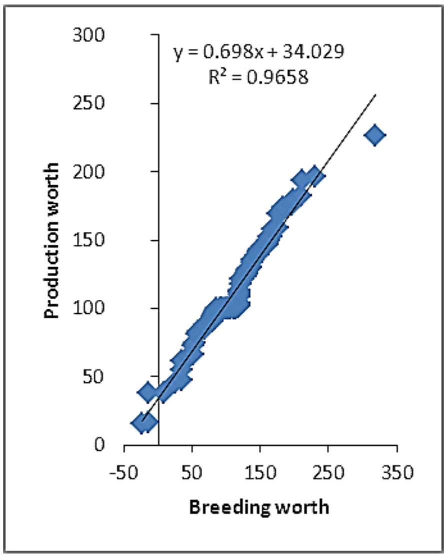


Figure 2-9: Scatter plot showing the relationship between breeding worth and production worth of the case animals (n=98)

Table 2-2: Summary statistics for the quality indices of the case animals

Indices	Breeding worth			Production worth		
Parity	0	1	2	0	1	2
n	2	81	15	2	81	15
mean	129.50	116.78	109.33	139	119.46	102.67
std	16.26	37.77	44.19	12.73	55.62	46.94
median	129.5	112	108	139	122	101
Q1		100	86.5		84	87
Q3		145	122.5		154	130
min	118	16	38	130	-23	-14
max	141	196	226	148	319	171
SE	11.50	4.20	11.41	9	6.18	12.12
95%ucl	152.04	125.00	131.70	156.64	131.57	126.42
95%lcl	106.96	108.55	86.97	121.36	107.34	78.91

The case frequency as a function of the observed body condition is shown in fig 2-10. The body conditions were arbitrarily assigned by the farmer into three ordinal scales: light, if the animals were under the acceptable weight; average, if the animals were at their suitable weight; extra, if the weight were above the normal. Three points along the cases' animal timelines were selected: before mating, upon return from grazier and before fracture. The condition before mating indicates the wellbeing of the case animals prior to pregnancy; the condition upon return from grazier indicates the food availability in the grazier and the body condition before fracture showed the general health status of the case animals. The three-time body condition observations could also show the fluctuation of the body weight of the cases over time. Fig 2-10 shows that 89% (92/103) of case animals before mating; 99% (99/100) of case animals upon return from grazier; 93% (93/100) of the case animals before fracture had an average to extra good body condition. The body condition of case animals did not deteriorate noticeably at any point of their time line. As a comparison to the other animals in the farm where the case animals were located, 68% of the case animals had been at par with the overall animals which had been classified as the non-case based on the case definition while, 7% were better than the farm average (fig 2-11).

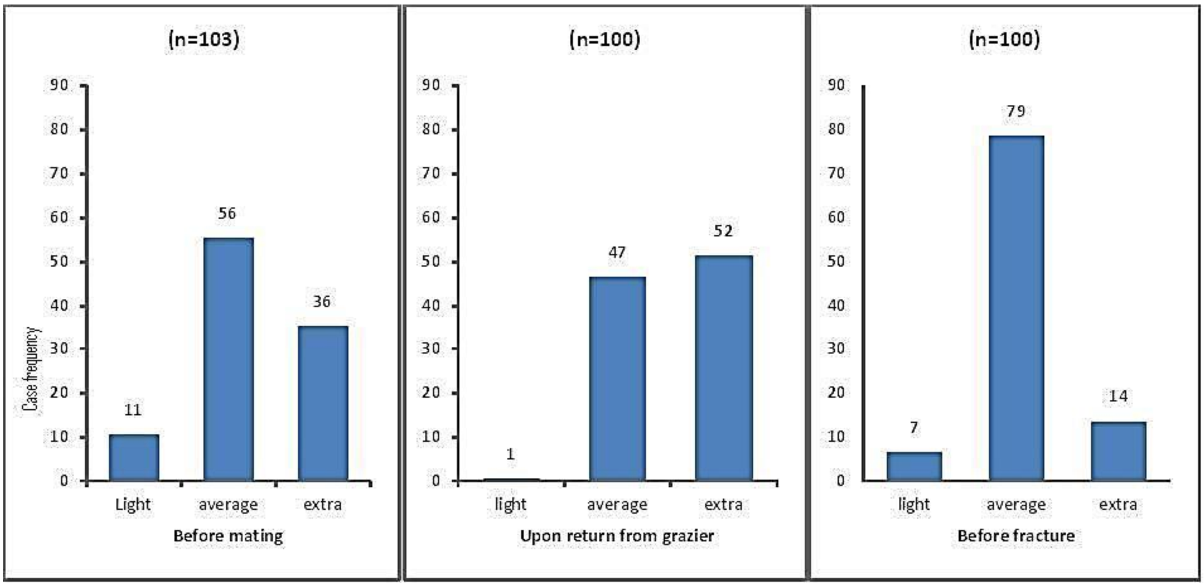


Figure 2-10: Case frequency as a function of the observed body condition

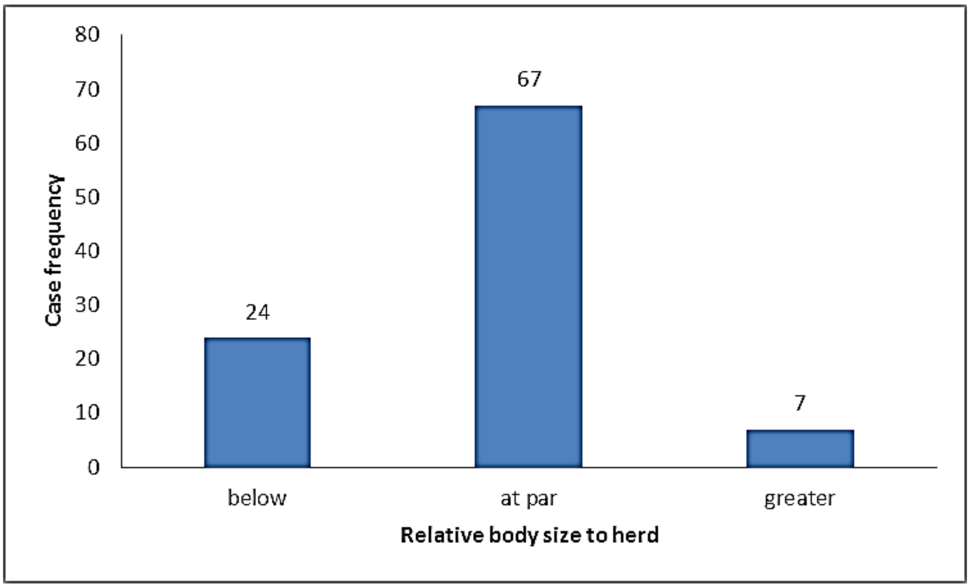


Figure 2-11: Cases frequency as a function of the relative body size to the whole herd (n=98)

The case animals, as well as the other animals in the case farm were raised in the grazier, which could be the other part of the farm or located in the other area outside the farm compound. At the age between 20 to 24 months, the animals returned to the farm either by walking or transported by truck. Fig 2-13 shows that 7% (7/103) of the case animals had been walked while the rest (93%, 96/103) had been transported from the grazier by truck. As the animal parity signifies the duration post transportation, parity 2 is further in the timeline as compared to the earlier parity. If the animal in parity 2 was discriminated due to the longer duration post transportation, the proportion of case animals transported by truck was still high (92%, 82/89).

Table 2-3: The count of affected front leg by the location (n=69)

		Affected leg			
Region Cat	Herd ID	both	left	rignt	Grand Total
N-North Island	1		1	1	2
	2	1			1
	3		9	9	18
	6		2		2
	7		1		1
	9			1	1
	10		3	1	4
	11		2	1	3
	12		5		5
	13		3	3	6
	14			2	2
	15		2	2	4
	16		1		1
	18			2	2
	21			1	1
S-North Island	4		1	1	2
	5		1		1
	22			1	1
South Island	8			2	2
	20			10	10
Grand Total		1	31	37	69

The affected humeri for the case animals are shown in fig 2-12. The right-side humeri recorded a higher percentage of fracture (53%, 37/69) as compared to the left (44%, 31/69) but the difference may not be significant as the 95% CI of the proportion of the null value is between 0.38 and 0.62.

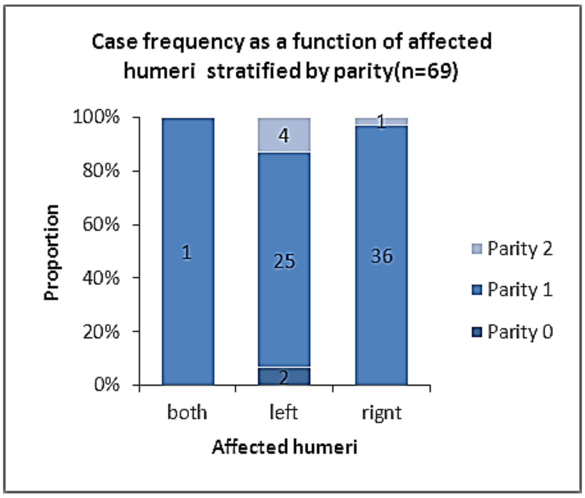


Figure 2-12: The proportion of affected leg stratified by the parity (n=69)

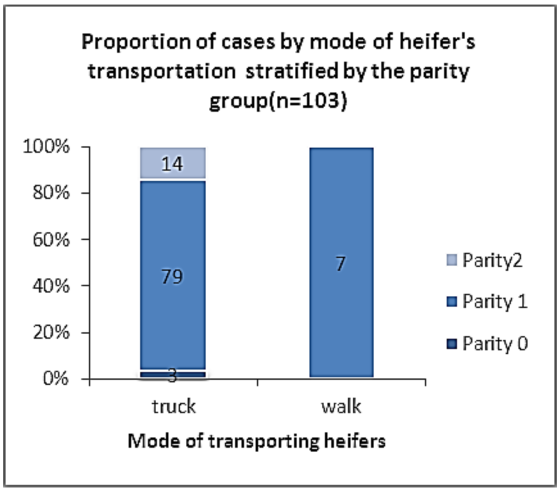


Figure 2-13: The proportion of the mode of transporting case animals stratified by the parity (n=103)

2.4.3 Temporal factors

The time line of the spontaneous humeri fracture cases over the five rearing season starting from season 2007/08 is shown in fig 2-14. The timeline plot shows that the spontaneous humeri fractures had a distinctive seasonal pattern; it occurred in every spring to summer except for season 2008/2009. Regrouping of the cases based on the absolute month of occurrences is shown in fig 15. The cases appeared in July until February, peaked in September (35/105) and October (34/105), declined by half after the peak month in every following month.

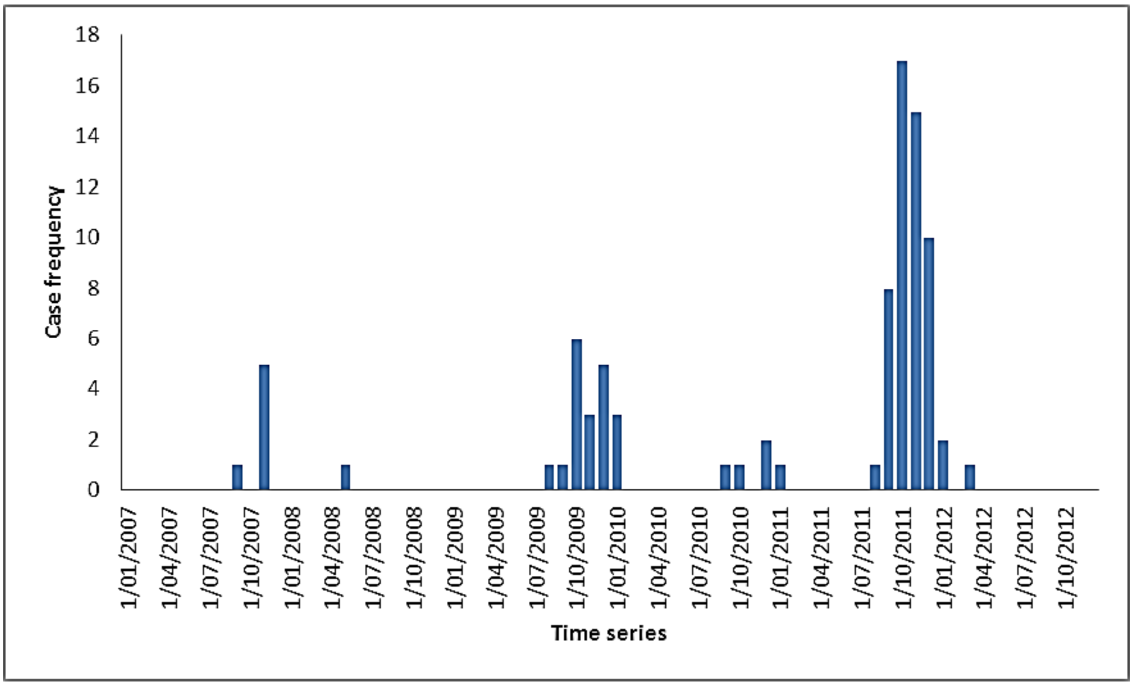


Figure 2-14: Time series of the spontaneous humeri fracture cases in dairy cattle from 2007-2012 (n=115)

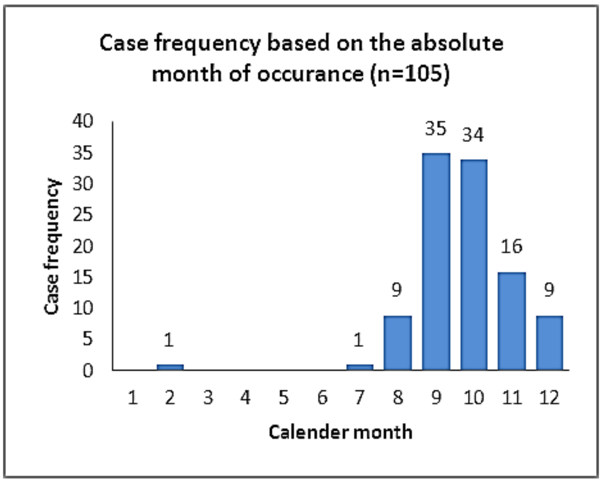


Figure 2-15: Case frequency based on the absolute month of occurrences (n=105)

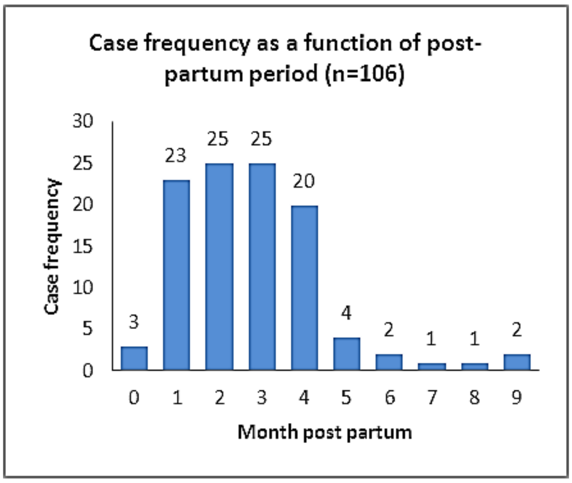


Figure 2-16: Case frequency as a function of post-partum period (n=106)

In relating to the drought experience of the case farms, 77% (88/115) case animals were from the farm which had recorded severe droughts that affect the vegetation in the farm within the observation period (fig 2-17). As the droughts were not a regular yearly event in the affected farms, the frequency in relation to the prior drought experience was produced (fig 2-18). The post drought to fracture duration for 88 case animals that were located in the drought-affected month ranged from 4 to 48 months, with the mod value of eight months.

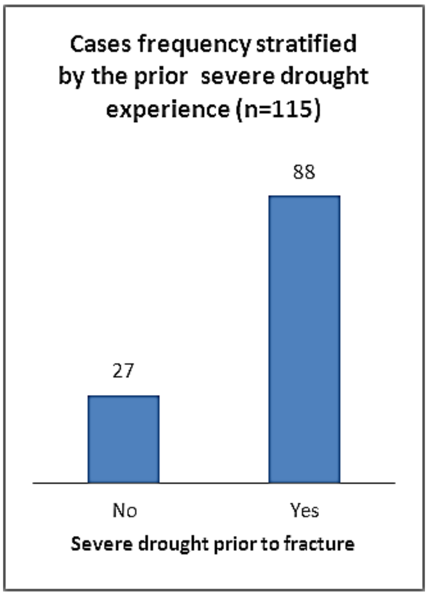


Figure 2-17: Cases frequency stratified by the prior severe drought experience (n=115)

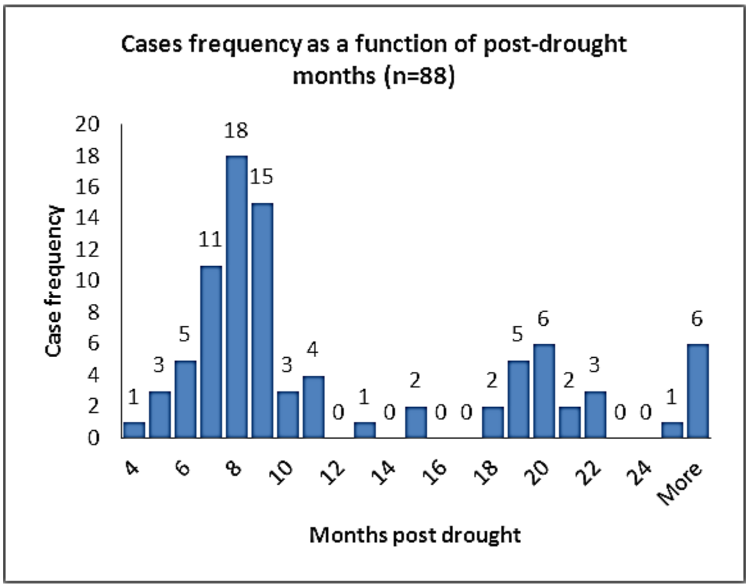


Figure 2-18: Cases frequency as a function of the duration between the last month of drought and the case occurrences (n=88)

2.4.4 Spatial factor

The case farms were centralised in six farming regions (table 2-4): Auckland, Bay of Plenty, Waikato, Manawatu, North Otago and Canterbury, whereby Waikato recorded the highest case count (64%) over the observation period. Based on the specific district in which the spontaneous humeri fracture occurred in dairy population 2007-2012, Morrinsville recorded the highest (27.5%, 41/149) while Pukekohe had the lowest (0.7%, 1/149) count.

Table 2-4: Count of cases based on the rearing seasons and the location

Herd code	Region cat	Farming Region	District	Rearing season					Total cases	Case %	Farming region %	Region cat %
				2007/ 2008	2008/ 2009	2009/ 2010	2010/ 2012	2011/ 2012				
21	North-North Island	Auckland	Pukekohe	0	0	0	0	1	1	0.7%	5%	73%
11	North-North Island	Bay of Plenty	Te Puke	0	0	4	0	0	4	2.7%	5%	
16	North-North Island	Waikato	Cambridge	0	0	0	0	2	2	1.3%	64%	
1	North-North Island	Waikato	Hamilton	0	0	0	0	2	2	1.3%		
15	North-North Island	Waikato	Hamilton	0	1	4	0	0	5	3.4%		
18	North-North Island	Waikato	Hamilton	0	0	0	0	2	2	1.3%		
9	North-North Island	Waikato	Morrinsville	0	0	0	0	1	1	0.7%		
10	North-North Island	Waikato	Morrinsville	0	3	2	3	5	13	8.7%		
17	North-North Island	Waikato	Morrinsville	0	0	4	7	16	27	18.1%		
7	North-North Island	Waikato	Ohinewai	0	0	1	0	1	2	1.3%		
12	North-North Island	Waikato	Ohinewai	0	0	0	1	4	5	3.4%		
3	North-North Island	Waikato	Putaruru	0	0	0	0	18	18	12.1%		
13	North-North Island	Waikato	Te Aroha	0	0	8	1	0	9	6.0%		
14	North-North Island	Waikato	Te Aroha	0	0	2	8	2	12	8.1%		
2	North-North Island	Waikato	Waharoa	0	0	7	0	6	13	8.7%		
6	North-North Island	Waikato	Waharoa	0	0	0	0	3	3	2.0%		
4	South-North Island	Manawatu-Wanganui	Dannevirk	0	0	0	0	2	2	1.3%	18%	18%
19	South-North Island	Manawatu-Wanganui	Foxton	0	0	1	0	3	4	2.7%		
22	South-North Island	Manawatu-Wanganui	Foxton	7	0	0	0	0	7	4.7%		
5	South-North Island	Manawatu-Wanganui	Palmerston North	0	0	0	2	3	5	3.4%		
8	South Island	North Otago	Oamaru	0	0	0	0	2	2	1.3%	5%	9%
20	South Island	Canterbury	Rakaia	0	0	0	0	10	10	6.7%	5%	
				7	4	33	22	83	149	1.00	100%	100%

The distribution of case farms is shown in fig 2-19, 2-20 and 2-21. Based on the farm cluster, the farming regions were re-categorized for gross comparisons; to see the persistence of risk factors in the case farms.

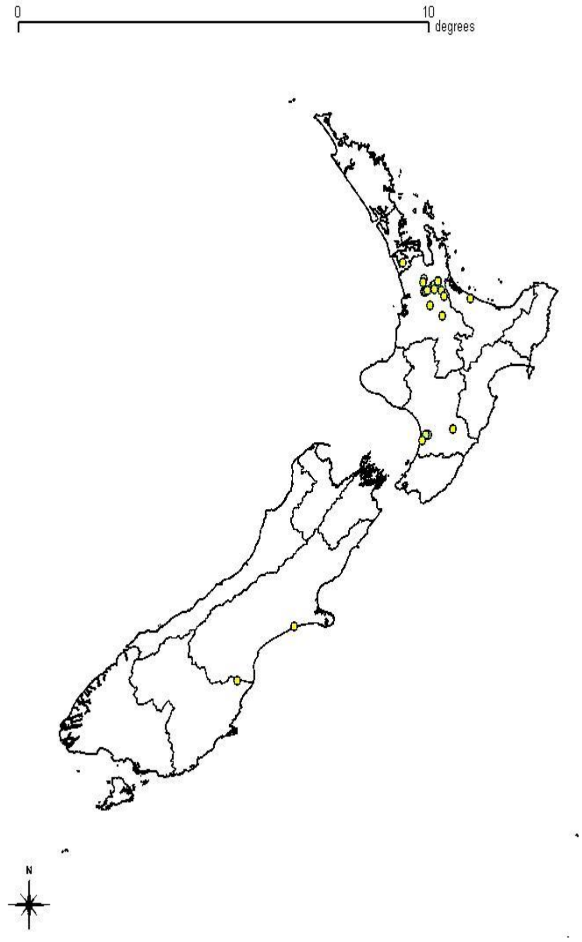


Figure 2-19: Spatial distribution of the case farms showing the geographical clustering of case farms into 3 major area: North-North Island, South-North Island and South Island

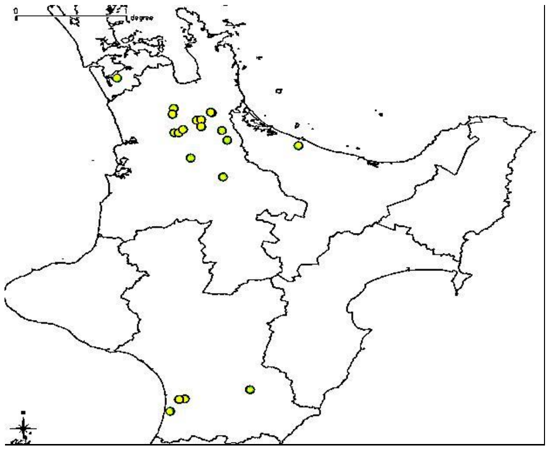


Figure 2-20: The spatial cluster of case farms in North Island

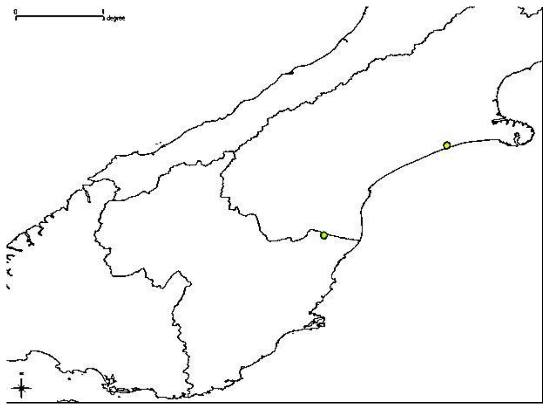


Figure 2-21: The spatial cluster of case farms in South Island

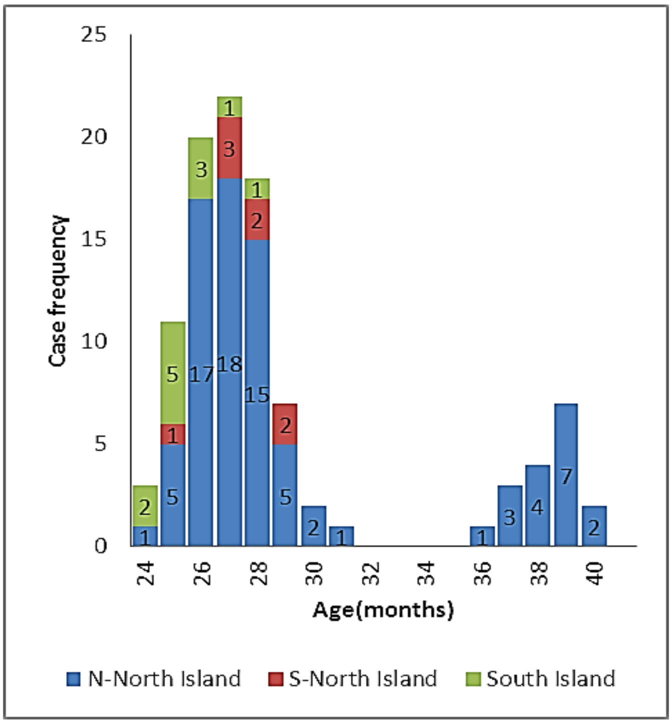


Figure 2-22: Case frequency as a function of age stratified by region (n=101)

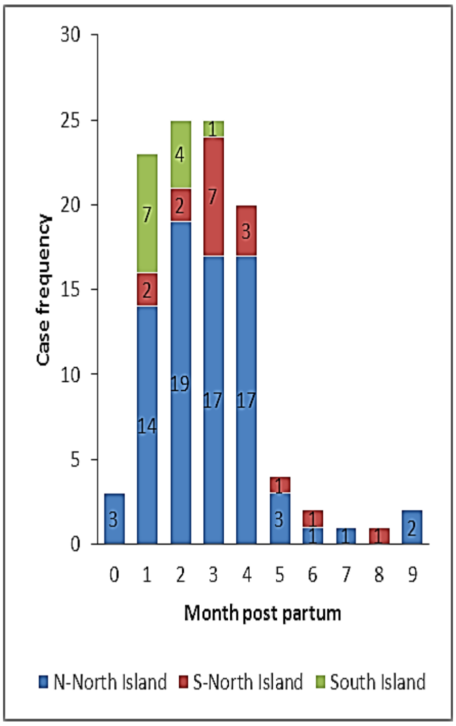


Figure 2-23: Case frequency as a function of post-partum period stratified by the region (n=106)

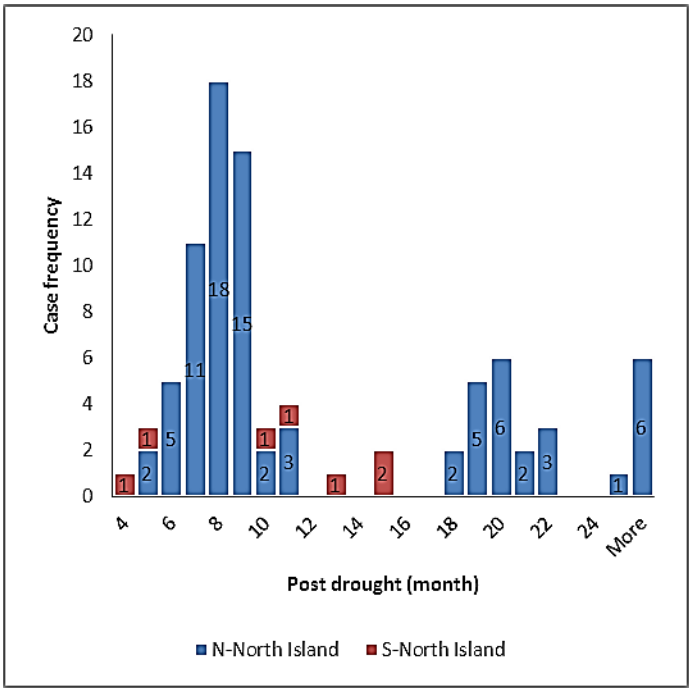


Figure 2-24: Case frequency as a function of post drought duration to case stratified by region (n=88)

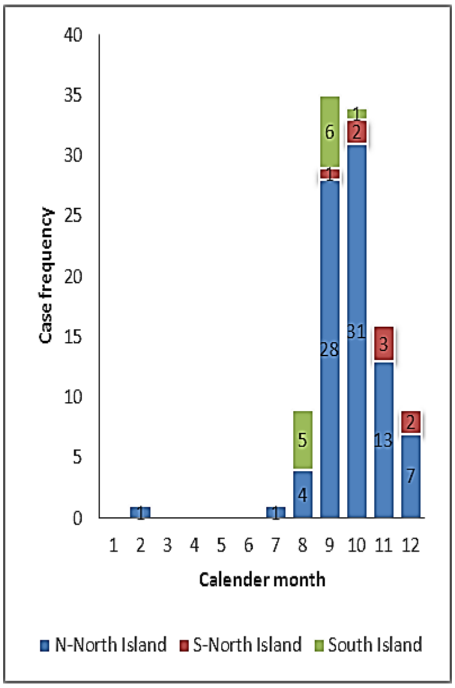


Figure 2-25: Case frequency based on the absolute month of occurrence stratified by region (n=105)

2.4.5 Spatial influence on animal factors

The farm locations were categorized according to the gross geographical location to investigate the similarities in case animal's attributes in different spatial factor. Stratification of the age of case animals by region that is shown in fig 2-22 stating the first age group of case animals(between 24 and 31 months) was common in all regions but the second age group of case animals (from 36 to 40 months) were dominated by the North-North Island region.

With regards to the post-partum period (fig 2-23), North-North Island (NNI) region was the only region that recorded late pregnancy spontaneous fracture in heifers (3/106) and contributed to the high case count in the first four-months post-partum. The South-North Island (SNI) region post-partum duration before fracture’s range was as wide as the North-North Island (range between one and eight-month post-partum) with the highest count was recorded in the 3rd months (41%, 7/17) . While the South Island (SI) region cases were recorded in the first three-month post-partum with the highest month was the 1st month (58%, 7/14).

The case frequency post draught experience, stratified by the region (fig 24) revealed that none of the South-North Island cases animals had experienced severe drought despite 23% (27/115) of the case animals were from that area. Most (92%, 81/88) of the drought affected case animals were located in the North-North Island.

Stratification of the case animal’s parity group by the region (fig 2-27) revealed SNI and SI had recorded cases that were in the first parity only. The NNI region recorded cases in all three parity groups with the 1st parity remains as the highest proportion (76%, 66/86).

The absolute month in which the case occurred, stratified by the region can be seen in fig 2-25. Generally, the cases' occurrences were distributed over a few calendar months of the year. In NNI, a higher count of cases (70%, 59/85) was observed in September and October. The SNI region, however, recorded an earlier peak of count (92%, 11/12) than NNI, which was in August and September, while the SI region’s peak counts (63%, 5/8) were recorded the latest in the calendar months (October and November) among the three regions.

The breed of the case animals based on the region category is shown in fig 2-26. Based on the breed proportion comparison between the North-North Island (NNI) and the South-North Island (SNI), the difference in the breed proportion was by chance only as a direct comparison of the breed proportion 95% confidence interval in the North-North Island overlapped with the same breed in the South-North Island (Breed/Region/95%CI): Crossbreed/NNI/0.42-0.63; Crossbreed/SNI/0.33-0.77; Jersey/NNI/0.18-0.36; Jersey/SNI/0.04-0.36; Friesian/NNI/0.14-0.31; Friesian/SNI/0.14-0.56). Breed proportion comparison against the South island was not produced as only one breed was recorded for the case animals: crossbreed.

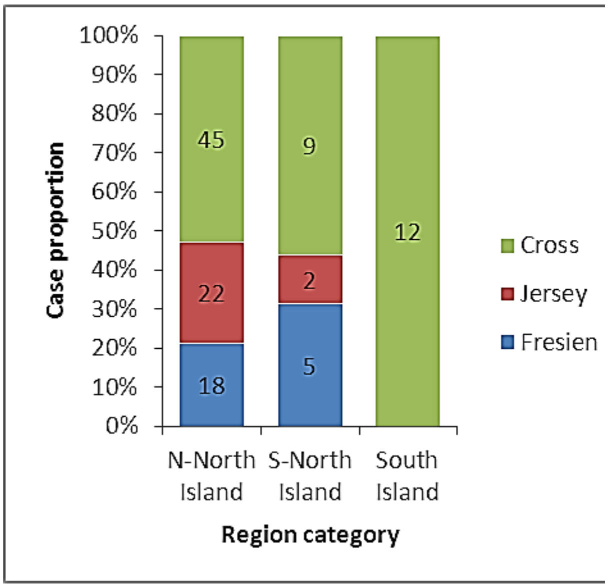


Figure 2-26: Proportion of cases in each region stratified by breed (n=113)

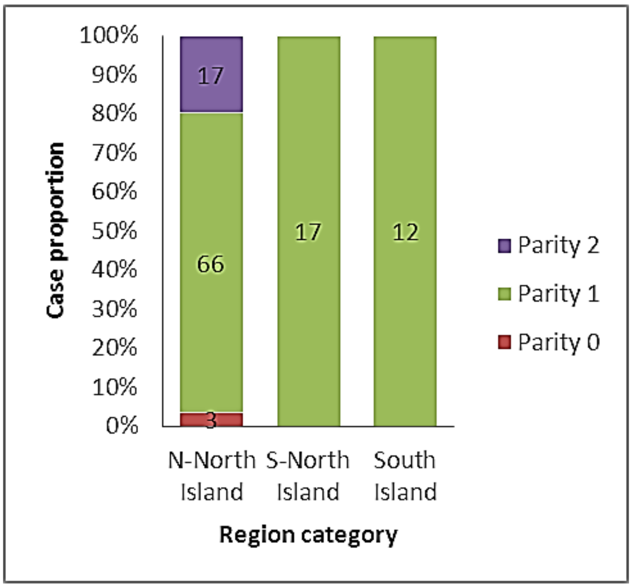


Figure 2-27: Proportion of cases in each region category stratified by parity (N=115)

The quality index of the case animals by the region category is shown in fig 2-27, with the summary statistics in table 2-5. From the aspect of BW, the NNI region had the highest measure of central tendency (mean=121.43, median=124.5) and the widest range (from 17 to 226). SNI appeared to have the largest BW variability (SD=50.62) while SI had the best measure of precision (SE= 4.19). From the aspect of PW, the SI region recorded the highest mean; NNI region recorded the highest measure of central tendency (median=127), variability (SD=58.91) and range (from -23 to 319). Conversely, the differences in the PW and BW statistics between regions may be due to chances alone as the 95% confidence interval of the means overlapped.

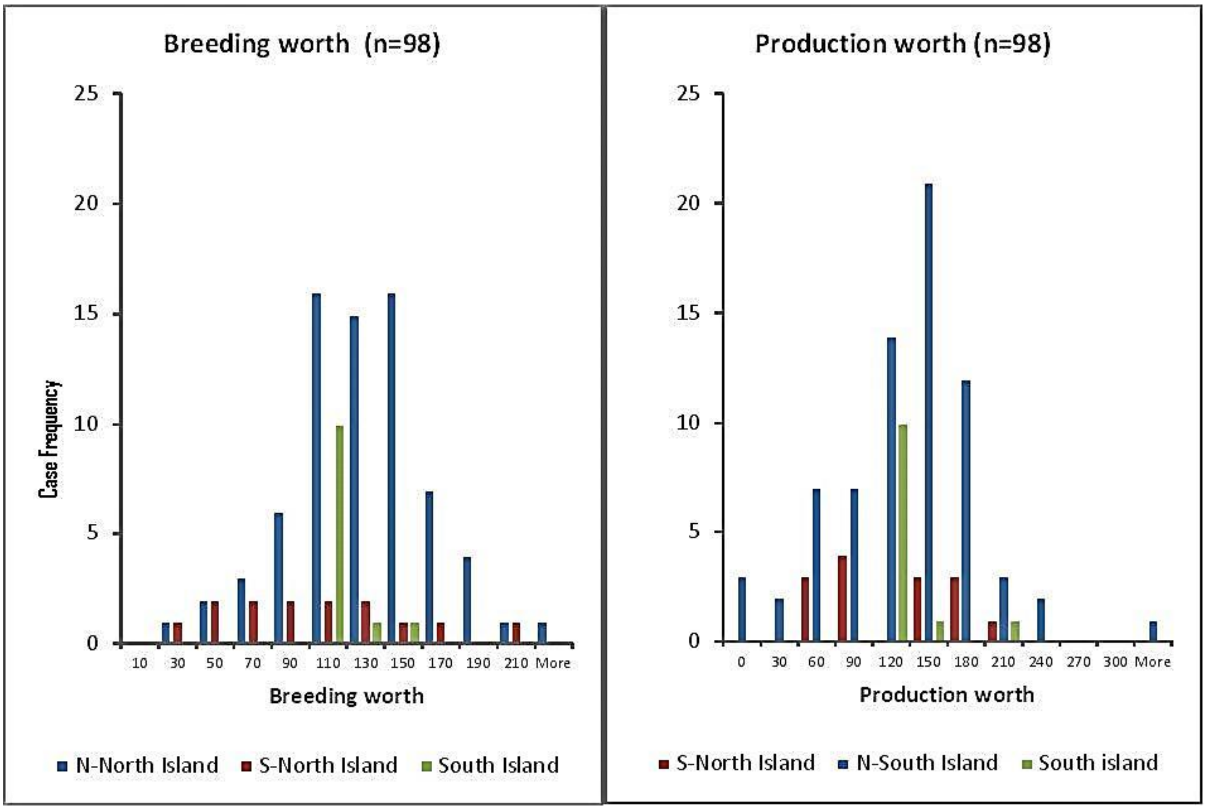


Figure 2-28: Breeding worth and production worth of the case stratified by region category

Table 2-5: The summary statistics of the quality indices of case animals by the region (n=98)

	BW				PW			
Region	**All**	**N-North Island**	**S-North Island**	**South Island**	**All**	**N-North Island**	**S-North Island**	**South Island**
n	98	72	14	12	98	72	14	12
mean	117.29	121.43	96.00	105.92	115.90	117.50	108.71	126.00
sd	54.02	37.19	50.62	14.53	38.37	58.91	49.06	17.61
med	120	125	87.5	100	113	127	103	120
min	-23	17	16	100	16	-23	44	120
max	319	226	196	146	226	319	197	181
Q1	84.25	100	65.25	100	98.50	84.25	69.5	120
Q3	148.75	146	125.75	100	142.75	151.25	150.25	120
Se	5.46	4.38	13.53	4.19	3.88	6.94	13.11	5.08
95%ucl	127.98	130.02	122.52	114.14	123.49	131.11	134.41	135.96
95%lcl	106.59	112.84	69.48	97.70	108.30	103.89	83.02	116.04

The timeline of case animals' body condition according to the region is available in fig 2-29. Generally, the case animals had achieved a desirable (average and extra good) body condition at every point of time under study except for SI region before mating as

83%(10/12) had been observed as underweight. Nevertheless, the body condition of the case animals in the SI region had improved as they returned to the case farms and before the fracture happened. As a comparison to the whole animals in the farm (fig 2-30), majority of the case animals in NNI (75%, 57/76) and SNI (80%, 8/10) maybe indistinguishable from the non-case. The case animals in SI, however, were noticed to be below the farm average despite having an average weight score before the fracture as pictured in fig 2-30. This could mean the non-case animal in the SI were having an extra good overall score.

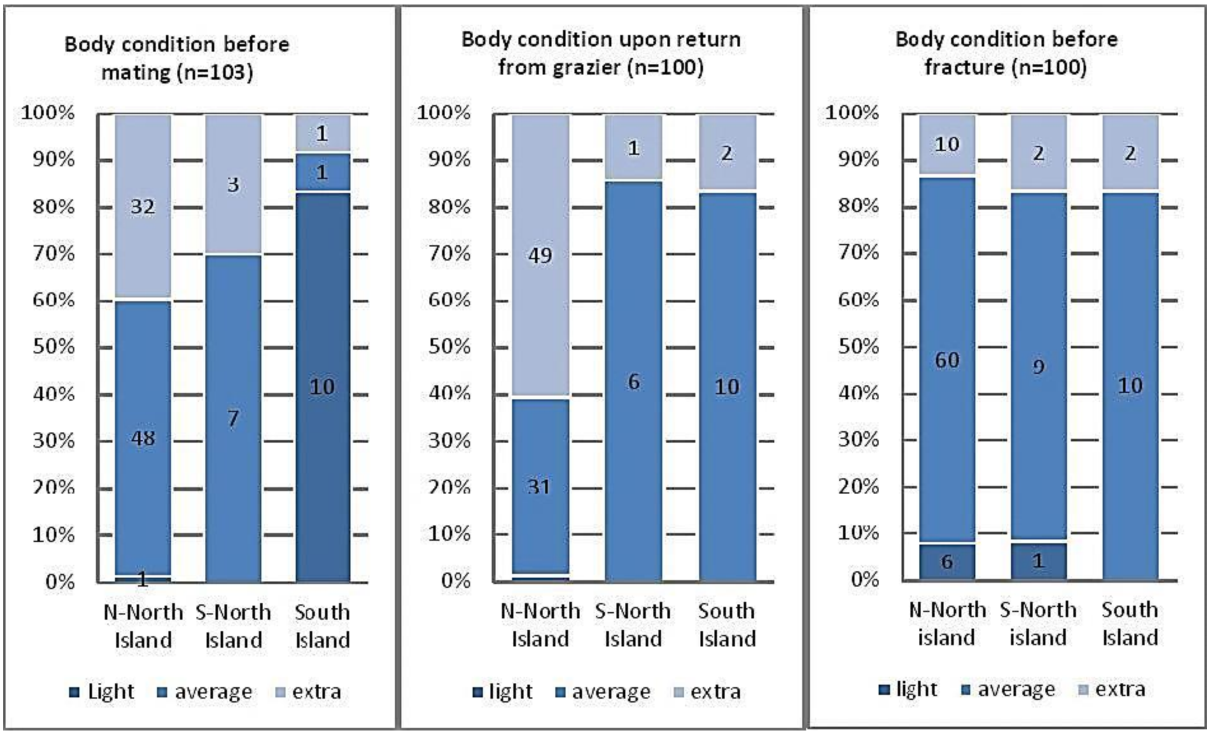


Figure 2-29: Cases proportion as a function of region stratified by the observed body condition

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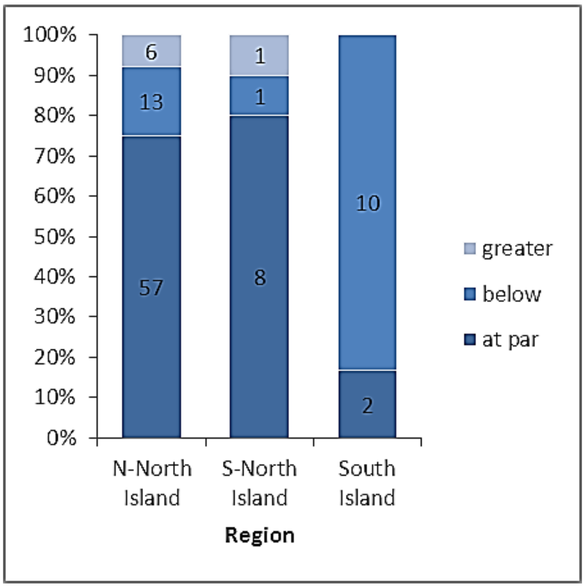

Figure 2-30: Proportion of cases by region stratified by the relative body size to the whole herd (n=98)

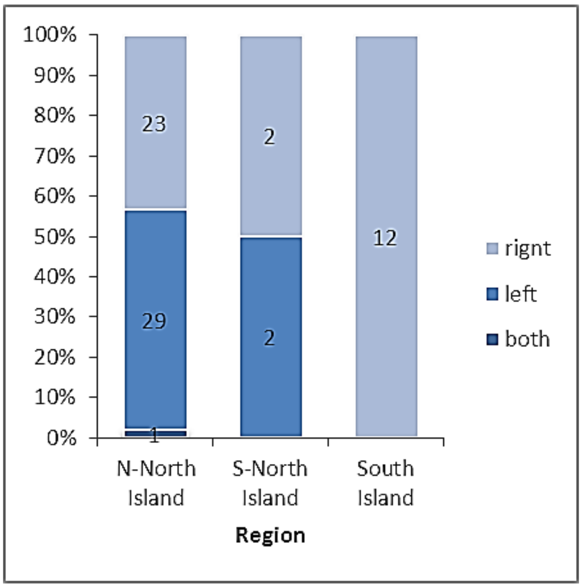

Figure 2-31: Proportion of cases by region stratified by the affected humeri (n=69)

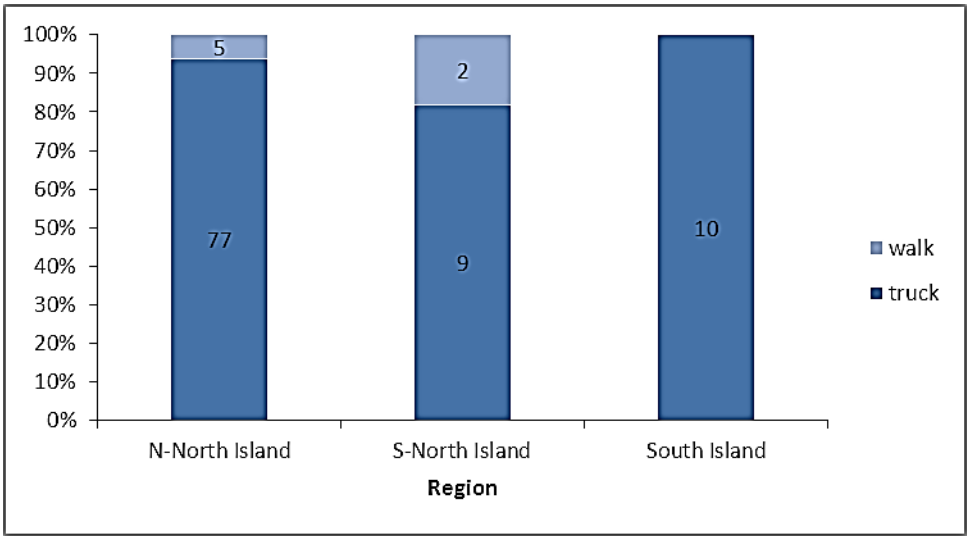

Figure 2-32: Proportion of cases by region stratified by the mode of heifer's transportation (n=103)

The proportion of the affected side of the leg appeared no different between NNI and SNI, but SI recorded 100% (12/12) cases involving right leg (fig 2-31). Transportation of heifers using a truck as the main method to move the case animals from their respective grazier was unanimous in the regions (fig 2-32).

2.4.6 Temporal trend on animal factors

The type of breed affected by the spontaneous fracture stratified by the rearing season is shown in fig 2-33. Contrary to the earlier pool data (fig 2-6) that showing the count of cases, in which cross breed animals had twice as many case counts than the other pure breed; no breed appeared to be consistently dominant in every rearing season over the other in all the observation periods of five rearing seasons, even though the cross breed consistently appeared in all observation year.

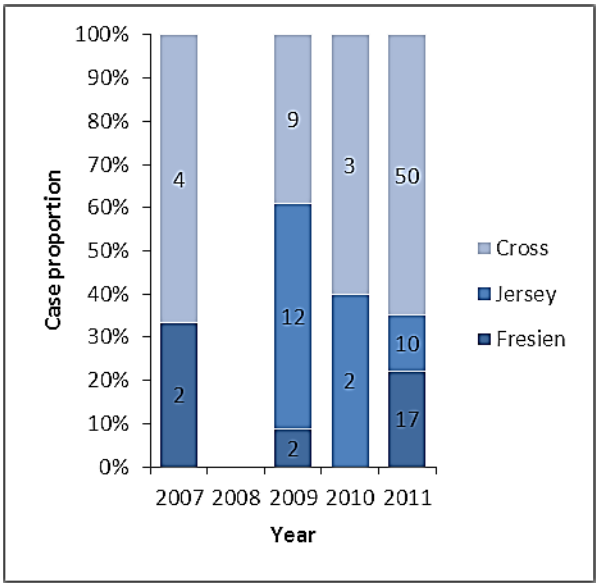

Figure 2-33: Proportion of cases over observation year stratified by breed (n=111)

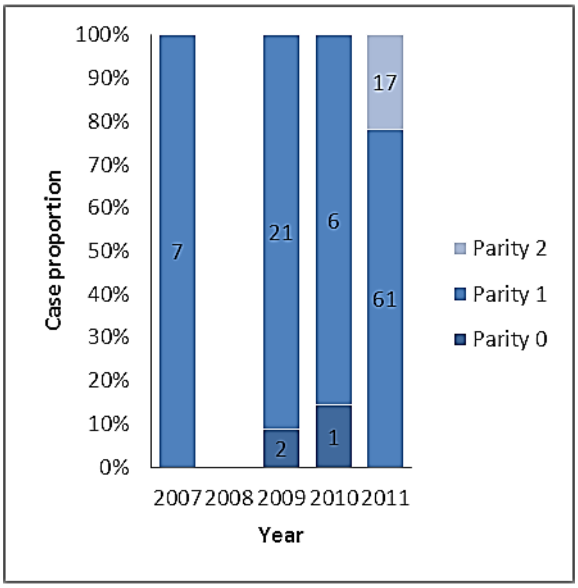

Figure 2-34: Proportion of cases over observation year stratified by parity (n=115)

Stratification of the case animals' age of fracture by the rearing season (fig 2-35) revealed that the involvement of the second age group (n=17, mean age= 38 months, age range= 36-40 month) was a new phenomenon as it occurred during the final observational rearing season of five. A further rearing season stratification of the case animals by the parity group (fig 2-34) revealed that the second age group were actually on their second parity, which comprises of 22% (17/78) of the cases in season 2011/12. Parity 1 was seen as the common and important parity for the spontaneous fracture as the proportions of parity 1 in every season were no less than 78%.

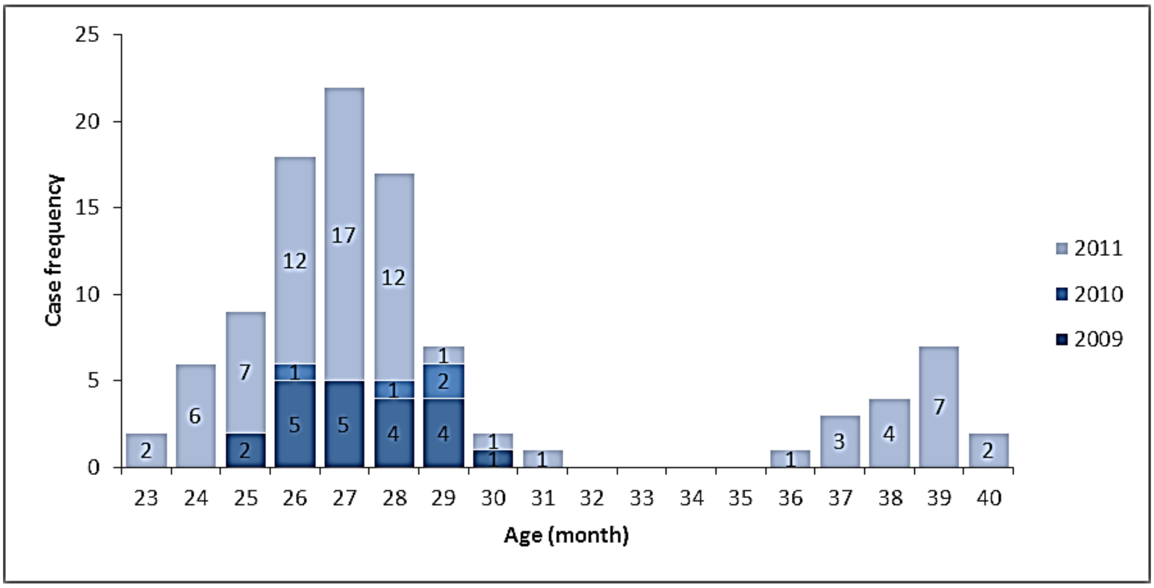

Figure 2-35: Case frequency as a function of age stratified observation year (n=101)

The scatter plots of the case animals' quality index (breeding and production worth) against the rearing seasons is shown in fig 2-36. A linear trendline using the least square method was fitted into the plot to examine the seasonal trend of the quality index. The PW plot shows that the fitted mean of PW in every observational rearing season (113.45) were constantly high from the beginning of the observation (R^2=0.0006). The BW of the case animal, even though it had already shown a high fitted mean from the beginning (90.99), grew by 7.4 unit on the following rearing season (R^2=0.05).

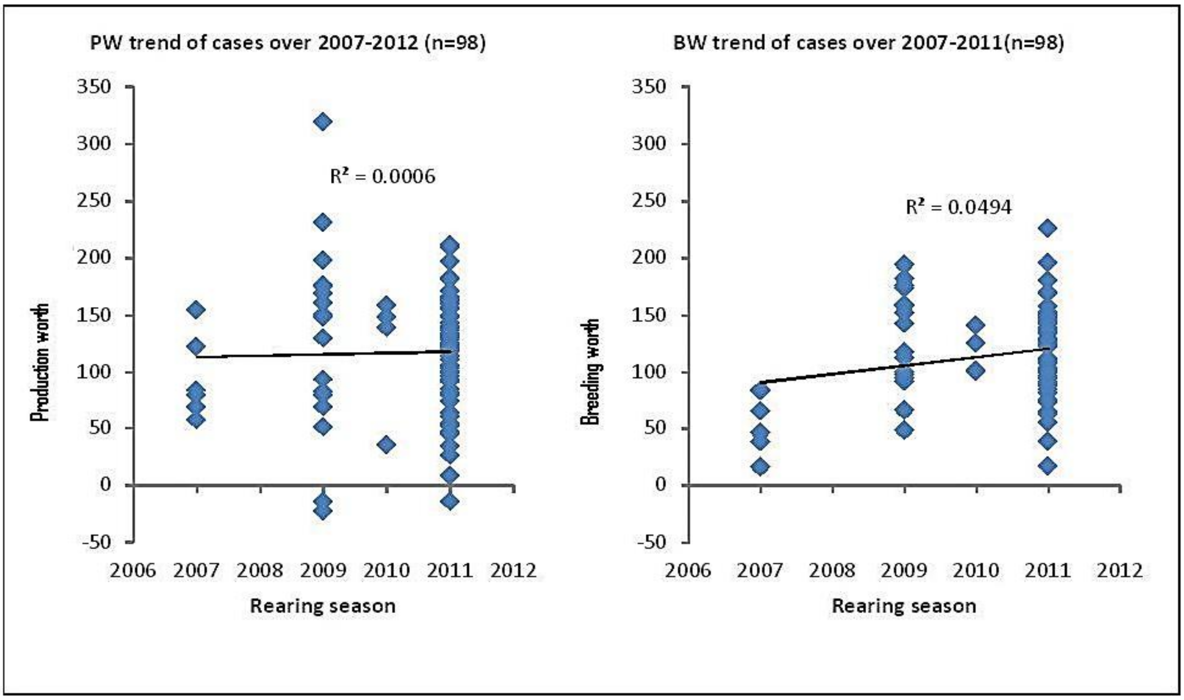

Figure 2-36: The trend of animal quality indices over five observation years

The timeline of case animals' body condition according to the rearing seasons in which the spontaneous fracture happened is available in fig 2-37. Generally, the case animals had achieved a desirable (average and extra good) body condition upon return from the grazier but dropped into the average score before the fracture happened. This may be due to the fact than the case animals were pregnant upon return; and had given birth or started lactating before the fracture; hence the body conditions upon return from grazier were seen superior than before the fracture happened. As a matter of comparison with the whole herd, more than 50% of the case animals in every rearing season were at par or greater than the herd average (fig 2-38). The proportion of affected side of the leg also appeared no obvious trend over the observational duration (fig 2-39).

The involvement of a truck as a transportation means for heifers from the grazier to the case farms was consistent throughout the observation period. Fig 2-40 shows that only on the last observation year, the case animals which did not use truck for the heifer transportation were included in the case count and a small fraction (10%, 7/73).

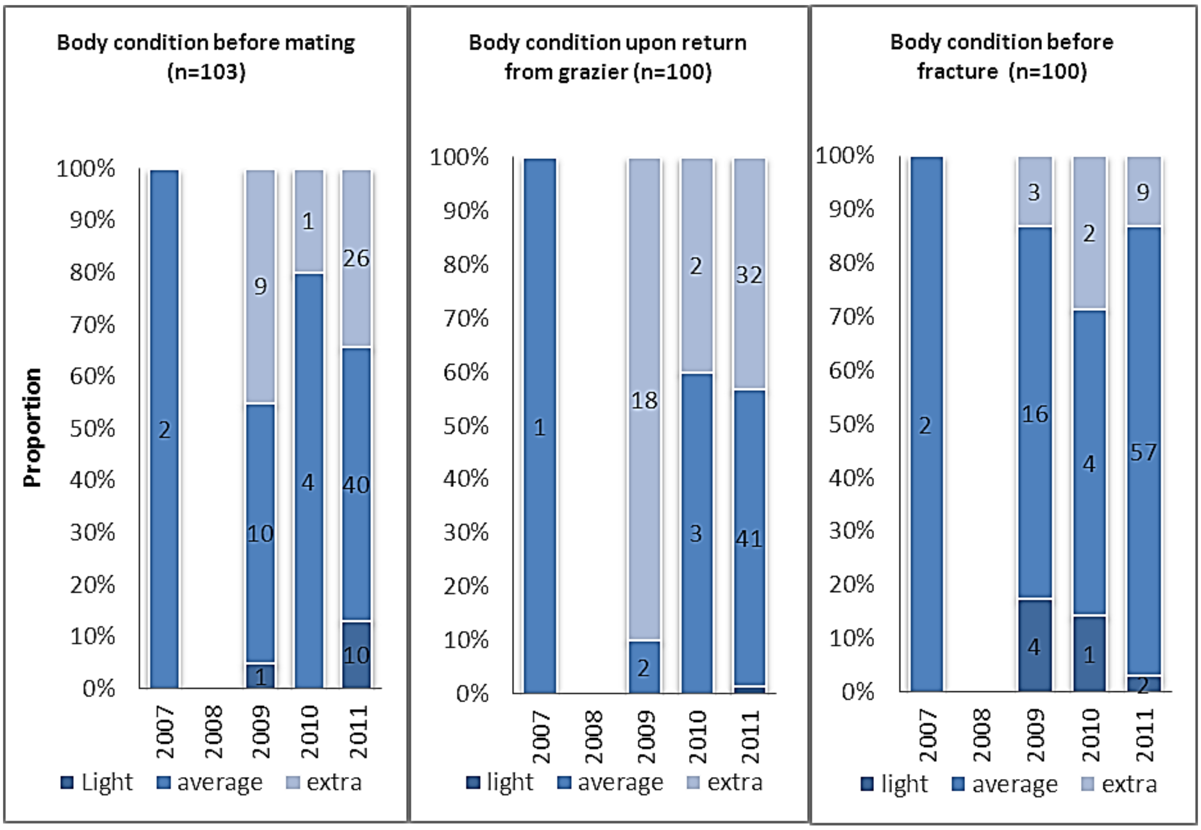

Figure 2-37: Proportion of cases by observation year stratified by the observed body condition

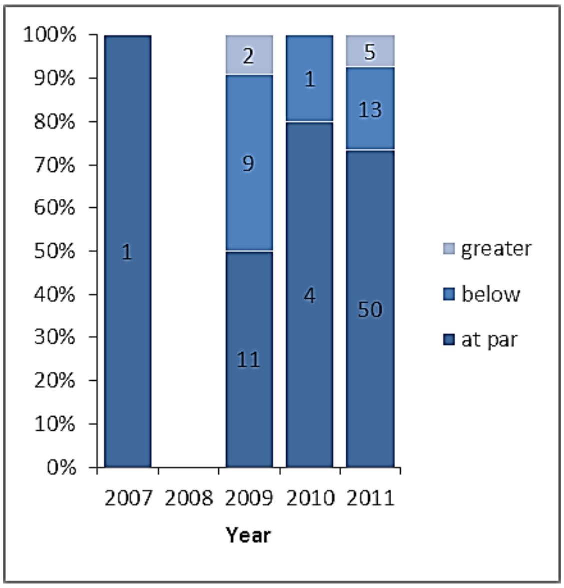

Figure 2-38: Proportion of cases by observation year stratified by the relative body size to the whole herd (n=98)

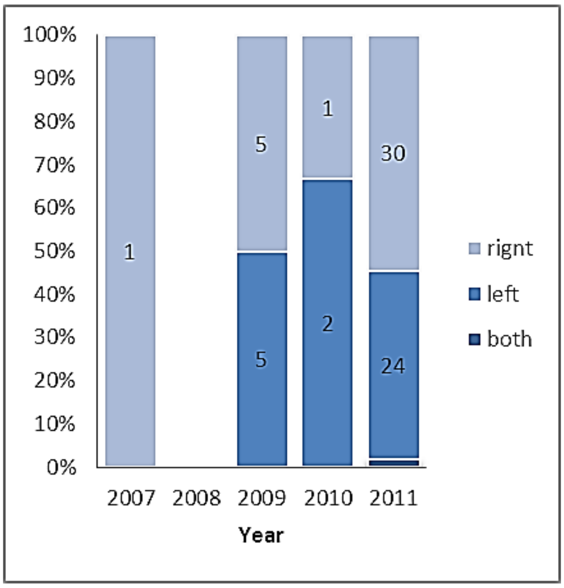

Figure 2-39: Proportion of cases by observation year stratified by the affected humeri (n=69)

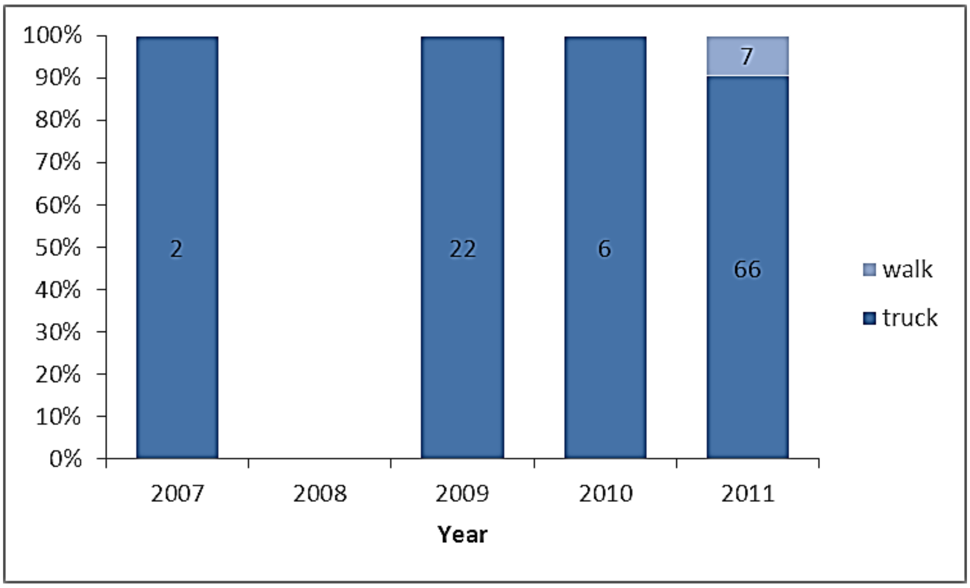

Figure 2-40: Proportion of cases by observation year stratified by the mode of heifer's transportation (n=103)

2.4.7 General farm factors

In general, the animals in the case farm had to walk daily to the milking platform to be milked. The summary statistic of the walking distance and speed is shown in table 2-6. The case frequency as a function of the average daily walk distance, four weeks prior to the fracture is revealed in fig 2-41. It seemed that the fracture cases were independent of the average daily walk distance as the plot pattern was random. The case frequency plot against the walking speed, however, as seen in fig 2-42 revealed an increasing trend. A linear plot fitted using the least square method shows that 12% of the variation in the case frequency can be explained by the walking speed (R^2=0.12).

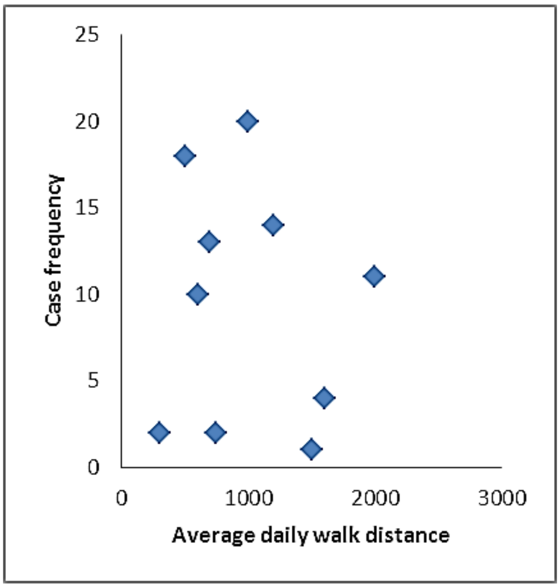

Figure 2-41: Case frequency as a function of the average daily walk distance in a month prior to the fracture (n=95)

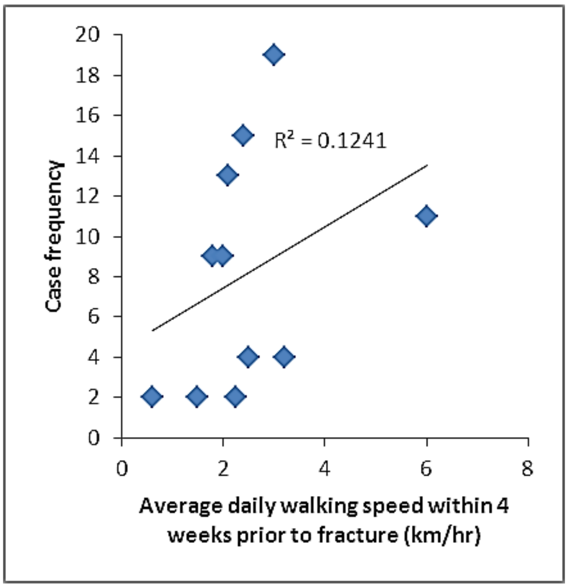

Figure 2-42: Case frequency as a function of the average daily walking speed within a month before fracture (n=90)

Table 2-6: Summary statistics of the walk speed and distance of the case animals within a month prior to the fracture

	Walk speed (km/hr)	Walk distance (metre)
n	90	95
mean	2.80	977.89
sd	1.31	479.12
med	2.40	1000
min	0.60	300
max	6.00	2000
Q1	2.10	600
Q3	3.00	1200

The feeding and supplementation profile of the affected farm were included in the study as to investigate the connection between the case incident and the feed in different parity. In this study, the proportion of cases in the first parity was noticeably higher (83%, 95/115) than the other parities and the parity group of higher than 2 were not affected. The feeding profile of case farms can be divided into four groups: calf, non-pregnant heifer, pregnant heifer and lactating cows. The summary of the proportion of case farms' basal feed in different animal groups is shown in table 7. It was noted that all animal groups had been fed with rye and clover as the main roughages. The other basal diet which exceeding the 50% mark (i.e. common in more than half of the case farms) were grass silage in pregnant heifer (68%) and lactating cows; maize silage (55%) and PKE (68%)in lactating cow. Figure 44 shows the graphical comparisons of the basal diet between the age group in the case farms (n=22).

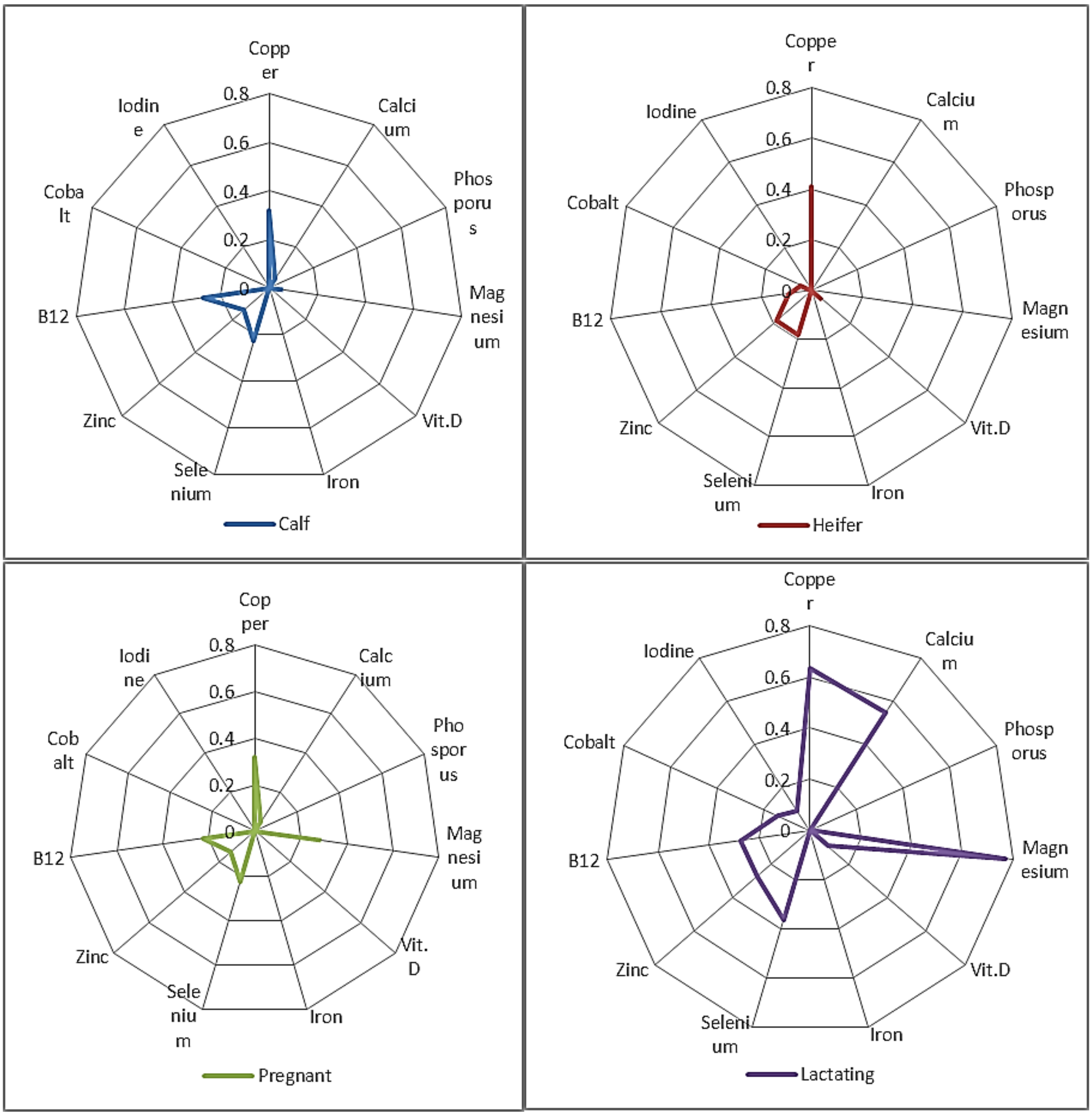

Figure 2-43: Nutrient supplement of different group in the case farms (n=22)

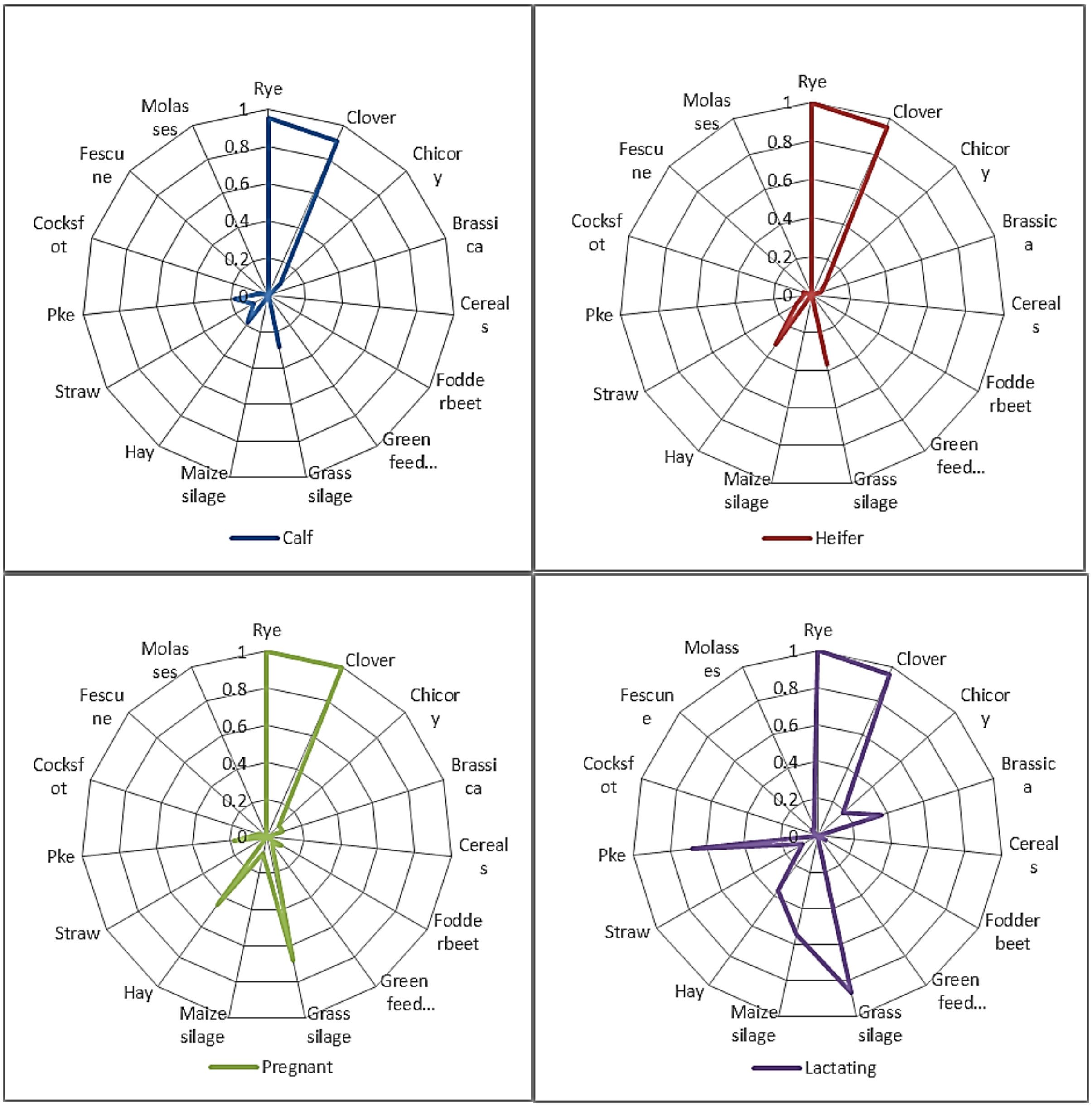

Figure 2-44: Basal diet of different age group in the case farms (n=22)

The summary of the proportion of case farms' mineral supplementation in different animal groups is shown in table 2-7. The supplemented minerals which exceeding the 50% mark (i.e. common in more than half of the case farms) were copper (64%), calcium (55%) and magnesium (77%) in the lactating cows. Figure 2-43 shows the graphical comparisons of the mineral supplementation between the age group in the case farms (n=22).

Table 2-7: The feeding and mineral supplementation profile for the case farms; the proportion figure stated in the table indicates the number of farms which has been given the listed item out of 22 case farms. If the figure stated 0.5, 50% of the case farms had given the item to all animas in the farm according to the age group.

	Rye	Clover	Chicory	Brassica	Cereals	Fodderbeet	Green feed oat	Grass silage	Maize silage	Hay	Straw	Pke	Cocksfot	Fescune	Molasses
Calf	0.95	0.91	0.09	0.00	0.00	0.00	0.00	0.27	0.00	0.18	0.09	0.18	0.05	0.00	0.00
Nonpreg	1.00	0.95	0.09	0.05	0.00	0.00	0.00	0.36	0.00	0.32	0.09	0.05	0.05	0.00	0.00
Preg	1.00	1.00	0.09	0.09	0.00	0.09	0.05	0.68	0.09	0.45	0.00	0.18	0.05	0.00	0.00
Lact	1.00	0.95	0.18	0.36	0.00	0.05	0.00	0.86	0.55	0.36	0.09	0.68	0.00	0.05	0.05

	Copper	Calcium	Phosporus	Magnesium	Vit.D	Iron	Selenium	Zinc	B12	Cobalt	Iodine
Calf	0.32	0.05	0.00	0.05	0.00	0.00	0.23	0.14	0.27	0.00	0.00
Nonpreg	0.41	0.00	0.00	0.00	0.05	0.00	0.18	0.18	0.09	0.05	0.00
Preg	0.32	0.05	0.00	0.27	0.00	0.00	0.23	0.14	0.23	0.00	0.00
Lact	0.64	0.55	0.00	0.77	0.09	0.00	0.36	0.27	0.27	0.14	0.09

2.4.8 Comparisons of study data with known references

The quality index of dairy cattle, as published by DairyNZ was compared to the quality index captured in the study and can be seen in figure 2-45. It was learned that the median of the case animals' quality index of were significantly higher (Wilcoxon rank test p<0.001) than the national average in 2011.

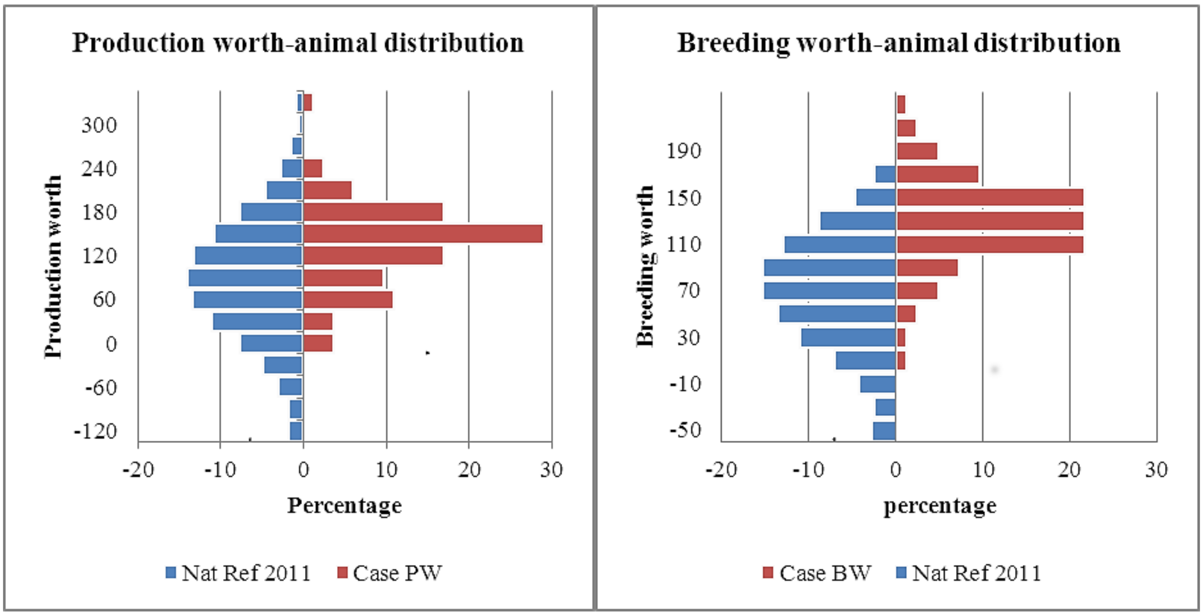

Figure 2-45: Comparison between case animals' quality indices with the national reference 2011 produced by Dairy NZ

2.5 Discussion

A total of 149 cases was reported by 22 respondents whose farm had been affected by spontaneous humeri fracture in five years observational studies, based on the proposed working definition. The figure was 37 times larger than the initial case report produced by Weston (2008). Even though the total number of affected cows is small as compare to the total cows in New Zealand, which is exceeding 4.5 million in 2011 (DairyNZ, 2011b), the facts that it had never reported in dairy cattle population elsewhere produces no precedents on how to effectively manage the problem. The trend of the incident over five-year observations (R^2=0.7) is distressing as the case count in the following years could potentially rise exceeding the last count in 2011/12, which was 83 cases. The case culling rate of 50% with the survivors had to be dried-off (Weston, 2008) justified the need to investigate the unique fracture syndrome as it produced nothing but loses to the affected dairy enterprise.

2.5.1 The case definition

With reference to the first case report by Weston (2008), a working definition that matched the earlier description of case animals was produced. Any cattle would be considered as a case if the following condition were met:

- sudden onset and severe non-weight bearing lameness on the front leg, and
- physical examinations suggest that the bone between the elbow and shoulder is fractured, and
- There was no sign of external trauma.

We were aware that the case definition could be more specific by including only cases that were physically examined by the veterinarian, but the sensitivity of the definition would be tremendously reduced as not every fracture case could be attended by veterinarians (Weston, 2008). Based on the case details we gathered, only 58% (67/115) were seen by veterinarians. Nevertheless, we are confident that fractured long bone in large ruminant is easy to diagnose as it impede the animals' movement (Nichols et al., 2010), and as the affected animals may undergo 'home kill' to salvage the edible part, the fractured bone can be seen.

2.5.2 The questionnaire

The questionnaire (annex 1) was constructed on mixed formats that combined the probing and open questions. The open questions or comments were meant to allow additional information to be reported besides the fixed variables in the questionnaire. However, not all variables were reported in this paper, especially the herd factors as it was prepared for the continuation of the current study such as case-control study.

The information bias that resulting from incomplete or missing information may happen in this study, especially with these variables (with the missing value percentage, n=115): wean age (56%), wean weight (43%) and affected leg (40%). In order to reduce information bias, the respondents were contacted after the questionnaire submission to further explain their responses. However, further explanations were not asked if the respondent could not remember the details as to reduce the potential recall bias. The other variables in the study (fig 1) recorded satisfactory responses.

2.5.3 The results

The study was conducted to find risk factors that were consistently existed before, and during the fracture happened. The risk factors were grossly divided into three major groups (individual, time, and place) in line with the convention of reporting sentinel event (Grimes & Schulz, 2002; Kempen, 2011) as to the best of our knowledge, little is known about the demographic features of the spontaneous humeri fracture syndrome in dairy population.

2.5.4 Temporal factor

In general, the time line of the spontaneous fracture cases (fig 2-14) suggested that there was a seasonal pattern of the case. Over the five-year observations, the cases consistently appear in the second half of the affected years. Stratification of cases according to the absolute calendar month confirmed our suspicious as the case appeared as early in July, and peaked in September and October. With the reference to New Zealand's season, the cases appeared in spring until summer, which was consistent with the common season of fracture cases elsewhere observed by Martens (1988) and Gangyl (2006). In relating to the widely practiced dairy management (DairyNZ, 2011b), spring is the season when pregnant cows start to calve and a new lactation cycle would begin.

Out of 22 farms that responded, 45% (10/22) had a repeated episode of spontaneous fracture in different years (fig 4) suggesting that the spontaneous humeri fractures in the case farms had the tendency to recur. Those case farms (table 4), which had been affected in the final observation year (2011/12), 56% (10/18), was affected for the first time. Judging from the repetitive tendency of the affected farms and the involvement of new farms, it was speculated that the risk factors were still present in the affected farms, and in the other presumably unaffected farms.

2.5.5 Spatial factors

The spatial distribution of the case farms is shown in fig 2-19, 2-20 and 2-21. The details of the location are available in table 2-4. Regardless the administrative boundaries, the case farms seemed to cluster in the northern part of the North Island, the southern part of the North Island and the middle part of the South Island, hence the case farms were regrouped as we were interested to know the persistence of the identified risk factor in the cluster of case farms. The reasons of the clustering of case farms remain unknown. If the remaining ten non-responding farms were included, the cluster may still have looked the same as three non-responding farm are located in Bay of Plenty while the rests are located in Waikato.

2.5.6 Animal factors

2.5.6.1 Breed

A crude evaluation on the cases' breed in fig 2-6 revealed that case counts in crossbred was twice as high the individual pure breeds. The New Zealand dairy breed breakdown (DairyNZ, 2011b) shown that the representation of the recorded case breeds in New Zealand was as followed: crossbreed (38.9%), Jersey (12.4%) and Friesian (40.0%). The findings clearly suggesting that the breed differences in case count was not influenced by the overall breed composition as if that were the situation, the proportion of cases in Friesian would be similar to the Crossbreed.

Stratification of case count by the region category (fig 2-26) which indicates crossbreed constitute more than 50% of the case count, concurred with the crude estimation of the overall case-breed proportion. Conversely, the stratification of cases against the rearing seasons (fig 2-33) revealed that crossbred was not always had the highest proportion. Nevertheless, the 95% CI of the breed proportion revealed the crossbreed contribution towards the annual cases was homogenous. The proportion of each breed that became the case in the observational period from 2007-2012 (except 2008) were: Crossbred (67%, 39%, 60%, 65%), Jersey (0%, 52%, 40%, 13%), Friesian (33%, 10%, 0%, 22%). The high overall count of crossbreed that became the case was contributed by the count of cases in season 2011/12 (50/77).

Distinctive features regarding the crossbreeds in New Zealand are: they have a live weight across the ages that are higher than Jersey but lower than Friesian, and (on average) were known as a higher producer of milk volume and milk fat than other breeds (DairyNZ, 2011b). Breed dominations in fracture cases were also observed in many fracture-related case series (Martens 1998, Gangyl 2006). In summary, breeds may be an important predisposing factor to the spontaneous humeri fractures and in New Zealand situation, crossbreed may be at a higher risk.

2.5.6.2 Quality indices

New Zealand dairy farmers in general, adopt performance indicators produced by NZAEL that are scaled into a 4500 kg DM/year as a unit of feed (NZAEL) and reported yearly in the New Zealand Dairy Statistics (DairyNZ, 2011b). Based on the information on the NZAEL info sheet, BW is a comparative ranking system that describes a cow's genetic ability to produce a profitable replacement. There are three major components (in a different weight contribution) that included in the calculation of BW: ancestry information, own lactation performance and progeny information on seven traits (four traits that increase BW: protein, milk fat, fertility, residual survival; and three traits that decrease BW: milk yield, live weight, somatic cells). While the contribution of each component varies with the increase in the lactation number, the ancestry information is always the biggest component of BW. A cow with a BW of 100 is expected to produce $50 extra profit per unit feed than a cow with a BW of 0, through the more efficient breeding daughter. On the other hand, PW is a ranking system that describes a cow's own ability to be a profitable and efficient lifetime producer; not what she is expected to pass on to the offspring. The component that made up the PW is similar to BW but the biggest contribution came from own lactation performance, which consists of four traits (traits that increase PW: protein, milk fat; and traits that decrease PW: milk yield and live weight). A cow with PW of 100 is expected to produce $100 extra profit per unit feed that a cow with PW of 0.

In this study, the case animals had a median (range) BW of 116 (16 to 226) and PW of 120 (-23 to 319). Stratification of the performance indicators according to the region category (tab 2-2) shows that the 95% confidence interval of the mean in each region overlapped. Hence it can be concluded that the performance indicator of the case animals in every region category was homogenous. Comparison of the performance indicator with the national average (DairyNZ, 2011b) of cows' BW and PW in 2011 (fig 2-45) using Wilcoxon rank sign test revealed that the case animals had a significantly higher median score ($p<0.0001$). The national average of the performance indicators in 2011 was used as a comparison because 70% of case details (77/111) were recorded in the year 2011/12, and as the performance indicator is on the increasing trend (DairyNZ, 2011b), comparison were made with the highest national average. The temporal trend of the case animals' performance indicator as shown in fig 2-36 suggesting that the case animals PW was consistently high (R^2=0.0006) and BW increased by 5% (R^2=0.05) throughout the five-year observation period. As the case animals BW and PW were found to be consistently and significantly higher than the national average, they could be risk factors to the spontaneous humeri fracture syndrome in dairy cattle in New Zealand.

2.5.6.3 Age, parity and duration post-partum

Out of 155 case details obtained from 22 affected farms, none were male. Gender-related event would plainly suggest that there were elements that the other gender (male) does not possess which lower the risk of getting the spontaneous humeri fractures' syndrome. Previously reported case series of bone fractures in large animals (Crawford & Fretz, 1985; Gangl et al., 2006; Nichols et al., 2010) did not regard gender as a common or important risk factor for bone fracture in their observation. Gender-related fracture, however, was described in human and swine population. In human, the increased risks of osteoporosis-related fracture were mainly post-menopausal (Ahlborg et al., 2003), and during pregnancy and lactation stage (Di Gregorio et al., 2000). Post-menopause's higher risk of fracture is related to the primary osteoporosis whereby the natural reduction of oestrogen has been identified associated with the event; while lactation osteoporosis is associated with a sudden increase in calcium demand that was met by the excessive mobilization of calcium reserve in bone. In swine, as described by Spencer (1979), marginal ration of calcium (5%) from weaning until the lactation stage produced osteoporotic sows in six weeks of lactation.

In this study, the case animals were relatively young whereby 83% (84/101) and 17% (17/101) were between 24 to 31 and 36 to 40 months old respectively. Hence, comparison with the post-menopausal osteoporosis in human may not be appropriate.

Another important feature of the case as reported by respondent was all the case animals were either in the late pregnancy, lactating or pregnant and lactating. The proportion of cases in the first parity (95/115, 95% CI: 0.75-0.88) was significantly higher than in the second parity (17/115, 95%CI: 0.09-0.22) or those pregnant heifers (3/115, 95%CI: 0.01-0.07). No cases were recorded beyond the second parity. The significantly higher case count in lower parity is uncommon in many dairy cattle common conditions that affect mobility such as lameness (Bicalho, Machado, & Caixeta, 2009) or heel erosions (Chapinal, Baird, Pinheiro Machado, von Keyserlingk, & Weary, 2010).

Stratification of the case animals by the month post-partum, regardless the parity, revealed that 88% (93/106) of those cases happened within the first four month of lactation. The first four-month post-partum is the duration for high milk production (García & Holmes, 2001; Wood, 1967). Therefore, it is postulated that the spontaneous fractures in dairy cattle were in line with the period of high milk output, which indirectly correlates with the calcium demand. Spontaneous fracture cases in the pre-partum period, even though the percentage was small (3%, 3/106), serve as an indicator that the bone calcium level was extremely low to compensate the dietary calcium to meet the developing foetus requirement in the third trimester which is estimated between 2.3g/day (gestation day = 190) and 10.3g/day (gestation day =280)(House & Bell, 1993).

2.5.6.4 Body condition

Body condition of dairy cows at any point of time is a general indicator of the past nutritional (Hady, Domecq, & Kaneene, 1994) or the health status (Heuer, Schukken, & Dobbelaar, 1999; Ruegg & Milton, 1995). Deficiency in feed or the feed quality usually can be translated into a lower body condition score (Burkholder, 2000). The presence of diseases such as internal parasitism may also lower the body condition even though the feed quality issue was non-arise (Sykes, 1994). The commonly used body condition score has five categories ordinal scale with the highest score of 5 indicating fat, while the lowest score of 1 indicating thin (Edmonson, Lean, Weaver, Farver, & Webster, 1989).

In this study, the body scores of the case animals were recorded in three different times whereby the case animals would normally be seen by the handler to reduce the information bias: before mating, upon return from grazier and during the fracture happened. Individually, the recorded body score may indicate the past state of the case animals, but together, they may serve as an indicator of the fluctuation of the body condition over an extended period of timeline. However, instead of using the common five score scale by Edmonson et al (1989), a simpler three ordinal score scale (light, average, extra good) were employed in this study with the categories that are generally known by New Zealand dairy farmers (based on the feedback received when we beta-tested our questionnaire on a farmer in Manawatu region). The lowest score (light) serves as an indicator that the case animals were below the acceptable weight to the occasion. The other two categories were the acceptable weight to the occasion but 'extra good' means the animals were overweight.

The timeline of case animals' body condition according to the rearing seasons in which the spontaneous fracture happened is available in fig 2-10. Generally, the case animals had achieved a desirable (average and extra good) body condition upon return from the grazier but a noticeable drop from extra good to the average was observed before the fracture happened. This may be due to the fact than the case animals were pregnant upon return; and had given birth or start lactating before the fracture happened, hence the body conditions upon return from grazier were seen superior than before the fracture happened. Similar change in the body condition scores relative to the parturition in normal cows was observed by (Dewhurst, Moorby, Dhanoa, Evans, & Fisher, 2000). As a matter of comparison with the whole herd, this study found that more than 50% of the case animals in every rearing season were at par or greater than the herd average (fig 2-38) which means physical appearance may not be able to discriminate the case from non-case.

The importance of the observations over the body weight which were recorded as a categorical score was to determine whether overweight factors consistent with the fracture cases. It was learned from the study that only 14% (14/100) of case animals were overweight hence weight may not be a good predictor for the spontaneous humeri fractures. Body weight is regularly used to describe the overall clinical symptom of disease condition. For example, in cases of ruminant copper deficiency, the case clinical description usually included growth retardation, lower immunity, scour, which collectively may lower the weight gain (Hidiroglou, 1980; Suttle, 1986) while calcium deficiency effect on the weight gain during the growing stage may go unnoticed (USDA 1954).

The affected side of the leg (humeri) were included in the study to investigate the predilection site for the spontaneous fracture. Earlier study by Neveux et al (2006) revealed that the weight distribution between fore and hind was not significant even though the forelimb consistently recorded handled a higher proportion of body weight. The ratio of weight distribution between front and hind of dairy cows were 54:46 while the weight distributions on the individual limb were as followed: right front (27.7-29.4%), left front (26.0-26.3%), right back (23.4-25.2%) and left back (21.7-22.5%) (Neveux, Weary, Rushen, von Keyserlingk, & de Passille, 2006). In this study, only the front limbs were seen affected in which 44% (31/69) was recorded on the left and 53% (37/69) was on the right sided.

Nevertheless, the differences were not significant as the 95 % CI for proportion of the affected leg overlapped with the null value (95% CI for null was 0.38 to 0.62). Stratification of the affected limb by the respective case farms (table 2-3) revealed that only two farms had recorded a noticeable unilateral high count of cases: farm 12 with five cases of left leg with no cases involving right leg, and farm 20 with 10 cases of right leg with no cases involving left leg. Stratification of the affected limb by the year of occurrence (fig 2-39) revealed the affected side of the front leg had no obvious temporal trend on the preferred site. It would appear that the fractured humeri may be random but caution has to be exercised to the conclusion as the missing value for the leg variable is quite high (40%), hence information bias may occur.

As the case animals were raised elsewhere during the growing stages and returned to the case farms at the age of 20-24 months, the return activity believed to be important as the return heifer activity happened prior to the occurrence of 83% (84/101) of the cases in the first age group (24 to 31 months). If case animals in the 2nd parity were excluded on the ground that the transportation activities had occurred for sometimes, as much as 92% (82/89, SE=0.05) of the case animals at risk had been transported by truck. The 95 % CI for the proportion of the transportation by truck did not overlap with the null value (95% CI for null was 0.40 to 0.61), hence the transportation by truck may be significant. Stratification of the means of transporting heifers back to the farm by the region (fig 2-32) and year (fig 2-40) showed a consistently high trend of the truck involvement. Therefore, transportation of heifer using a truck may be an important risk factor to the spontaneous humeri fracture.

2.5.7 General farm factors

As the accumulation of micro damage as a result of repetition of stress on the bone could lead to the weakening of the bone strength (Fyhrie et al., 1998), we investigated the effect of the physical activities of the case animals in four weeks prior to the fracture. The physical activity parameters of interest were the walk distance and the walk speed of the case animals. Fig 2-41, in which the case frequency was plotted against the average walk distance underwent by the case animals, shows a random pattern. Therefore, it could be stated that the average daily walk distance in four weeks prior to the fracture was not consistent with case animals' count. The walk speed of the case animals in four weeks prior to the fracture (fig 2-42) shows an increasing trend of case frequency with the increase in the walk speed. A fitted linear trendline using the least square method showed 12% variation in the case frequency could be explained by the increase in the walk speed four weeks prior to the spontaneous humeri fracture (R^2=0.12). Rubin et al (1982) found that increased in the moving speed could increase the micro strains on the long bones (C. T. Rubin & Lanyon, 1982), hence it could be stated that the increase in walk speed of the case animals may be a risk factor for the spontaneous fracture syndrome dairy cattle.

2.5.7.1 Drought

Water stress is known to have effect on the dry matter yield but the chemical compositions usually remain unchanged (Karsten & MacAdam, 2001) hence nutritional deficiencies as a result of drought are mainly as a consequence of the reduced feed availability. We investigated the effect of drought on the count of case animals. As the droughts were not a regular yearly event in the affected farms, the count of cases in relation to the prior drought experience was produced. In this study, it was revealed that 77% (88/155) of case animals were located in drought affected farms (fig 2-17). Further investigation of the post draught fracture event (fig 2-18) revealed that the duration between the last month drought to the month of fracture ranged between 4 to 48 months (mod=8 months). The finding suggested that there was a long lag between the recorded drought and the fracture event hence it is doubted that the drought had a meaningful contribution to the case. The body condition score (BCS) of the case animals that were located in the drought affected farms were majority (94%, 74/79) on the desired level (average and extra good), support the thought that the recorded drought had little impact on the case animals.

2.5.7.2 Different nutritional profile

Feeding the dairy cattle is a critical area as it represents 27% of the total operating cost of a pastoral based rearing system in New Zealand(DairyNZ, 2011a). Therefore, strategizing the feeding regime that would increase profit has been a priority in dairy enterprises by the introduction of supplementary feed to the pastoral dairy system (Deane, 1999; Macdonald, 1999). Supplementary feed would not just be providing a low-cost energy source to dairy cattle (E.S. Kolver, Roche, Miller, & Densley, 2001), it can be used to break the pasture feed barrier set by the weather related low dry matter yield (Gray & Lockhart, 1996). The feeding and supplementation profile of the affected farms was investigated in the study in an attempt to link the feed with the fracture cases as the nutritional element deficit have been implicated in many previous studies (Weston, 2008).

The feeding profiles of the case farm were divided into four animal groups: Calf (newborn to six months), non-pregnant heifer (6 to 15 months), pregnant heifer (15 to 24 months) and lactating cow (>24 months). Whereas the first three feedings profiles were not repetitive as the animals grow older, the last feeding profile (lactating cow feeding profile) was repeated in each breeding cycle.

Basal diet of the 22 case farms which recorded a total of 149 fracture cases is shown in fig 2-44 and table 2-7. It was learned that the main basal diets in all 22 case farms were rye and clover. The other basal diets which were common in more than half of the case farms were grass silage in pregnant heifer (68%), grass silage in lactating cows (86%), maize silage in lactating cows (55%) and PKE in lactating cows (68%). Mineral supplementation profile of the 22 case farms is available in fig 2-43 and table 2-7. The mineral supplement that was common in more than 50% of the case farms were: copper in lactating cows (64%), calcium in lactating cows (55%) and magnesium in lactating cows (77%). The findings as illustrated in radar charts (fig 2-43 and 2-44) indicate that the lactating group received a better ration amongst the four animal groups.

The lactating group received a better ration possibly because of the additional requirement for energy and minerals to support the lactating phase (E. S. Kolver & Muller, 1998). The ration differences observed in the study may also explain the distinctive features of the spontaneous humeri fracture syndrome which was very high in the first parity (83%) then reduced greatly in the second parity (15%) and none in the subsequent higher parity. It is suggested that the nutrition content in the growing stages did not prepare the bone for the normal dynamic of bone remodeling during in the final trimester to meet the foetal growth and in the lactation stages (Braithwaite, 1983). The basal diet could only supply between 0.42% (rye) to 1.19% (clover) of calcium in dry-matter of feed (Harrington, Thatcher, & Kemp, 2006) with a maximum absorption efficiency of 66% (Braithwaite, 1983). In the 3rd trimester, the amount of calcium needed for foetal growth is an additional 2.3g/day (190th day of pregnancy) to 10.3g/day (280th day of gestation) (House & Bell, 1993) and the lactation calcium requirement varied is between 1.22 to 1.45 g/kg milk (National Research Council, 2001). If the calcium deficit in the feed were prolonged, calcium from the bone will be mobilised to meet the demand of maintaining the narrow plasma calcium level between 8-10mg/dL (Goff et al., 1991). In the second and subsequent parities, the susceptible animals were getting a better nutrition supplement (calcium, magnesium and copper) from the previous lactation cow ration hence it was speculated that the lactation ration had prepared the bone for the next massive bone remodeling in the lactation stage. The lactation ration may not be able to address the bone calcium extraction appropriately in the first parity probably because of the expected negative net balance of bone calcium in the early lactation stage (Braithwaite, 1983; Oliveri et al., 2004). Additional calcium in the feed during the early lactation stage may be of less beneficial as additional vitamin D is needed to actively transport the intestinal calcium through the calcium-ion canal in the gut epithelium (Bronner, 2003). Unfortunately, normal vitamin D level during lactation is lower than the gestation stage (Kovacs & Kronenberg, 1997) therefore, the normal bone calcium deficit may not be able to be compensated through calcium in feed, especially when the lactating cow is expected to produce milk. It is also speculated that as the susceptible cows in the case farm becoming pregnant for the second time, the elevation of serum Vitamin D in 2nd trimester (Oliveri et al., 2004) on top of the surplus of calcium in the lactation ration may prepare the bone for the subsequent normal negative net balance of bone calcium. Braithwaite (1983) observed that the bone replenished the mineral deficit in the mid-lactation. In this study, the speculation is supported by the finding of fewer cases (15%, 17/115) in the 2nd lactation and none in the subsequent.

2.5.8 Plausible cause of spontaneous humeri fracture in dairy cattle

This study found that the selective calcium supplementation given to the lactation group in the case farms may be responsible in suppressing the incident of spontaneous humeri fractures in the second parity and higher. The similarity in the individual case attributes which serve as distinctive features that associate spontaneous humeri fractures' syndrome with the high calcium demand:

- It occurred in late pregnancy or lactating stage.
- It occurred mainly (87%, 93/106) within the first four-month post-partum.
- The high quality indices (BW & PW) of the case animals suggesting that they had the potential to produce more output (milk) as compared to the median of cows in New Zealand.

Evidence from the other observations revealed that:

- Mobilisation of bone mineral in late pregnancy and early lactation occurs in ewe regardless the dietary calcium and phosphorus content (Braithwaite, 1983).
- Prolonged lactation period increase the risk of lower bone density in female (Sowers M & et al., 1993)

- Calcium deficit in gilt during the growing, continued to the lactation stage increase the risk of spontaneous fracture in less than seven weeks of lactation (Spencer, 1979).
- Pregnancy related transient demineralisation of bone during the 3rd trimester had been reported in human (Curtiss & Kincaid, 1959).

Together with the age group and gender specificity, as well as the absence of other classical syndromes that would relate the case animals to the popular believe of copper deficiency such as lowered immunity, lower body weight, bowed leg, scour; we believe that transient osteoporosis to pregnancy and lactation was the most likely causative agent of the spontaneous humeri fractures in dairy cattle in New Zealand form 2007-2012.

2.5.9 Limitation

To our knowledge, this is the first study that comprehensively describes the occurrences of spontaneous fracture of humeri bone in dairy cattle in New Zealand. While bone fractures in ruminant have been widely reported (Gangl et al., 2006), the affected bones were not specific. Case reports that specifically describe the occurrences of specific long bone (femur) fractures in 10 years (Nichols 2010) only manage to gather 26 cases.

The study was purposive in nature; to investigate the incident of spontaneous humeri fractures in dairy cattle. Hence, candidate farms were chosen based on the presence of the outcome of interest. Recommendation from veterinarians, researchers and even farmers whose farm had become or once were having the fracture syndrome, were obtained to ear-mark the possible candidate of the study. As the recommendations from the above-mentioned personnel were pursued independently, the suggested farms overlapped. The final list of farms which were believed to be affected by the fracture syndrome contained 30 farms, which were contacted between December 2011 and January. Out of the 30 farms suggested, only 73% (22/30) replied. This may potentially underestimate the actual count of cases under study, but we believe; form the response of two farmers who wished not to be included in the study, the remaining count was small. From 22 farms that returned the questionnaire, only 77% (115/149) cases reported in the questionnaire managed to be gathered, which may introduce bias to the observation. Nevertheless, the study was the largest retrospective study on spontaneous fracture in dairy population in New Zealand involving 115 cases in five years.

This study is categorised as a case series as it was lacking of control populations. Such observational study was chosen as, to the best of our knowledge; the syndrome was relatively new. The only reference available for the spontaneous humeri fracture in dairy population was the case report produced by Weston (2008). Even though in general, case series were known to tender a low level of evidence (Wang & Attia, 2010), the contribution of the case series cannot be denied. An example of a case series of an exceptionally rare pneumocystis pneumonia among homosexual men in Southern California (Centers for Disease Control, 1981), contributed to the identification and recognition of the epidemic of the previously unknown acquired immunodeficiency disease syndrome (AIDS)(Steinbrook, 2012).

2.6 Conclusion

The study managed to achieve the objective of describing the features of spontaneous humeri fractures in New Zealand dairy population. The initial intuition that the spontaneous fractures that involving a specific long bone were not random was concurred in the study; there were observable patterns in the case animals and farms. This study is categorised as a case-series as it was describing the case animals in five consecutive rearing seasons but lacking of control population. As such, no conclusions were attempted on the cause of the event except to provide some clues to the associated risk factors that constantly present in a high proportion. The merit of the study was to alert the interested parties about the similarities of the risk factors that were present prior or during the occurrence of the spontaneous fracture in dairy cattle. A continuation of the study with other studies that able to tender a higher level of evidence is highly recommended. This study prepared the information regarding case properties, spatial and temporal factors as well as the herd properties of the case group, which can be used for case-control studies. Based on the information gathered on the case animals, it is hypothesized that the spontaneous humeri fracture syndrome that affecting New Zealand dairy cow population from 2001-2012 was a transient osteoporosis to pregnancy and lactation, as it was gender specifics and occurred during the period of increased calcium demand. Similar events in swine and human heighten our suspicion on the protagonist of transient osteoporosis in explaining the spontaneous fractures' syndrome dairy cattle. Other risk factors that were consistently present with the case animals and/or case farms, which may be associated with the fractures: lack of the dietary calcium in the growing stages; breed of the dairy cows; high in the quality index (breeding and production worth); increased in the walk speed of the lactating cows; involvement of a truck in the transportation of heifers back from graziers.

2.7 References

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3 Appendix

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

Investigation of front leg fractures in dairy heifers

Questionnaire

December 2011

PLEASE RETURN (in the reply paid envelope) TO: Zul Bahar Abdul Rashid, Epicentre, IVABS, Massey University, Private Bag 11222, Palmerston North.

If you need help with filling in this questionnaire please contact your local veterinarian. Further information about the investigation can be obtained from:

- A/Prof Cord Heuer, Epicentre, (06) 350 5948 (Email: c.heuer@massey.ac.nz)
- Prof Keith Thompson, (06) 356 9099 ext 7621 (Email: K.G.Thompson@massey.ac.nz)

Institute of Veterinary, Animal & Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North

QUESTIONNAIRE ON FRONT LEG FRACTURES IN DAIRY HEIFERS

Preface

In recent years, there have been an increasing number of young dairy heifers suffering a spontaneous fracture of the humerus (bone in the front leg between shoulder and elbow), usually early in their first lactation. We are interested in gaining more information on this syndrome in an effort to determine risk factors. The aim of this questionnaire is to record such cases and establish a database that could provide a starting point for further investigation.

This questionnaire is divided into 2 parts. The first part relates to information about your farm and the second part should be filled in for each case that you have had on your farm. Please complete and return as many of the second questionnaires as you are able.

Confidentiality

All information provided will be treated with confidentiality. Access to the information will only be available to the investigation team.

I. Person completing this questionnaire (tick all that apply):
- ☐ Farm owner
- ☐ Farm manager
- ☐ Stockperson
- ☐ Veterinarian
- ☐ Other: ___________________

II. Date of questionnaire completion:___________________________

III. Study consent

a. Would you be happy to participate in any follow-up studies on the above topic (tick one that applies)
- ☐ Yes
- ☐ No

b. If yes, what would be your preferred method of communication:_________________________

1) Contact details

a) Farmer's name:____

b) Trading name:____

c) Farm address:____

____

____

____

d) Contact no; Ph: _____________________ Mobile: ___________________Email:_____________________

e) Vet practice/ vet involved: ____

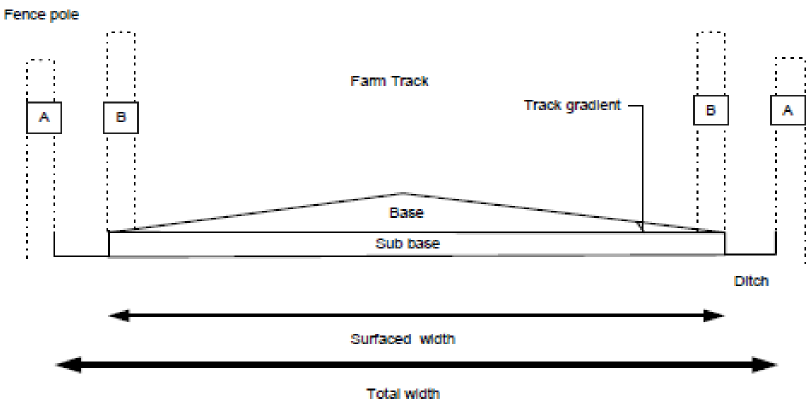

Fig 1. Schematic drawing of farm track

v. Main farm track description; refer to figure 1:

a. Width in total (metres): ____________________

b. Surfaced width (metres): ______________________

c. Surface cover materials(tick all that apply):

□ Gravel □ Sand □ Crusher dust

□ Rotten rock □ Limestone □ Other (state): __________________

d. Any ditch(s) at the side (tick one that applies):

□ Yes □ No

e. Tracks become muddy in bad weather (tick one that applies):

□ Yes □ No

f. Track surface design (tick all that apply):

□ Crowned □ Flat □ Raised on one side

□ Other (state) _________________________________

g. Track gradient sideways slope (tick one that applies):

□ <3% □ 3-5% □ 5-8% □>8%

h. Fence pole location (tick one that applies):

□ A □ B

Comment:____

____

____

____

____

d) Physical hazards

i. Any obvious physical hazards on the farm that the cows may be exposed to? (tick all that apply)

- ☐ Sharp curve in walkways
- ☐ Concrete/metal feeding trough
- ☐ Narrow alley in the milking shed
- ☐ Bottle-neck (e.g. underpass)
- ☐ Projection of object from the tracks
- ☐ Slippery concrete floor
- ☐ Other (state):

____

____

____

e) Mob's feeding management

i. Have any of these weeds detected in the pasture? (tick all that apply)

☐	Thistles (Scotch)	☐	Dock	☐	Dandelion
☐	Thistles (Californian)	☐	Ragwort	☐	Hawkbit
☐	Buttercup	☐	Stinking mayweed	☐	Oxeye daisy
☐	Kikuyu	☐	Fathen	☐	Paspalum

Comment:____

____

____

ii. Mineral/vitamin supplementation management for the whole mob

Type	Given to (*tick √ where appropriate*)				Specific name (if known)
	Calf	Heifer		Cow	
	(3-12 month)	Before pregnancy (13-15 months)	During pregnancy (16-24 month)	Lactating	
Copper	☐	☐	☐	☐	
Calcium	☐	☐	☐	☐	
Phosphorus	☐	☐	☐	☐	
Magnesium	☐	☐	☐	☐	
Vitamin D	☐	☐	☐	☐	
Iron	☐	☐	☐	☐	
Other:________	☐	☐	☐	☐	
Other:________	☐	☐	☐	☐	
Other:________	☐	☐	☐	☐	

Comment:____

____

____

____

iii. Base diet (tick all that applies):

Type	Given to (*tick √ where appropriate*)				Specific name (if known)
	Calf (3-12 month)	Heifer		Cow	
		Before pregnancy (13-15 months)	During pregnancy (16-24 month)	Lactating	
Pasture					
Rye grass	☐	☐	☐	☐	
Clover	☐	☐	☐	☐	
Other: ________	☐	☐	☐	☐	
Other: ________	☐	☐	☐	☐	
Crops					
Brassicas	☐	☐	☐	☐	
Cereals	☐	☐	☐	☐	
Fodder beet	☐	☐	☐	☐	
Green feed oat	☐	☐	☐	☐	
Italian rye	☐	☐	☐	☐	
Other: ________	☐	☐	☐	☐	
Other: ________	☐	☐	☐	☐	
Derivatives					
Grass silage	☐	☐	☐	☐	
Maize silage	☐	☐	☐	☐	
Hay	☐	☐	☐	☐	
Straw	☐	☐	☐	☐	
Palm kernel (PKE)	☐	☐	☐	☐	
Other: ________	☐	☐	☐	☐	
Other: ________	☐	☐	☐	☐	

Comment: (for example PKE: 5kg/day in peak lactation)

____

____

____

Case details: One form for each case

3) **Case details** (Please indicate 'NK' if the details are not known)
   a) **Case description**
      i. Lifetime ID: ____________________
      ii. Which of the following would best describe the case (tick one that applies)
         - ☐ Sudden, severe lameness in a front leg thought to be a fracture high up in the leg
         - ☐ Confirmed as a humeral fracture (bone between shoulder and elbow) by a veterinarian
         - ☐ Suspected partial / greenstick fracture of the humerus – confirmed by a veterinarian
      iii. Date of occurrence:____________________
      iv. Left or right front leg (if known): ______________
      v. Breed:____________________
      vi. Date of birth:____________________
      vii. Weaning age and weight:____________________
      viii. Sire (name or identification):____________________
      ix. Body condition at fracture (tick one that applies):
         ☐ Light ☐ Average ☐ Extra good
      x. Relative body size to mob average (tick one that applies):
         ☐ Below ☐ At par ☐ Greater
      xi. Number of animals in the group:____________________
      xii. Production worth:____________________
      xiii. Breeding worth:____________________
      xiv. When and where the case was found:
         ____
         ____
      xv. Any other relevant information (past illness, worm infestation, drenching records etc):
         ____
         ____
         ____
         ____
   b) **Reproductive status at the time of fracture (tick one that applies)**
      - ☐ Calved
        If yes, state calving date: ____________________
      - ☐ Pregnant
        If yes, state the duration of pregnancy (month): ______________

      Comment:____
      ____
      ____

c) **Management of the case**

i. During the rearing period, the heifer was (tick all that apply)

□ raised on milking platform (till what age): ___________________

□ run-off (from what ages): _______________________

□ Grazier (from what ages): _______________________

ii. Body condition at mating/ artificial insemination (tick one that applies)

□ Light

□ Average

□ Extra good

iii. If raised by grazier,

i. Grazier's name & address:

____

____

____

j. Soil type at grazing block:____

k. Age at return:____

l. Mode of transport to return to home farm:

□ Cattle truck

□ Walked home

□ Other:__________________

m. Body condition at return (tick one that applies)

□ Light

□ Average

□ Extra good

iv. What was the average daily walking distance to the milking shed in the 4 weeks before the fracture (if applicable): ______________________

v. What was the time required by the case to walk to the milking shed on average? ________________

vi. Comment:____

____

____

____

4) **Any further comment/ observation:**

____

____

____

Thank you for your cooperation.