Bovine ephemeral Fever as a model for antigen /antibody interaction as the basis for Milk Fever of cows.

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INTRODUCTION

In the latter part of the 19th century two diseases of cattle emerged from relative obscurity to cause economic havoc . Both have a paralysis as the major presenting clinical sign. The first of these was bovine ephemeral fever (BEF). It was first described in southern Africa, Egypt and various parts of Asia (Fig 1). The first epidemic in Australia began in 1937, and succeeding epidemics have swept north to south at irregular intervals to reach northern Victoria on occasion. (Uren *et al* 1987, St.George 2008). Cows, heifers, bulls and steers are affected. The cause is bovine ephemeral fever virus (BEFV), a rhabdovirus that exists as a single serotype world-wide. The only effective methods of transmission are by the direct inoculation of blood from a clinical case of BEF or by mosquito transmission, BEFV does not traverse the lymphatic system to reach the bloodstream. (St.George 2008). Immunity is life long following a single attack.

Figure 1 A cow with ephemeral fever completely paralysed

The second disease is Milk Fever (MF). The disease has been known since the 18th century. The day a calf is born it has a small appetite for milk on the first day of life Also, the cow used to require the presence of the calf to let down milk. In cities, the cow and calf was taken around the streets to the customer. This practice still exists in some Asiatic cities. In Europe and America, the rise of modern dairying in the latter part of the 19 th century that saw MF prevalence and mortality rise. Farmers began to feed cows more nutritious food to increase milk yields. Calves were separated from cows at birth and cows milked by teams of hand milkers and then machines. The mortality from MF increased in parallel. MF occurs usually within 2-3 days of calving. First calving cows (heifers) very rarely suffer from MF at calving. (Hibbs 1950).The many theories have been reviewed by Gray *et al* (2007).

The aetiology of MF remains unresolved. In this paper,the many parallels in the clinical signs, haematology and biochemistry of BEF are used as a guide to the underlying pathogenesis of MF. The story begins 5 days before the veterinarian sees the paralysed cow with MF close to death.

CLINICAL EXPRESSION OF THE DISEASES

BEF

BEF is a biphasic disease. The first phase is mild. The affected animal displays inappetence, depression, separates from the herd. Milk production of lactating cows falls (Davis *et al* 1984). This phase lasts approximately half a day. The second phase develops abruptly with a rapid rise of body temperature with lameness and reluctance to move. Heart and respiratory rates are increased. Cows lie in sternal recumbency and in some cases proceed to progress loss of reflexes (including smooth muscle of rumen and oesophagus), lateral recumbency, coma and death. Many cattle cannot swallow if offered water. In most cases recovery is spontaneous and quite sudden after 2-3 more days. They are then immune for life. Lactating and non-lactating cows, bulls, draught oxen are equally affected. The pattern of onset of the two phases varies with individual cattle. There is an interval of

normality between the two phases of experimental BEF of 2-8 hours. However, when the times of onset in experimental cattle are pooled and analysed the picture is clearer.

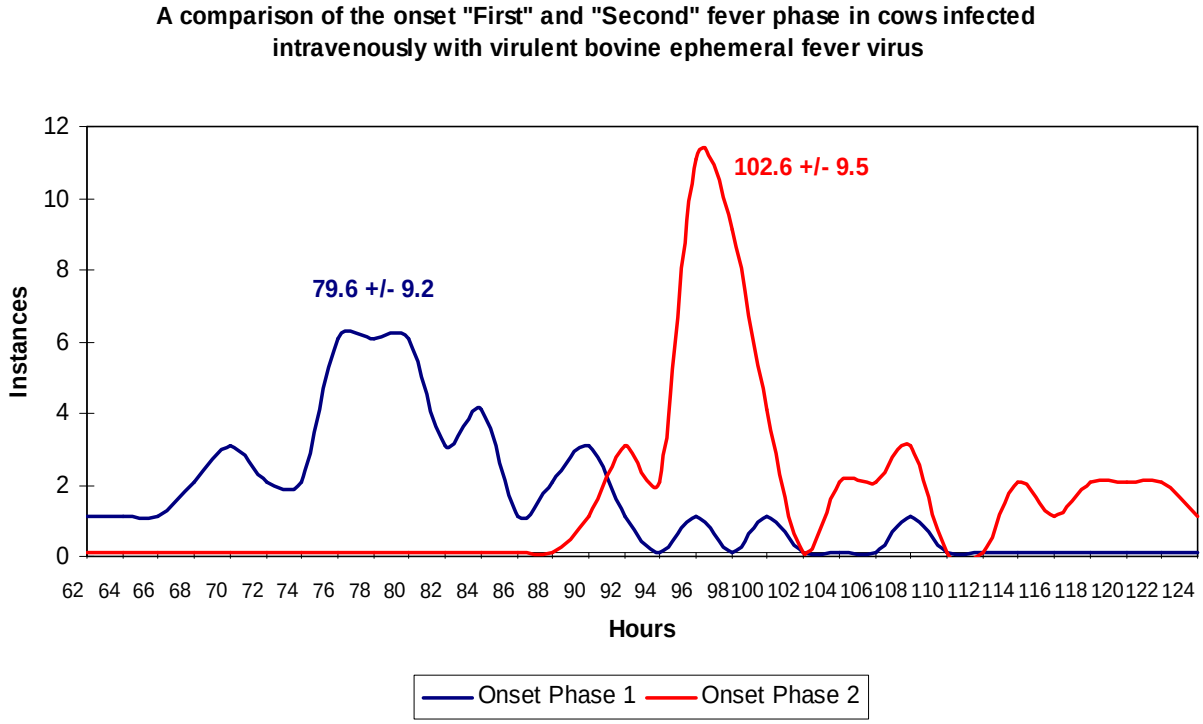

Figure 2 The onset of fever with experimental BEF in the two phases of 47 cows under two-hourly observation shows distinct biphasic peaks with a similar standard deviation. BEFV antigen peaked at 96 hours in one of the 47 cows.

The first trace of antibody detected by neutralization tests in experimental infections with BEFV is at approximately 8, days 2-3 days after recovery has begun. The titre rises slowly to a plateau at 3-4 weeks. This is a primary response and begins with IgM the main antibody class.

Kimberley virus (KIMV) is a mosquito- borne rhabdovirus in the same group of ephemeroviruses as BEFV. It has been found widely distributed in the world Africa, Asia, and Australia (St.George 2005).A series of serum samples was collected in 1983- 1984 from sentinel cattle. Most were infected with KIMV approximately 11 before an epidemic of BEF. The combined effect of the two viruses is to produce a marked anamnestic response of neutralising antibodies to both these viruses, with BEFV infection. A much lower antibody response to both ADEV and BRHV has also developed in the same time

frame as for BEFV. This type of response did not occur in the cows that did not experience earlier infection with BEFV. Their antibody response to BEFV plateau 3-4 weeks later.

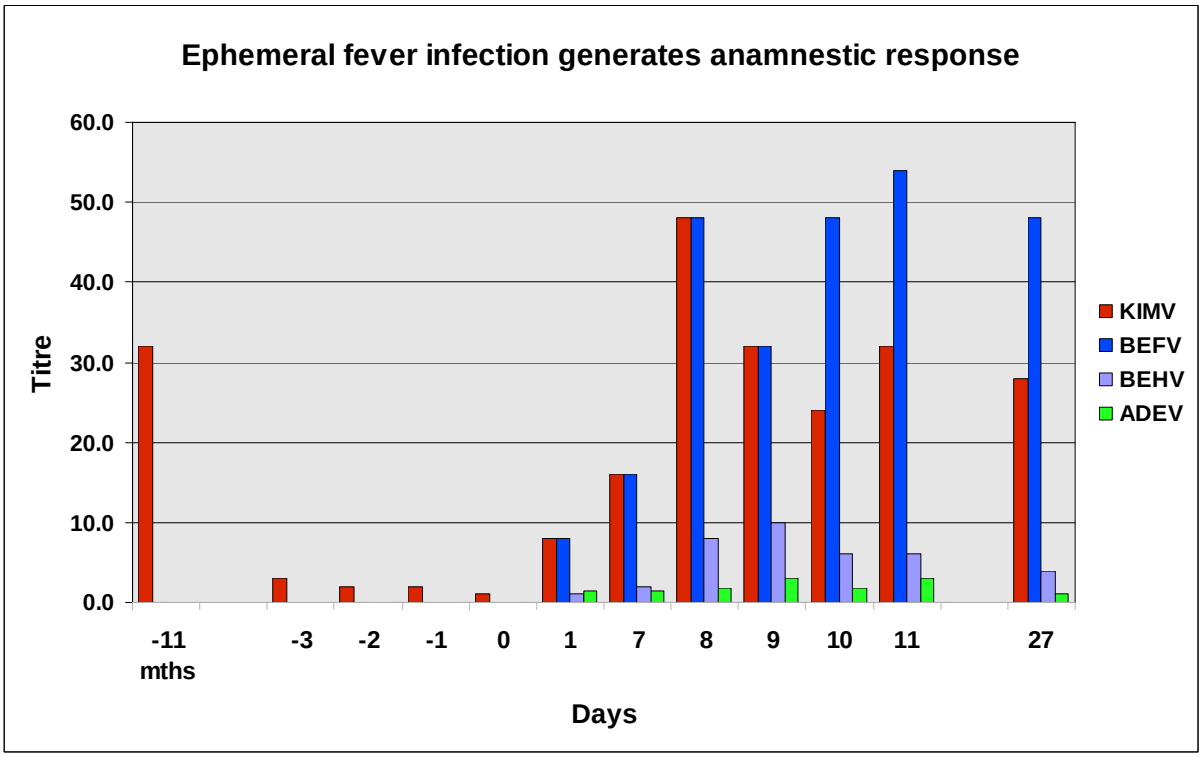

Figure 3 Preinfection with KIMV causes an anamnestic antibody response to four ephemeroviruses the day after the onset of acute bovine ephemeral fever.

M F

This disease is associated closely with calving and onset of lactation in cows having their 2nd or later calf. Premonitory signs are an uneasiness and unwillingness to move. Later the cow lies down and the head may be turned to one flank. Reflexes are lost progressively.Lateral recumbency; coma and death follow, if untreated, in the present time. Heart rate and breathing are rapid. The smooth muscle of the rumen is not moving so calcium cannot be absorbed. Water usually cannot be swallowed because of the loss of the swallowing reflex. Milk continues to be secreted into the udder draining ionized calcium and water from plasma. Body temperature becomes sub-normal. Without treatment, most would die in the present time.

Heifers (1st calvers) and non-lactating cows do not suffer from MF. before calving. Moodie and Robertson (1961) studied the effect of parity on feed consumption before and after calving. They divided their cows into young and old cows. Fig .4 The younger cows did not lose appetite in the 4 days before calving whereas a decline began between the 4th and 3rd day before calving in the older group. The effect of the number of calvings on feed consumption is shown in Fig.5 adapted from their results. Heifers remained at the level established up to and including the day of calving. Two cows whose food consumption was measured for a sufficient time before calving (St.George and Murphy 2000) were added to the graph produced Fig 5. They were in harmony.

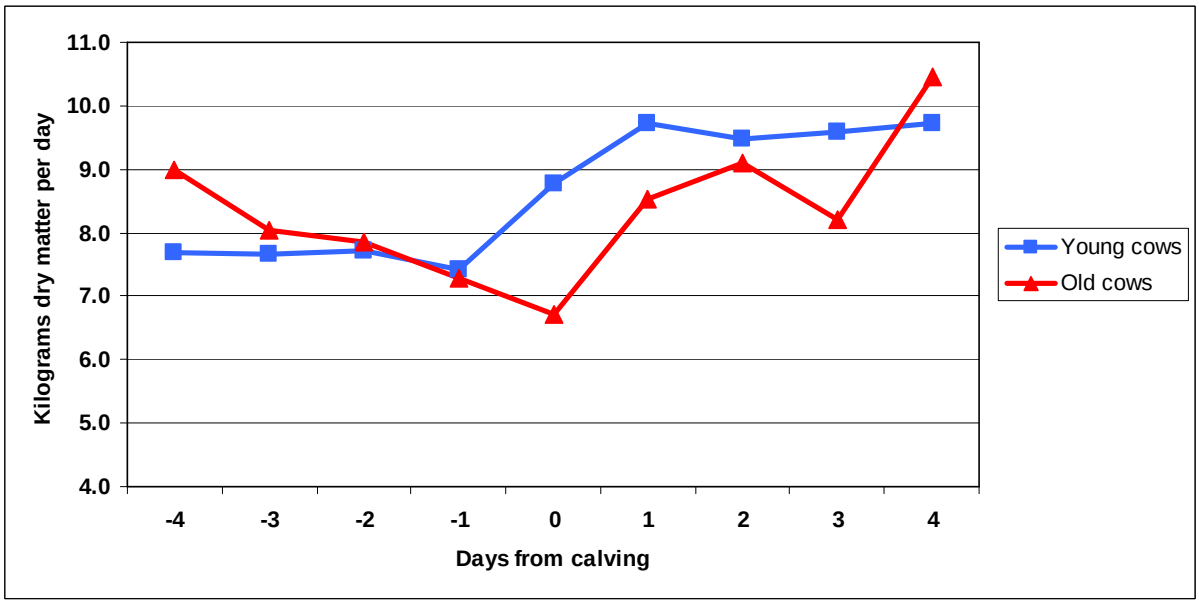

Figure 4 The loss of appetite in older cows before calving in contrast to younger cows is graphed from the tabulated data in Moodie and Robertson (1961).

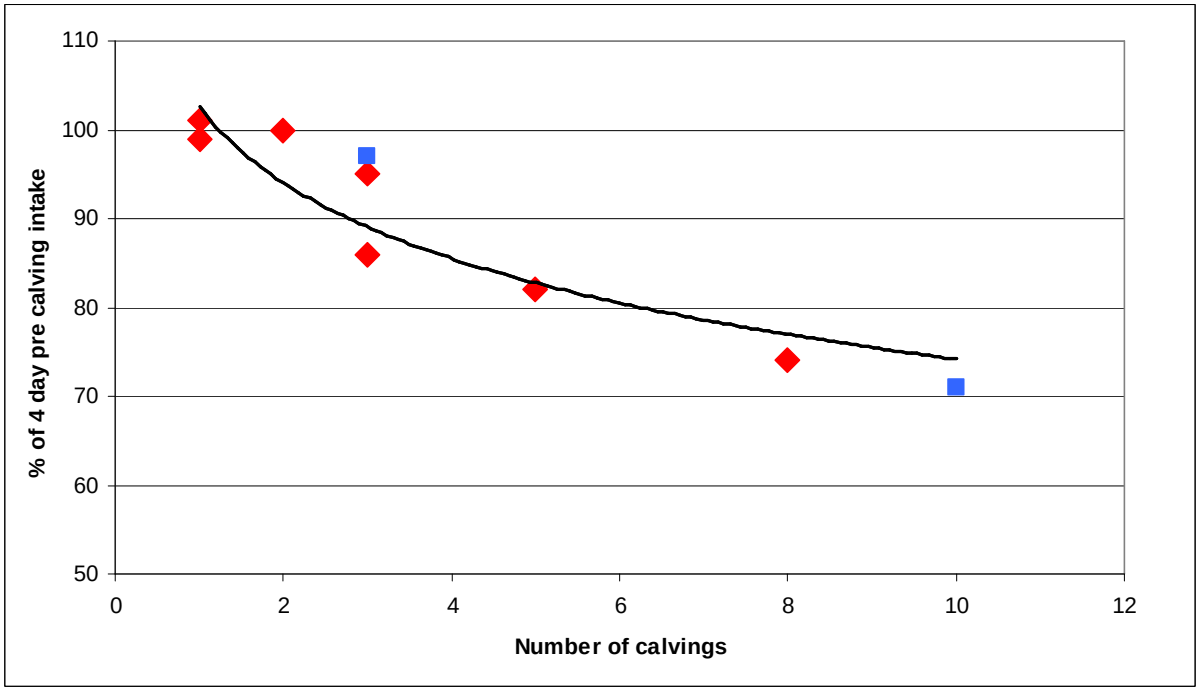

Figure 5 The relationship between parity and appetite reduction before calving was demonstrated by Moodie and Robertson (1961). Two compatible observations (blue points) have been added to their data (St George and Murphy 2001), without changing the slope of the line.

BLOODSTREAM COMPONENTS

The departures from normal haematology and plasma biochemistry are remarkably similar in BEF and multiparous cows in the periparturient period.

BEF

The most relevant of the components of the bloodstream to the paralysis is calcium. This exists in two main forms, ionized and bound to serum albumin. Thus an immediate reservoir of calcium is circulating in the blood to maintain homeostasis of the ionised fraction. Normal values for total calcium range between 2.1 and 2.8 mM/litre. In 8 experimental cases monitored 2-hourly, the mean total plasma calcium fell from 2.35 to 2.10 mM/litre .Hypocalcaemic clinical signs occurred in half the group. The fall was more marked in the ionized fraction that fell from 50% to 35 % of the total, approximately 40 hours after the onset of fever. Plasma zinc and phosphate levels fell while glucose levels rose. (Uren and Murphy 1985, Uren *et al* 1992).

MF

In MF, only cows with total calcium values below 2.0 mM/Litre are considered hypocalcaemic in virtually all publications but there is no doubt that hypocalcaemic signs occur above this arbitrary level. My experience with acute MF fever cases is that values ranging from 0.04 to 2.1 mM/litre were present in cows before treatment with calcium borogluconate was successful in banishing paralysis. Mobilization of calcium from bone takes 6 hours in heifers and cows. Excellent studies by Kronfeld (1971), Kronfeld *et al* (1973) have demonstrated how inflows of calcium from, diet, bone, are balanced by out-flows to foetus, milk, urine and faeces. Plasma zinc was also below the expected range in all the MF cases (St.George *et al* 1995). Hyperglycaemia also occurs.

BEF and MF

Haematology and cytokines

BEF

The haematology of BEF has been illustrated by St.George *et al* (1984, 1985) It is characteristic of an inflammatory reaction with a neutrophilia and oesinopaenia. The pathology has been described by Young (1979) for experimental BEF. Blood clots fail to contract normally during the febrile stage of BEF. (St.George 2000). A similar effect in severe cases of MF was attributed by Fenwick (1969), but wrongly attributed to calcium deficiency. Interferons" (interleukins) were demonstrated as being present in natural and experimental BEF (St.George *et al* 1986, Uren and Zakrzewski 1989)

MF

The haematology of multiparous cows in the periparturient period and MF cases was poorly described until carried out by St.George *et al* (1995). Also, all cows with MF, sampled before treatment, showed a relative neutrophilia. In contrast, a single heifer did not show a neutrophilia or an eosinopaenia in the corresponding period. The single case of MF, under

hourly observation displayed two distinct neutrophil responses, one before calving and the second 24 hours after the first when clinical signs were apparent. (St.George *et al* 2005). Jonsson *et al* (in press) have described a different pattern of cytokines between heifers and multiparous cows in the periparturient period.

LONG TERM SEQUELLAE

BEF

The cows, bulls, steers and draught oxen that do not return to complete normality after 3 days of illness are also termed “downers”. They are usually bright and can eat and drink but cannot control their hind limbs or rise to their feet. They may eventually recover completely after days, weeks or months from onset. Many therapies have evolved to support such long term cases to prevent muscle deterioration or secondary complications from pressure. A proportion does not recover and are euthanased .Mackerras *et al* (1940) reported one “downer” in 256 experimental infections with BEFV, where long term paralysis occurred .I observed only one in approximately 200 experimental infections. In contrast, in epidemic BEF it is quite common to see several cattle that have passed the febrile stage but are unable to rise. Their plasma calcium levels are normal and they can eat and drink. The recovery of many of these resembles the situation that occurs in Guillain Barré syndrome, where remyelination is occurring.

Guillain Barre Syndrome is a complication of some virus infections in humans, with an onset of paralysis of muscles affecting lower limbs first then rising. The loss of nerve function resolves in the opposite order if the patient is nursed appropriately. Onset is usually 19 or more days after the first signs of the initiating virus disease. (Ropper1992, Vaughan *et al* 1992, Gerdts *et al* 1999).At first sight the different time intervals would seem to be incompatable, 19 days versus 5 days from the initiation of BEFV infection. However, since the class of antibodies involved is IgG, these appear as an anamnestic response 14 days earlier in natural BEF.

MF

In general terms, one episode of MF means an increased chance of other occurrences associated with later calvings. The trend of higher and higher milk production means a greater chance of MF, except for heifers. Heifers produce approximately 11% less milk than they do at the second lactation. The production of a heifer in 2012 is much higher than a cow at the peak of production in 1930 but they remain immune to MF.

"Downers" are those cases of MF that do not fully respond to intravenous calcium replacement. Fenwick (1969) defined a "downer" as a cow with MF that does not rise within 4 hours of treatment with injected calcium. Again, they may rise to their feet in days, week or months. They usually have a normal plasma calcium level and can eat and drink after treatment with calcium borogluconate. Valuable cows may be floated in water baths for weeks, to relieve pressure on muscles.

RESPONSE TO TREATMENT

BEF

The treatments evolved over a century are to "wait and see" in the acute stages as most cattle or buffalo with BEF recover uneventfully. Rest and the provision of food, water and shade in the recovery period are important .Draught oxen die if worked too soon. Valuable cattle were treated with single injections of a range of steroidal and non-steroidal anti-inflammatory drugs, with varying success. In 1973, the clinical appearance of BEF reminded me of those in MF. I began to use injections of calcium borogluconate on acute cases. These cows and bulls responded in a sequence that would be expected in treatment of MF. Usually, they rose to their feet in less than 15 minutes. Later, valuable bulls were treated for the expected duration of natural disease with considerable success.

Under experimental condition, cattle under hourly or two hourly observations and sampling could have the clinical signs of BEF completely suppressed if given phenylbutazone 8 hourly for the expected duration of

fever. Viraemia still occurred. (St.George *et al* 1986, Uren *et al* 1989). The cattle looked and behaved normally, in contrast to the untreated controls. When challenged a month later, they were resistant whereas the controls developed typical BEF disease. This treatment remains the only instance of complete suppression of the clinical expression of an arbovirus disease.

BEFV must induce the clinical disease through mediators and not by cell destruction as response is almost immediate. Non steroidal anti-inflammatory drugs are now widely used in BEF epidemics with calcium injections where the circumstance or dehydration requires them to drink.

Milk Fever

The first effective treatment was evolved in Denmark in 1897. A 1 % solution of potassium Iodide was injected up the teat canal of the four quarters of the cow's udder. Tie- offs prevented the solution escaping. Water was later found as effective .Finally, in 1901, air was pumped in from bicycle pumps instead of fluids. Mortality was reduced from 60-70 % to about 15 % (Hibbs 1950). Back pressure inhibits the formation of milk in the udder and also allows movement of calcium ions to return calcium to the plasma within 6 hours.

The success of Grieg (1930) in demonstrating the effectiveness of calcium gluconate lead to the current theories that a derangement of the control of plasma calcium occurred between first and second calving although no direct evidence has ever been found. The theories of the "derangement "of control of calcium "evolved in the 1920s by Dryerre and Grieg (1928) were accepted as confirmed by the success of specific treatment with Calcium gluconate (Grieg 1930.In recent years, other electrolytes have been added to calcium borogluconate. Spontaneous recoveries did occur in the 19th century. This means that the initiating circumstance did not persist for more than a few days. Today, recovery without treatment is very unusual in high producing cows.

WHY IONIZED CALCIUM IS DEPLETED

BEF

In an unpublished experiment (GM Murphy and TD St.George 1985), the intake and excretion of calcium was monitored in four steers in metabolic measurement apparatus in an air-conditioned room. The calcium intake in diet and output in urine and faeces was measured. After a period of stabilization the steers were infected with BEFV. Intake of food and water decreased or ceased during fever. The total plasma calcium level fell before their rumens ceased movement. There was no increased excretion of calcium during fever. In other words, the calcium was displaced from its normal availability during BEF, but not excreted.

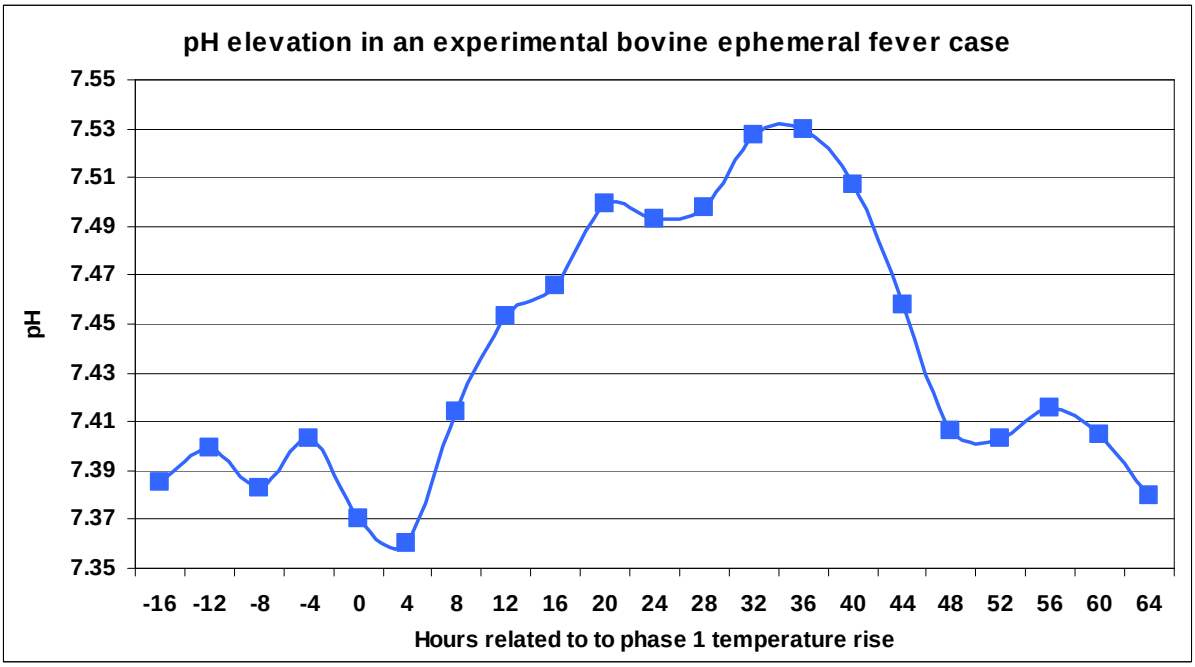

Figure 6 The prolonged rise of plasma pH in a steer with experimental Bovine Ephemeral Fever.

Young (1979) described the pathology of increased vascular permeability and other signs he attributed to an antigen/antibody reaction. There is evidence from natural cases that this is occurring. Direct evidence of the peak of BEFV antigen at the peak of severe BEF disease was detected by the use of an ELISA test developed by H Zakrzewski (unpublished data 1992).

Luttrell and Hennicker (1991) consider that antigen/antibody complexes bind calcium ions. This would make part of the calcium in plasma unavailable although still in the circulating blood. The elevated plasma pH would inhibit the transfer of calcium from the available reserve bound to albumin. A change in the balance between the total and ultra filterable (ionised) components as described by Cockran *et al* (1988) has occurred. The formula developed by them is that the ionised fraction of the calcium is depressed by 1.2% for each 0.02 pH unit rise. In the acute stage of BEF, when sufferers are breathing very rapidly, the pCO2 is lowered and as a consequence plasma pH is raised by a mean of 0.1 units at the same time (Uren *et al* 1992) (Fig.6). This restricts the movement of calcium from the bound to the ionized form. The rise in pH also effects the movement of magnesium ions from the bound to ionised form (Wang *et al* 2002), They concluded that the rate of change of ionised concentration with pH change (0.12 mMol/L per pH unit) is significantly less than that of ionised calcium(0.36 mM/L per pH unit).

This has implications for the balance between ionised calcium and magnesium. A similar pattern with a larger pH rise in a multiparous cow with MF has been illustrated by St.George (2001). Thus the rise in pH in the plasma inhibits the transfer of calcium from the circulating reserve to maintain the homeostatic level for optimum control of smooth muscle movements and reflexes. One of the immediate effects of treatment of BEF and MF with calcium is slowing of respiration and consequent loss of carbon dioxide.

MF

Almost the whole of research in MF prevention is aimed at diet manipulation to stimulate the movement of calcium from bone before calving and by adding micronutrients. The effect of adding nutrients that effect the biochemistry of the multiparous cow in the periparturient period are effective in lowering the risk of MF if initiated 3 weeks before calving (Lean *et al* 2006).Thus the limits of natural calcium mobilisation and pH rise are circumvented. This remains unnecessary in heifers.

DISCUSSION

The success of treatment of BEF with anti-inflammatory drugs proves that that the clinical signs of this virus disease are not the direct effects of BEFV. Viraemia remains through out the expected course of the disease. The short term paralysis is directly resolved by injected calcium borogluconate, as it is for MF.

The many similarities between the two diseases with a major presenting sign a reversible paresis or paralysis indicate a common response mechanism. Lactation per se cannot be the fundamental basis for the clinical signs of MF. The syndrome existed before modern dairying practice raised its prevalence. Also, most BEF cases and some MF cases have total plasma calcium levels that indicate a circulating reserve that has not been depleted. Importantly, in BEF, males as well as females exhibit the same hypocalcaemic signs as multiparous cows with MF. The haematology and biochemistry of multiparous cows is in the periparturient period almost the same as BEF, varying only in amplitude. It is quite clear that multiparous cows are experiencing effects on appetite 3-4 days before calving, in contrast to heifers.

Exposure to calf antigens is happening as the complex placenta detaches in the days before calving, from personal observation. The consequent inflammation would account for the neutrophilia and the eosinophilia that has been observed.The neutrophilia probaly contributes to ooncreased vascular permeability (Lewis and Grunger 1986). An interaction between the calf antigen and circulating heterotypic antibodies could be responsible for the reduced appetite that is directly related to increasing parity. In contrast, milk production in contrast increases at first with parity and then declines.

As has been developed for naturally occurring BEF, a two phase model for MF is advanced. The time span between exposure to antigen to the onset of phase 2 in both diseases is approximately 5 days.

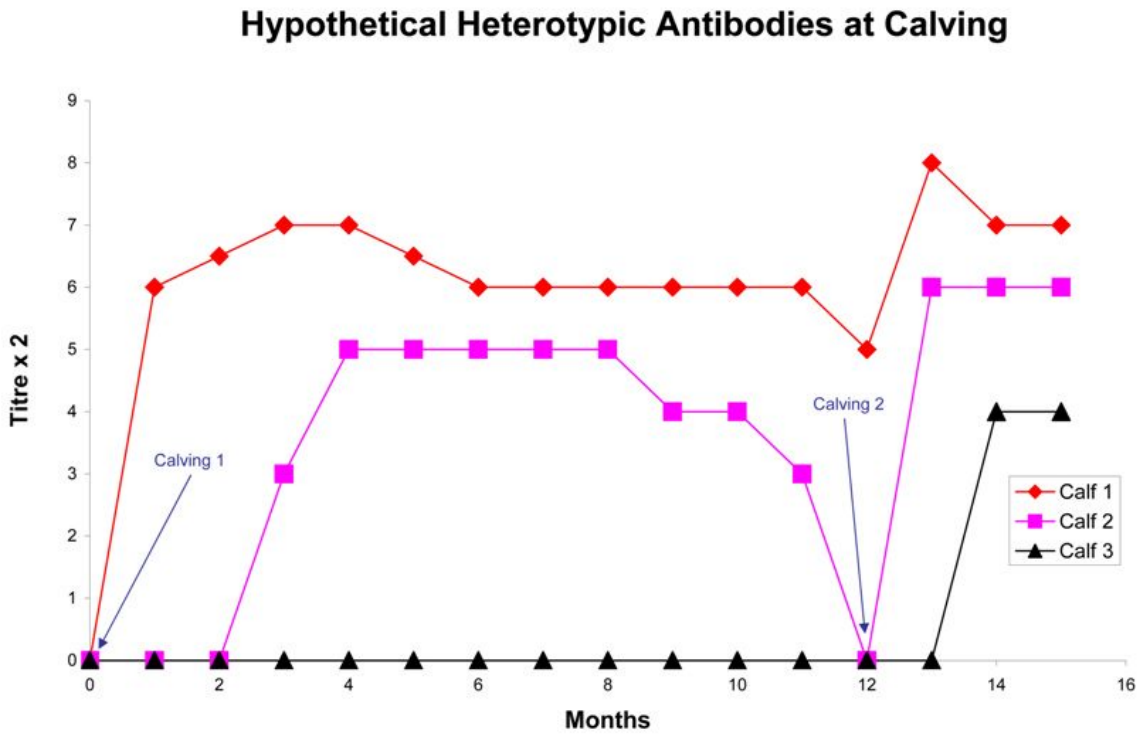

Figure 7 This figure is adapted from the acute naturally occurring ephemeral fever where KIMV preconditions a heterotypic antibody response to BEFV from a vector at 5 days prior to exposure to second calf antigen. The model could be expanded further to take in the more distant relationships to other antigens as shown in Fig 3 for BEF.

Preliminary

Heifer calving

*Exposure to calf 1 antigens during calving. A spectrum of antibodies generated against calf 1 antigen that is IgG class by the time of second calving.

Multiparous Cow calving with MF

Phase 1

*Interaction of calf 2 antigens with heterotypic antibodies generated by calf 1 as placenta detaches 2-3 days prior to calving thus binding calcium. Rumen movements slowed. Inflammation causing neutrophilia lasting until 1 day post-calving. Cytokines generated.

Apparent normality from calving with cessation of foetal calcium demand until 5th day post-exposure to calf antigens

Phase2

*Interaction of anamnestic calf 1 IgG antibodies with calf 2 antigens absorbed during second calving, binding calcium.(Fig.7). Acute hypocalcaemia and hypovolaemic vascular shock if peak demand from udder for milk secretion exceeds immediate availability of calcium ions,

CONSEQUENCES OF ANTIGEN/ANTIBODY MODEL FOR MILK FEVER

- The range of plasma calcium levels at which hypocalcaemia occurs in MF and BEF is explained by the raised plasma pH affecting the ionized fraction.
- Hypovolaemic vascular shock in MF can be recognized as a consequence of vascular permeability
- The number of calvings increases the probability of generation of heterotypic antibodies.
- Decline of appetite of multiparous cows in the periparturient period that correlates with parity is explained by mild antigen/heterotypic antibody/calcium interaction prior to calving effecting appetite as happens in natural BEF.
- “Downers ‘compare directly with Guillain Barré syndrome with demyelination and remyelination processes.
- Heifers have the same physiological mechanism for mobilisation of calcium as multiparous cows
- Heifers will continue to have immunity to MF as milk yields increase in the future

This model can be tested by further haematology and biochemistry of heifers in comparison with heifers in the periparturient period. Haematology of MF cases can be expanded to add to the evidence of inflammation already known from the neutrophilia and elevated fibrinogen levels .Histopathologic examination of untreated MF cases especially of peripheral small blood

vessels and lungs for evidence of increased permeability is essential. The “downer “syndrome begins concurrently with hypocalcaemia in both BEF and MF .An objective examination of “downers” in both MF and BEF in terms of neural effects of Guillain Barré syndrome should become routine.

Addendum

A recently published paper by Blasdell KR *et al.* (2012) has found that Malakal virus and Kimberley virus are geographical variants of the same virus.

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The observations and blood samples, at times daily, in a commercial dairy herd were entirely due to the unstinted help by Terry and Mavis Hunt. Gerry Murphy provided belief and excellent biochemistry for the studies of calving cows. Nic Jonsson, a sceptic of conventional MF dogma began investigation of cytokine profile in the periparturient period of heifers and cows. Many others assisted the tedious observations and collection of samples in the field and on a 24 hour basis for experimental disease.

REFERENCES

Blasdell KR, Voysey R, Bulach DM, Trinidad L, Tesh RB, Boyle DB and Walker PJ. (2012) Malakal virus from Africa and Kimberley virus from Australia are geographic variants of a widely distributed ephemerovirus. Virology. Aug 24. [Epub ahead of print]

Cochran M, Rumbelow B and Allen G (1998) the relation between the Ultrafiltrable calcium fraction and blood pH and concentrations of total plasma calcium, albumin, and globulin. Clinical Chemistry 44: 1559-1562.

Cybinski DH (1987) Homologous and heterologous antibody reactions in sera from cattle naturally infected with bovine ephemeral fever group viruses. Vet Microbiol 13: 1-9.

Dryerre H and Grieg JR (1928) Further studies in the etiology of milk fever Vet Rec 8 721-726

Fenwick DC (1969) The downer cow syndrome. Aus Vet J 45: 184-188.

Geber BD, Zanni MP, Uguccloni M, Loetscher M, Mackay CR, Pichler WJ, Yawalker N, Baggionlini M and Moser B (1997) Functional expression of the eotaxia receptor CCR3 in T lymphocytes colocalizing with eosinophils. Current Biol 1: 836-843.

Gerdts R, Jensen D, Fauchald P and Skjedal O (1999) Guillain-Barré Syndrome Current treatment principles in the light of clinical aspects. Tidsskrift-Norsk Laegeforen 119: 506-509.

Gray CP, St.George TD, Jonsson NN (2007) Milk Fever in dairy cattle: A novel hypothesis for an immune mediated aetiology. Cattle Practice. 15: 277-282.

Grieg JB (1930) Calcium gluconate as a specific in milk fever Vet Rec 10 115-120

Hibbs JW (1950) Milk fever (parturient paresis) in dairy cows - a review. J Dairy Sci 33: 758-789.

Jonsson NN, Fortes MRS, Wittek T, Vankan DM and Piper EK (2011) Comparison of metabolic, haematological and peripheral blood leukocyte cytokine profiles of dairy cows and heifers during the periparturient period. In press.

Kronfeld DS (1971) Parturient hypocalcaemia in the dairy cow. Advances Vet Sci 15: 133-157.

Kronfeld DS, Mayer GP and Romberg CF (1973) Production disease in farm animals. (Ed. Payne JM).

Lean IJ, DeGaris PJ, McNeil DM and Block E (2006) Hypocalcaemia in dairy cows: meta analysis and dietary anion difference theory revisited J Dairy Sci 89 669-684

Lewis RE and Grunger HJ (1986) Neutrophil-dependent mediation of microvascular permeability. The endothelial cell in regulation of permeability to Molecules. Federation Proceeding 45: 109-113.

Luttrell BM and Hennicker AJ (1991) Reaction coupling of chelation and antigen binding in the calcium ion-dependent antibody binding of cyclic AMP J Biol Chem 266: 21626-21630.

Mackerras IM, Mackerras J and Burnet FM (1940) Experimental studies of ephemeral fever in Australian cattle. Bull Council for Scientific and Industrial Research, Melbourne, No. 136.

Moodie EW and Robertson A (1961) Dietary intake of the parturient cow Res Vet Sci 2 217-226

Ropper AH (1992) The Guillain Barré Syndrome. New England J Med, 23 April 1002, 1130-1136.

St.George TD, Cybinski DH, Murphy GM and Dimmock CK (1984) Serological and biochemical factors in bovine ephemeral fever. Aus J Biol Sci 37: 341-349.

St.George TD (1985) Studies on the pathogenesis of bovine ephemeral fever in sentinel cattle. 1. Virology and serology. Vet Microbiol 10: 493-504.

St.George TD, Uren MF and Zakrzewski H (1986) The pathogenesis and treatment of bovine ephemeral fever. Arbovirus Res Aus 4 303-307.

St George TD, Murphy GM, Burren B, and Uren MF (1995) Studies on the pathogenesis of bovine ephemeral fever .IV Vet Microbiol 46 131-142

St.George TD (2000) Effect of ephemeral fever of cattle on the appearance and contraction of blood clots. Aus Vet J 78 857-858

St.George TD and Murphy GM (2001) An alternative view on the hypocalcaemia of cows. Proceedings of the Australian association cattle veterinarians, Melbourne Conference, Brisbane Australia, 30-39.

St George TD (2005) Chapter 100 Bovine ephemeral fever in Disease of Livestock in Africa, Capetown

St.George TD (2008) Evidence that mosquitoes are the vectors of bovine ephemeral fever virus Arbovirus Res Aus 10 151-154

Uren MF and Murphy GM (1985) Studies on the pathogenesis of bovine ephemeral fever in sentinel cattle. II. Haematological and biochemical data. Vet Microbiol 10: 505-515.

Uren MF, St.George TD and Zakrzewski H (1989) The effect of anti-inflammatory agents on the clinical expression of bovine ephemeral fever. Vet Microbiol 19: 99-111.

Uren MF and Zakrzewski H (1989) mechanisms of immunity to BEF Arbovirus Res Aus 5 274-276

Uren MF, St.George TD and Murphy GM (1992) Studies on the pathogenesis of bovine ephemeral fever in experimental cattle. III. Virological and biochemical data. Vet Microbiol 30: 297-307.

Vaughan VC, Mckay RJ and Behrman RE (Eds.) (1992) Guillain-Barré Syndrome. In: Nelson - Textbook of
Pediatrics. 11th Edition, (Eds. W.B. Saunders Company, Philadelphia).

Wang S, Mc Donnell EH, Sedor FA and Toffaletti JG (2002) pH effects on measurements of ionized calcium and ionised magnesium in blood Arch Pathol Lab Med 126 947-950

Young PL (1979) Studies of the pathology and pathogenesis of bovine ephemeral fever virus infection of cattle. Thesis - PhD, University of Queensland.