

VLA MONTHLY SCANNING SURVEILLANCE REPORT MARCH 2009

HIGHLIGHTS

Fasciolosis continuing to cause disease in cattle, sheep and alpaca

The national data indicates the highest incidence of this disease for ten years which supports the anecdotal evidence from the field. It will be damaging for individual farm economies. Continuous exposure to infective metacercariae on pasture throughout the winter is likely to be associated with climate change and will require recognition in the formulation of future herd and flock health plans. Farmers appear to be unaware of, or reluctant to implement the various avoidance strategies which can be used to help prevent the disease. The use of molluscicides to control snail populations is rarely if ever used and would benefit from some further research. The efficacy of chemoprophylaxis may be compromised by increasing triclobendazole resistance in the future. Sustainable control of this disease will depend upon developing and promoting avoidance strategies and snail control alongside chemoprophylaxis rather relying entirely on the use of anthelmintics. Further study of the epidemiology of the disease in relation to recent climate and husbandry changes, using VLA scanning surveillance data, would also advance our understanding of recent changes.

Onion toxicity in a calf

A further case of onion poisoning, this time in a calf underlines the need for cattle farmers to do their homework before feeding unusual feedstuffs or by products of the food industry.

Clostridial diseases causing losses in unvaccinated sheep

This was reported in the previous month and underlines the need for vaccination to be a cornerstone of flock health planning

Border disease causing abortions in sheep flocks

This disease can be transmitted between flocks via the purchase of persistently infected stock. One of the two outbreaks described was related to the purchase of breeding stock

Listeriosis causing abortion and encephalitis in sheep

This infection is often related to the feeding of silage which has undergone secondary fermentation. This may be related to the feeding of the poorer quality silage at the end of the winter.

Atypical necrotic enteritis diagnosed in commercial layers

This is an unusual manifestation of this condition. This fact together with the absence of the usual predisposing factors indicates that further monitoring is indicated.

CATTLE

Reproductive diseases

Abortion

Sutton Bonnington diagnosed IBR as the cause of abortion of a mid term fetus from a heifer. The diagnosis was confirmed by histopathology which demonstrated multifocal to coalescing areas of hepatocellular necrosis and scattered foci of kidney necrosis with subsequent demonstration of BHV-1 by IHC in the lung and kidney.

Preston diagnosed abortion due to *Campylobacter spp.* in a small suckler herd in which two cows had aborted at five months gestation. Post-mortem findings in both fetuses were similar, the most striking feature being a fibrinous perihepatitis. Pure cultures of *Campylobacter* organisms were identified in foetal stomach contents in both cases and these isolates are now awaiting subspeciation.

Langford isolated *Bacillus licheniformis* in pure culture from the fetal stomach contents of a prematurely born calf which was born alive but died soon after. The dam was one of a group of 20 suckler cows one of which had also recently aborted.

Shrewsbury also identified *Bacillus licheniformis* infection in two abortion incidents. A single abortion was reported in a 30 cow suckler herd in north Wales and three abortions in 6 weeks were reported in a 160 cow Shropshire dairy herd.

Alimentary tract diseases

Colienteritis

Penrith diagnosed six incidents of enterotoxigenic *E.coli* infection in neonatal calves associated with K99 +ve *E.coli*, all on separate premises. All calves were aged less than 72 hours old.

Fasciolosis

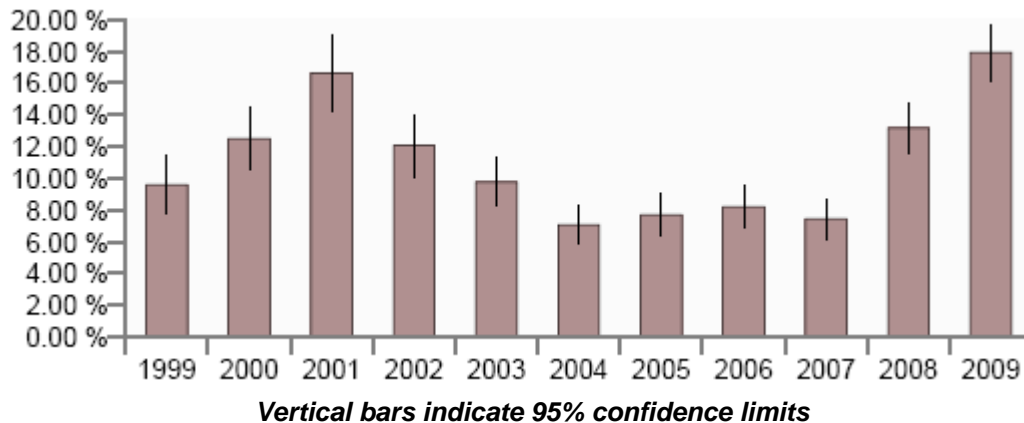
An ongoing problem of condition loss in yearling beef heifers was investigated initially as a suspect BVD infection. Blood samples submitted to Preston were unremarkable and it was advised to widen the scope of the investigation, with faecal samples being requested. Examination of these revealed a complex of diseases present including patent fasciolosis, severe endoparasite infestation (6,250 trichostrongyle eggs per gram) and salmonellosis Dublin.

Preston remarked that the number of cases of ill thrift associated with fasciolosis continued to rise with fifteen incidents diagnosed during the month. They reported that although practitioners are very aware of the issue of resistance to flukicides, very few are being asked for comprehensive advice in the approach to fluke control.

Starcross also remarked on the continuing rising incidence of fasciolosis having diagnosed 24 incidents. Rumen fluke, *Paramphistomum cervi*, infection was also identified on two farms.

National figures for England and Wales confirm the continuing rising incidence of fasciolosis.

Figure 1
VIDA Incidents of Bovine fasciolosis (as a percentage of diagnosable submissions)
Jan-Mar 1999-2009



Respiratory Diseases

IBR

Penrith diagnosed two incidents of IBR by fluorescent antibody test of nasal/ocular swabs. The first affected dairy cows which displayed typical upper respiratory disease and milk drop and the second affected a group of crossbred bulls of which 20 from a group of 50 were said to be showing clinical signs.

Starcross also diagnosed IBR on demonstrating significant rising titres in paired serum samples from four adult dairy cows which had demonstrated milk drop, respiratory signs and pyrexia when first sampled.

Pasteurellosis

Newcastle diagnosed Pasteurellosis in a 4-month-old Limousin-cross calf which died following signs of acute dyspnoea. *Pasteurella multocida* was recovered in pure growth from consolidated lung tissue.

Leahurst also investigated an outbreak of respiratory disease affecting 6 out of 20 7-10 day old calves born on a dairy farm over an 8 weeks period. Necropsy of one 8-day-old Holstein x beef calf showed extensive cranioventral consolidation of both lungs and *Mannheimia haemolytica* was isolated. Histological examination confirmed that there was no evidence of viral involvement. Assessment of colostral antibody status of calves aged 2 to 7 days, using the ZST test was recommended, as well as an assessment of housing, ventilation and hygiene.

Mannheimia haemolytica

Langford diagnosed acute severe pneumonia caused by *Mannheimia haemolytica* affecting five milking cows in a herd of 200 which developed a severe milk drop and pyrexia. The affected group were treated quickly and responded to antibiotic therapy.

Bibersteinia trehalosi

Carmarthen diagnosed *Bibersteinia trehalosi* septicaemia in a three-year old Holstein Friesian cow. The animal had been calved ten days and was the only one affected in a 200-cow high yielding group. A severe fibrinous

bronchopneumonia affected all lung lobes. There was interlobular fibrin exudate and accumulation of fibrin fluid in the mediastinum ventrally. Many lung lobules were dark purple in colour with fibrin tags on the visceral pleura. *B. trehalosi* was cultured from lung, spleen, mammary gland and uterine contents.

Other Diseases

Dermatitis

Two heifers from a 120 cow dairy unit had recently developed a foul-smelling, inflamed area of skin, approximately four centimetres in diameter at the junction of the cranial udder and stifle. Both heifers were 100 days post-calving. The cubicles appeared comfortable with non-abrasive material used for bedding. The clinical description was similar to a number of previously described conditions including necrotic dermatitis (Mastitis Control in Dairy Herds, Blowey and Edmondson 1995) digital dermatitis of the udder (Boyer P, & Singleton G, Veterinary Record 1998 142: 147-148). Culture of a swab taken from a typical lesion revealed *Arcanobacterium pyogenes* and *Corynebacterium minutissimum*, both of which have also been described in a paper by Beattie. (Cattle Practice (2000) 8:4 377-38) *C. minutissimum* is considered to be a cause of skin disease in man, specifically erythrasma, a superficial infection of the body folds. No human skin disease was reported to be associated with this case.

Urea poisoning

Bury investigated a case in which nine out of 10, loose yarded two-year-old beef cattle, which were observed to be in good health in the morning, were found dead late in the afternoon. The tenth was recumbent and groaning. Necropsy of three animals revealed a high rumen pH and strong smell of ammonia in two, with little else of note. Samples taken on farm identified urea in the drinking trough and its portable water tank supply, which had also been used the previous day to supply water to a urea fertiliser tank. Urea had refluxed into the supply tank causing acute urea toxicity within three hours of its connection to the drinking trough. The tenth animal made a full clinical recovery by the following day.

Onion Toxicity

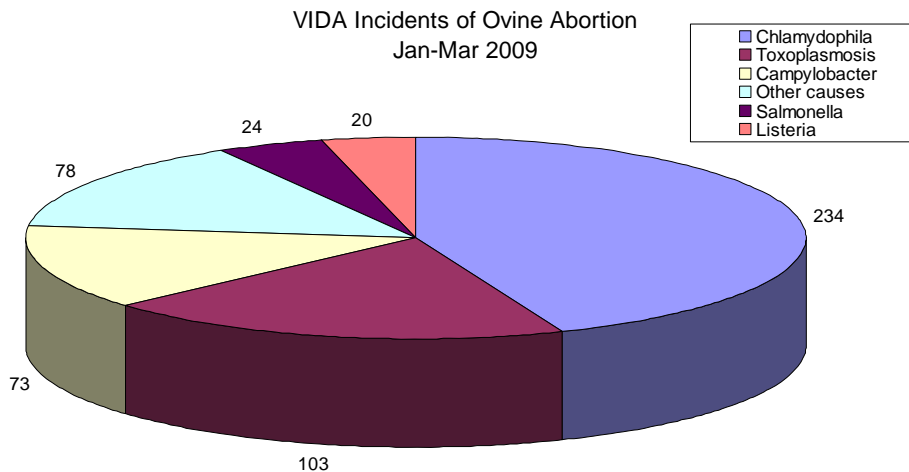
Onion toxicity (toxic principles: *n*-propyl disulphides and thiosulphinate compounds) was suspected to have caused deaths in seven, ten-week-old calves in a group of 140 suckler cows and calves. The principal clinical signs were opisthotonus and convulsions. The affected group were fed ad-lib onions, sprouts and potatoes and also silage twice weekly. No further clinical signs were seen following withdrawal of the onions from the feed.

SMALL RUMINANTS

Reproductive diseases

Figure 2 shows the proportions of the causes of ovine abortion in the first quarter of 2009. The data is unremarkable when compared to the previous year.

Figure 2.



Campylobacter Abortion

Two aborted fetuses were submitted to Thirsk as part of an investigation into an abortion outbreak in a group of 35 ewes. *Campylobacter fetus* was isolated from the foetal stomach contents of both fetuses.

Langford also diagnosed *Campylobacter* abortion in two flocks by the recovery of *C. jejuni* in pure culture from the fetal stomach contents. In one of these scanning had revealed many barren ewes in a flock of 200 and some scanned to show twins subsequently only producing single lambs. Additionally a number of weak or dead full term lambs had also been born.

Listeriosis

Listeria monocytogenes was isolated from material submitted to Starcross: from two farms. In the first case 10/400 ewes had aborted and three ewes had also been found dead. On the other premises where one out of 37 ewes had aborted *Listeria monocytogenes* was isolated from the liver of aborted twins.

Border disease

Luddington investigated a flock of 400 ewes in which eight to ten shearlings aborted out of a group of 240. The affected animals had been purchased from a single farm as ewe lambs, and had been kept as a separate group from the older ewes. A near term aborted fetus was presented for necropsy which had an abnormal coat and was very poorly grown for the stage of development, weighing only 900 grams. PCR testing of foetal spleen for BDV proved positive, supporting a diagnosis of Border Disease.

The disease was also diagnosed by Aberystwyth in an incident in which over 30 ewes had aborted out of a group of 200, on a farm with approximately 4,000 sheep. Foetuses submitted for necropsy were all near term and no gross lesions were observed. Border disease virus was detected by PCR in the spleens from twin lambs. No other potential causes of abortion were demonstrated following several submissions.

Alimentary tract diseases

Parasitic gastroenteritis

A ewe that had recently died was received at Starcross for post mortem. It was one of 14 animals in group of 180 which had been affected by marked loss of weight. Another three had already died. The group had received anthelmintic and fluke treatment in November. Post mortem examination revealed 23,000 worms in the abomasum (*Ostertagia circumcincta* 50%, *T. axei* 30%, *O. trifurcata* 20%) and 12,000 worms in the small intestine. A rectal faeces sample had a worm egg count of 14,950 trichostrongyle-type epg. It was also affected by pneumonia and *M. haemolytica* was isolated from the lungs.

Clostridial Enterotoxaemia

Penrith investigated two incidents of Pulpy kidney disease (Clostridial enterotoxaemia), the first affecting unvaccinated 10-month-old Swaledale lambs that had been turned out on to turnips. Gross post-mortem findings were typical, but no toxin could be demonstrated in intestine. The diagnosis was confirmed on brain histopathology.

The second case involved a 1-month-old Texel-cross lamb from a flock that did vaccinate the ewes. Post-mortem findings were typical and included “tiger-stripe” haemorrhages on the diaphragm (see Figure 2). Epsilon toxin was demonstrated in intestinal contents. The single case suggested the possibility of inadequate colostrum intake by the affected lamb.

Shrewsbury also diagnosed the disease in two unvaccinated flocks in one of which five lambs had been found dead with no previous signs of illness in a group of 170 lambs with 110 ewes.

Lamb Dysentery

The carcase of a three-day-old lamb was submitted to Thirsk with a history of dysentery affecting four lambs shortly after birth. No clostridial vaccination was practised. Post-mortem examination revealed purple discolouration of the intestinal tract from the terminal ileum distally with watery blood stained contents. *Clostridium perfringens* beta and epsilon toxins were detected, and anaerobic culture identified *Clostridium perfringens* in intestinal contents.

Figure 3
“Tiger stripe” haemorrhages on the diaphragm of a Texel lamb with Clostridial enterotoxaemia



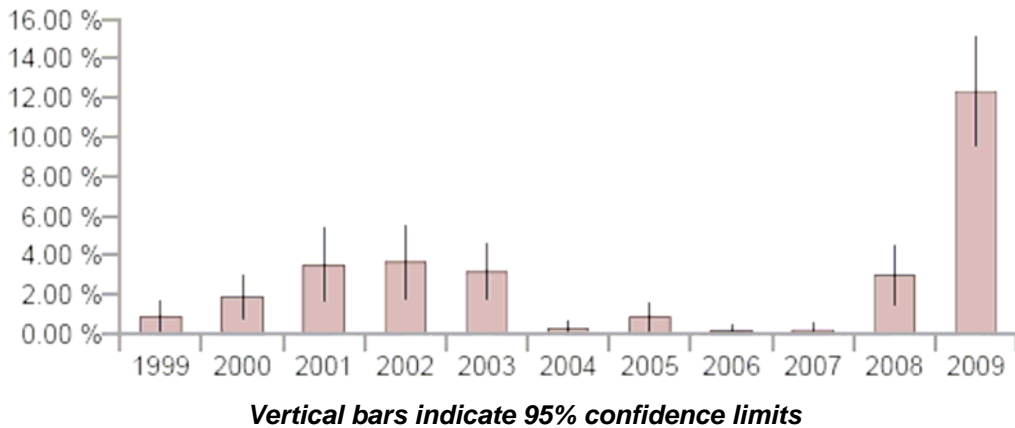
Fasciolosis

Patent fluke infestations continued to be diagnosed during this month and usually presented as cases of wasting, ill thrift and apparent sudden death.

Preston investigated a flock in which 30 out of 300 Suffolk cross hogs which had been housed for 6 weeks had died. Severe chronic fasciolosis with concurrent trichostrongylosis was diagnosed in the two sheep submitted for post mortem examination. No clinical signs prior to death had been noted by the farmer.

Aberystwyth reported evidence of recently migrating immature fluke in the liver of two ewes submitted for necropsy indicating that exposure to metacercariae on pasture had continued through the winter. This is supported by national data which shows the largest increase in acute disease in the first quarter year for over 10 years.

Figure 4
VIDA Incidents of Ovine acute fasciolosis (as a percentage of diagnosable submissions) Jan-Mar 1999-2009



Carmarthen diagnosed chronic fasciolosis in a ewe which was one of five to have died out of a group of 28. They had been treated with levamisole after lambing but no flukicide had been given during the winter. The ewes reportedly became thin, anorexic and died in two to three days. The liver was enlarged, fibrotic and massive numbers of adult liver fluke were seen in the bile ducts and gallbladder. Immediate treatment with flukicide was recommended.

Other diseases

Yersiniosis

Yersinia pseudotuberculosis was cultured from the aspirate of a mass in the parotid region of an adult goat. The goat had ocular lesions around two weeks prior to developing the mass in the parotid region. This condition (likened to Parinaud's oculoglandular syndrome in man) first presented in this way on this premises approximately one year ago. It is possible in this case that the initial ocular lesion was caused by *Yersinia* and it has spread to cause lymphadenitis in the parotid region.

Nephrosis

The occurrence of 20 deaths in a group of 290 twin lambs resulted in a lamb being submitted to Winchester for examination. The gross post mortem revealed enlarged pallid kidneys consistent with nephrosis, a diagnosis supported by the finding of raised urea concentrations in aqueous humour. There was evidence of concurrent coccidiosis and the attending veterinarian had identified that the water troughs present were too high for lambs to get to the water, both of which could have predisposed to disease.

Metabolic diseases

Hypocalcaemia

Thirsk diagnosed two outbreaks of hypocalcaemia in sheep approximately two months off lambing. In both cases, ewes presented with clinical signs which were at first assumed to be attributable to pneumonia. Two live ewes were submitted for necropsy in both cases and in neither case had any treatment been administered. Affected animals had been found recumbent with tachypnoea and a mucoid/green nasal discharge. In one case, signs of hypocalcaemia were thought to have been triggered by a recent move in all ewes from a distant pasture into their lambing accommodation. Plasma calcium levels in the affected animals were found to be between 0.64 and 0.79 $\mu\text{mol/l}$ (reference range 2-3 $\mu\text{mol/l}$)

Ketosis

A heavily pregnant four-year-old dairy goat which had ataxia was euthanased and submitted to RVC for post-mortem. The clinical history indicated that approximately 25% of the 200 does that had already kidded to be affected with varying degrees of ataxia and recumbency, and a mortality of about 20%. Animals were affected at various stages, both before and after parturition. Ante-mortem blood results provided by the submitting veterinarian revealed: a mild hypoproteinaemia (58.5g/L ref range 64-70g/L); hypocalcaemia (1.86 mmol/L ref range 2.23-2.93 mmol/L); and ketosis with a BHB of 4.31 mmol/L (ref range 0.47-0.63). Gross post mortem findings were largely unremarkable, supporting the hypothesis that metabolic disease was most likely responsible for the losses seen on this farm.

Nervous Diseases

Listeriosis

Listeriosis was frequently reported during the month. In one case described by Shrewsbury the carcasses of one live and one dead ewe were presented for post-mortem examination from a flock in which six ewes had died with a history of neurological disease and rapid progression to death. Neurohistopathological findings in both carcasses included severe acute multifocal to coalescent necrotising and purulent encephalitis orientated on the caudal brain stem associated with intralesional gram positive bacteria. *Listeria monocytogenes* was isolated from one of the two brain samples.

Luddington described an incident involving six-week-old lambs, one of which was submitted for post mortem examination. It was one of two lambs in a group of 210 showing acute onset nervous signs, such as torticollis leading to recumbency. Gross necropsy findings were unremarkable, but both culture and histopathological examination of the brain stem were consistent with meningoencephalitis caused by *Listeria monocytogenes*.

PIGS

Reproductive Diseases

PRRSV was confirmed as the cause of reproductive disease and neonatal mortality on a predominantly indoor 500-sow unit producing weaners. Sows were vaccinated with modified live PRRSV vaccine two-weeks after each farrowing and were also vaccinated for erysipelas and parvovirus. Over three to five weeks, approximately five of 15 sows per week were affected with lethargy, inappetence and pyrexia with early farrowings, increased numbers of still-born piglets, low viability in neonatal piglets and low litter sizes. Respiratory disease was reported to have affected most of the herd in January 2009. Piglets were submitted from two litters, both from first parity sows and PRRS virus was detected by PCR in the spleens of four fetuses tested.

Alimentary tract diseases

Clostridial enteritis

Losses of neonatal pigs on an outdoor breeding herd of 330 sows was investigated by Bury. Approximately 25% of newborn pigs were dying and six were submitted for post-mortem examination which revealed the intestinal lesions comprising, segmental haemorrhage, occasional gas bubbles on the serosal surface, diphtheresis of the mucosal surface from the mid to terminal small intestine and fluid large intestinal contents. Gram stained smears demonstrated large numbers of clostridial-like organisms and clostridial enterotoxaemia due to *Clostridium perfringens* type C was confirmed by detection of alpha and beta toxins in small intestinal contents in one piglet.

Respiratory Diseases

PRRS

Bury investigated a case in which approximately half of a group of 980 14 - 15-week-old housed finishers showing malaise, lameness, respiratory disease and recumbency were suspected to be affected with PCV2-associated disease. Fixed tissues were submitted to the laboratory, including lymph node and lung. Histopathology revealed no evidence of PCV2 involvement in the disease, however a moderate bronchointerstitial lymphocytic pneumonia was present and PRRS virus was detected by immunohistochemistry.

Musculoskeletal Diseases

Streptococcal dermatitis

The carcasses of three pigs of different ages were presented to Shrewsbury for post-mortem examination. A major herd problem was reported with severe lameness developing in the hind legs with initial signs of swellings around the hock area shortly before weaning at 3-4 weeks of age. There had also been significant numbers of abattoir condemnations associated with severe pleurisy and pneumonia. Post-mortem examination revealed bilateral thickening of the periarticular skin surrounding the hocks and mild diffuse consolidation of the lung tissues. Bacterial cultures yielded *Streptococcus dysgalactiae* subsp. *equisimilis* from the periarticular tissues and *Mycoplasma hyorhinis* from the

lung tissues. Histopathological findings confirmed the presence of a chronic cellulitis associated with mild necrotising dermatitis to a sub-acute to chronic purulent and necrotising cellulitis. There was also a mild acute broncho-pneumonia with a lymphoplasmacytic interstitial component consistent with *Mycoplasma* infection. One of the major predisposing factors in this case was considered to be the abrasive flooring which was likely to cause problems early in the pre-weaning period when the infection was established.

BIRDS

Broiler Chickens

Spiking mortality syndrome

Spiking mortality syndrome (probable hypoglycaemia) was thought to be the main cause of sharp and transient increase in mortality in a flock of 25 day-old broilers. Post-mortem examination findings included congested subcutaneous fat and livers, pale spleens with blood splashes and absence of food in the upper digestive tract. A definitive diagnosis for this condition depends on the demonstration of blood glucose levels below 150mg/dl. However microscopic finding of numerous lipid droplets in renal tubular epithelium and myocardial cells which are demonstrated by Oil Red 'O', supports the possibility of a metabolic problem e.g. hypoglycaemia resulting in the mobilisation of fat stores as an alternative energy source.

Broiler Breeders

Septic Arthritis Associated with *Staphylococcus* Infection

Increased mortality and an increase in the number of culls due to leg problems were reported in 27 week old broiler breeders at a unit with 7,000 birds in each of the four houses. Culled and 'found dead' birds were submitted for post-mortem examination. In the culled birds there was subcuticular swelling above the hock and in the associated tendons from which a coagulase positive *Staphylococcus* was isolated. Signs of egg peritonitis were seen in the found dead birds.

Commercial Layers

Necrotic enteritis

An unusual case of necrotic enteritis was diagnosed in a flock of 10000, 29 week old free range layers with a history of sudden slight increase in mortality. Typical lesions were found in two of the three birds submitted for post mortem examination and involved almost the entire intestinal tract, sparing a small area around Meckel's diverticulum. Lesions were confirmed histologically as large areas of coagulative necrosis in which there were numerous colonies of large Gram positive bacilli.

Necrotic enteritis in layers of this age is very unusual. In broilers there is sometimes an association between coccidial infection and necrotic enteritis. Coccidiosis is occasionally seen in layers in the early part of lay, although no evidence of it was found in these birds. No possible predisposing factors were discovered in this case.

Erysipelas

Acute Erysipelas was diagnosed in a flock of 40 week old organic free range layers with cumulative mortality since point of lay of 20% following a recent flock diagnosis of *E coli* peritonitis. Post mortem findings included swollen livers and spleens, ovarian regression and in one carcass lesions of vegetative endocarditis. Routine cultures of livers, spleens and heart valve produced heavy pure growths of *Erysipelothrix rhusiopathiae*.

Backyard Flocks

ILT

Winchester reported a case in which a new cockerel had been introduced to a small flock of 50 chickens, and eight days later respiratory signs began to be seen in the group. Post mortem examination of a bird that died revealed an acute tracheitis and a small amount of lung congestion. Virus isolation work carried out on the trachea revealed the presence of a herpes virus typical of infectious laryngotracheitis (ILT). This disease is often seen after being introduced to susceptible flocks through the introduction of purchased birds.

MISCELLANEOUS SPECIES

Alpaca

Fasciolosis

Death due to chronic fasciolosis was diagnosed by Penrith in a 7-month-old female alpaca despite treatment in November with triclabendazole. The liver was fibrosed and shrunken with degenerate fluke evident in thickened bile ducts. The apparent lack of efficacy of triclabendazole could be due to several factors including, true resistance, inadequate metabolism of the product in the liver, or inadequate dosing. Camelids require a higher product dose rate of 15mg/kg body weight, and are also prone to spitting out drench.

Camelostongylus mentulatis

The carcase of one approximately three-year-old female breeding alpaca was submitted to Thirsk for post-mortem examination after the animal died following a short illness. Reportedly, the animal had suddenly stopped eating and lost condition over a period of five days. Gross post-mortem revealed a total worm count of C3 stomach 7, 900 *Camelostongylus mentulatis* worms. The albumin content of a pre-mortem blood sample was 18.5 g/l (reference range 25-42 g/l) leading to suspicions of a protein-losing enteropathy supporting a diagnosis of parasitic gastritis. Further investigation in this case revealed histopathological evidence of encysted L4 larvae in gastric crypts in the C3 stomach suggesting the possibility of disease being caused by eruption of arrested larvae in this animal which would explain the rapid onset of clinical signs and may explain the low albumin level which may be occurring as a result of protein loss through this damaged mucosa. *C. mentulatis* has been reported by the VLA previously (Welchman, *Veterinary Record*, **162**: page 832), although specifically type 2 disease has not been reported.

Johne's diseases

Johne's disease was diagnosed in an adult male alpaca which died with a history of anorexia and elevated liver enzymes and a short period of watery

diarrhoea prior to death. At necropsy the mucosa of the terminal jejunum and ileum were found to be thickened with the mesenteric and submandibular lymph nodes enlarged and containing white caseous foci. Smears of the lymph nodes contained numerous acid-fast organisms. Due to the involvement of the submandibular lymph nodes, the DVM was notified of the suspicion of tuberculosis. Subsequent culture of the lymph nodes resulted in the growth of *Mycobacterium avium*. Histopathology confirmed lesions consistent with Johne's disease in the mesenteric lymph node and intestines.

Water Buffalo

Ergotism in water Buffalo

Langford reported a case involving a herd of water buffalos which experienced the deaths of 20 animals over a short period of time. The majority of these animals had gangrenous lesions of the fetlocks and some had ear and tail tip necrosis. An additional observation was that the bulls appeared to be sexually overactive. The cause of illness and death was identified as ergot poisoning as ergots were found within the grass silage which had been fed. This had been made late last year once the grass had seeded and become ergotised.

Horses

Lead poisoning in horses

Blood and hair samples were submitted from a thoroughbred mare from a race yard to investigate laryngeal and oesophageal dysplasia and bilateral ptosis. Lead poisoning was confirmed with both blood and hair lead levels elevated. The yard included an area of pasture where environmental contamination by lead was suspected as the likely source.

WILDLIFE

A red squirrel (*Sciurus vulgaris*) in thin body condition with a noticeable dermatitis in the ventral abdominal area was found dead in a wood. *Staphylococcus sciuri* was isolated from the liver and the infection may have come from the skin lesion. *S.sciuri* is present on the skin of healthy red squirrels but may occasionally become invasive as an opportunistic systemic pathogen.