

# The dam and her calf, before, during and after parturition

Marcel A. M. Taverne

*Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands  
m.a.m.taverne@uu.nl*

## ABSTRACT

Foetal and perinatal mortality rates in dairy cows may reach levels which represent serious problems, both from animal health/welfare and economic points of view. This is even more relevant for cows pregnant from in vitro produced embryos and has stimulated several groups in the past years to pay more close clinical and research attention to the pathophysiology of pregnancy and parturition in cows. In this contribution to a workshop on perinatal mortality in cows, some methods and recent results are reported, by which several (patho)physiological functions of the dam, the placenta and her fetus have been investigated or monitored during pregnancy, parturition and the immediate postpartum period. These include: in vivo measurements of uterine bloodflow and myometrial activity, cervical ripening and dilatation, placental hormone and proteins production, fetal and placental growth and development, foetal heart rate, oxygenation and mobility. Some recommendations for future approaches are given as well.

## KEYWORDS

Cow, pregnancy, parturition, puerperium, fetus, placenta

## INTRODUCTION

After a positive pregnancy diagnosis has been made, clinical attention paid to individual pregnant dairy cows generally subsides, until the moment the animal is dried off or the first signs of the calving process become visible. Yet, there are several reasons for a more intensive monitoring of cows during their pregnancy. Firstly, there is a substantial rate of prenatal foetal deaths (25), which may even reach extreme levels in cows pregnant from in vitro produced embryos (22). Secondly, an increased rate of so far unexplained perinatal losses in dairy cows has been reported in the past few years from different countries (for references see: 30, 41). Although this particularly affects first calving animals, the absence of a clear association with dystocia leads to the suggestion that at least part of these losses might find their origin during gestation, i.e. during the period of placental and foetal development. Thirdly, there is a substantial and increasing amount of observational and experimental studies, mainly in species other than the cow (but including humans), which indicate that external factors to which females are exposed at the start or during the course of a pregnancy, may affect and program (metabolic, endocrine, behavioural) functions and performance (growth) of their offspring (for examples see: 32,49).

The calving process itself constitutes a crucial part of the cow's reproductive cycle and usually supervision and some form of assistance by the farmer and/or the veterinary surgeon is necessary to reduce calf losses that would otherwise be caused by dystocia. But the signs for impending parturition are not always clear or noticeable, intervention might take place at a too early stage, or the type of obstetrical help might not be appropriate. To attack these shortcomings, many different aspects of the physiology of the calving process are still to be explored. For example, the functional role of the calf's motility in attaining the correct posture and position, the regulation of the softening and dilatation of the cervix, and the factors

influencing foetal oxygenation and viability during the different stages of parturition, belong to many processes which are only poorly understood in cows.

Postpartum uterine involution is a prerequisite for a timely new conception and pregnancy. But processes like loosening and separation of the foetal placenta, regulation and rate of cervical closure and characteristics of (early and late) postpartum uterine contractility have only been scarcely explored. Despite the widespread use of a variety of drugs to stimulate early postpartum uterine contractility and to speed up uterine involution, there is still little solid evidence that a routine use of uterotonic drugs has a positive effect on subsequent fertility parameters at the farm level.

So, gestation, the calving process and the early puerperium each represents a period that still deserves more attention by clinical and basic researchers. The present paper aims to shortly review some of the methodologies and technologies that could or have already been explored for the in vivo investigation of several maternal, placental and foetal functions in individual animals during each of these three periods.

## Bovine pregnancy

*Measurements of some maternal functions associated with pregnancy*

Uterine blood flow determines the amount of nutrients and oxygen that can potentially be passed through the placenta from the mother to her conceptus. Repartitioning of blood flows within the maternal organism is crucial for a normal development and growth of the placenta and foetus. This is, for example, nicely illustrated by experiments in which very young, prepubertal ewes were overfed during a pregnancy that was established following hormonal treatment and embryo transfer. Apparently these young mothers used the extra feed for their own rapid growth, but at the expense of growth of the placenta and foetus, which was associated with a dramatically reduced uterine blood flow (for references, see review: 49). Uterine blood flow in pregnant cows, as measured by invasive techniques increases steadily from the fourth month of gestation onwards (17). At term pregnancy, uterine blood flow reached levels of some 5.5 L/min (Herefords) and 7.8 L/min (Charolais). Twin pregnancies in cows results in significantly lower weights of the calves at birth and this is associated with significantly reduced uteroplacental bloodflows (17). The importance of an adequate uterine circulation became also clear from experiments in which pregnant cows were fed with ponderosa pine needles (15). Vasoactive substances in these needles causes a change in uterine vascular resistance, resulting in a steady decrease of uterine blood flow and premature calvings. The non-invasive Doppler technique for measuring uterine blood flow, as explored by Bollwein and his group (for review see: 21) appears to be an excellent approach to investigate the effects of external factors, such as nutritional deficiencies, supplements, toxins and drugs, on uterine perfusion in pregnant cows.

Despite the presence of elevated maternal plasma progesterone levels throughout the entire course of gestation (38), the myometrium of the cow's uterus is not completely immobilized during pregnancy. Like in other ruminants, so-

called contractures occur, representing long lasting episodes (in cows on average 12 minutes) with slightly elevated intrauterine pressure and persisting electromyographic activity, and with a mean frequency of 13.6 per day (40). Because contractures temporarily decrease uteroplacental blood flow (sheep: 20), they affect a variety of fetal physiological functions, such as blood PO<sub>2</sub> levels, body, eye and breathing movements, and hormone secretion (35). A hormonally induced, prolonged hyperfrequency of these contractures in late pregnant sheep also affects cardiovascular maturation of the fetus and its response to hypoxia (36). One may wonder if stillborn calves, which were delivered without any sign of dystocia, might already have been weakened in utero by an abnormal level of myometrial activity during gestation.

#### *Monitoring of placental functioning.*

Besides its role in exchanging metabolic substances and respiratory gases between mother and fetus, the placenta also functions as an endocrine organ. Like in most other mammals, a variety of steroids, peptide hormones, growth factors and proteins (such as pregnancy-associated glycoproteins, also described as pregnancy specific proteins) are synthesized and secreted by the bovine placenta. Apart from their (potential) use for pregnancy diagnosis, their concentrations in maternal blood can also indirectly reflect a normal/abnormal placental development and viability of the calf in utero (12,27,28,30). A typical example was reported by Heymann and coworkers (22), who demonstrated that in bovine pregnancies obtained with cloned embryos, abnormally elevated maternal plasma levels of pregnancy specific B during gestation were associated with pathology of the conceptus. Because of the rather large variability of plasma levels of hormones and peptides, especially between individual pregnant animals, repeated blood sampling is usually necessary to identify the abnormal development of a conceptus. Predictions on the basis of determinations in a single plasma sample are even more difficult.

Because of the (sometimes highly) elevated rate of fetal losses and complications during pregnancies with in vitro produced embryos, much more research has been performed in recent years on the development of the bovine placenta. These studies used non-invasive ultrasonographic morphometry of placentomes in situ (see for example: 6,22,29) and histology and gene expression of placental tissue from sacrificed animals (see for example: 16,29,33). In general these reports indicate that marked deviations in placental development may occur in such pregnancies. In fact such pregnancies provide an "ideal model" to apply and test methods for maternal, placental and fetal monitoring. To enable longitudinal correlation studies between maternal biochemical/clinical chemistry parameters and placental development during the same pregnancy, it would be very helpful to develop a placental biopsy technique by which placental tissue can be obtained repeatedly from the same cow, without interruption of the pregnancy. We have previously demonstrated that repeated, ultrasound-guided collection of fetal fluids is possible during bovine pregnancy (46).

#### *Monitoring of foetal viability: heart rate, foetal movements.*

Based on ultrasonographic measurements, growth curves of several fetal dimensions, such as crown-rump length, the largest diameter of the fetal trunk, the eyeball, the braincase, the stomach and the length of several long bones are available for bovine fetuses (13,19,26). Yet, Breukelman (12) questioned whether such measurements are able to identify deviations in fetal growth already during the first four months. We have preliminary evidence that countings of fetal heart rates (on-line or off-line from videotaped scannings) is a much more promising tool to detect compromised fetuses during early

pregnancy, because fetal heart rate (FHR) is still rather stable during the first months. At later stages of gestation, the large size and intrabdominal position of the pregnant uterus and the limited penetration depth of ultrasound beams may limit access to the fetal heart by a transrectal or even transabdominal route. Nevertheless we recently demonstrated that Doppler recording of FHR heart rate from the flank is feasible during the last month of gestation, but prolonged measurements are necessary because of the highly variable FHR at this stage (14). This technique could be used to evaluate possible cardiovascular effects in the fetus of drugs that are given to their mothers during pregnancy. The use of this approach might also be of interest for investigating pregnant cows in herds with high levels of unexplained perinatal mortality. A very recent field study on an extensively managed herd of Friesian dairy cows and heifers in the U.K., suggested that intrapartum asphyxia, associated with myocardial degeneration and necrosis of the left ventricle, was the primary cause of the fetal deaths (34). It would be interesting to know if antepartum FHR recordings would be able to detect such abnormal fetuses already before the onset of calving.

#### *Foetal movements*

From both invasive and non-invasive studies on fetal body movements in large domestic animals, including the cow, it is known that during late pregnancy fetal position and posture may change rapidly, while fetal presentation (predominantly anterior) remains the same. There is evidence to suggest that anterior presentation favours the normal kinetic development of the bovine fetus: a significantly percentage of malformations, including the neck and legs, have been found in fetuses in posterior presentation (18). Given the frequently occurring, obstetrical problems associated with malpresentation, malposition and malposture of the calf (18,24), fetal behaviour and motility and the factors influencing fetal movements during late pregnancy and around calving, still deserve more investigation in the future.

#### **The calving process**

##### *The parturient dam*

Lack of supervision during calving can lead to an increased risk of unattended dystocia and associated puerperal disorders (39). Hormonal induction of calving is not an ideal tool to facilitate a better supervision and frequently results in cows with retained placenta. Because an accurate early recognition of the start of the parturition process appears not always easy under farm conditions, methods have been explored to predict the onset of calving. These include: measurements of changes in body temperature, changes in plasma progesterone, colostrum and observation of behaviour of the dam (for references, see: 8). These methods still require further improvement and adjustments before they can be easily implemented on farm to assist the farmer in his calving management.

A substantial percentage of the dystocias in cows is associated with abnormal dilatation of the cervix (37,47) and there is also the risk that calving assistance is provided too early during the dilatation stage (i.e. during a still normally progressing calving process). Because of the nearly lack of information on the ripening and dilatation of the cervix in pregnant and parturient cows, our group has intensively explored these aspects during the past years (9,43). These studies revealed that biochemical preparation of the stroma layer of the cervix (which include connective tissue, fibroblasts, smooth muscle cells and invading white blood cells) can be divided in two stages: a gradual ripening, which already starts during the final months of pregnancy, and a more rapid final ripening during parturition itself, which shows many characteristics of an inflammatory process. By using stromal biopsies, repeatedly taken from the caudal cervix in the same

animal from mid pregnancy up to and including parturition, we started to unravel temporal changes in the expression, localization and possible interactions of several collagenases, collagenase inhibitors, cytokines, and hormones between the tissue components (for details see: 1,10,45). These studies aim to find clues for future therapies that could improve cervical ripening in cases with cervix-derived dystocia.

During another series of *in vivo* experiments with pregnant cows, provided with myometrial electrodes (for electromyography: EMG), we installed ultrasonic crystals on the cervix to measure dilatation during prostaglandin induced calving (9,11). This approach revealed a specific sequence of physiological events: after a rapid drop of the maternal plasma progesterone levels during the first 10 hours after injection, uterine EMG activity started to increase (on average) at some 13 hours after PG injection. Yet it took another 15 hours before the caudal cervix started to dilate progressively, at a mean rate of 2.6 cm per hour. Calves were vaginally delivered at some 9 hours after onset of dilatation. Although comparable measurements still need to be performed in spontaneous calving animals, this methodology will allow us to investigate factors (dystocia, stress; drugs) that might influence cervical dilatation and to combine it with studies on biopsies from the cervical stroma.

#### *The calf during parturition*

The regular and more powerful uterine contractions during parturition are (most likely) associated with pronounced decreases of uteroplacental blood flow. However during the intervals between contractions, blood flow is restored so that a proper oxygenation of the fetal calf is usually maintained. Yet at the final stage of expulsion, when traction or compression of the umbilical cord may start to develop and maternal abdominal straining forces are added to the pressure generated by the myometrium, fetal oxygenation may become compromised. The latter may also be the case when dystocia or a (partly) separation of the fetal placenta has developed. It is in those cases of importance to obtain information on the condition of the calf during the birth process, especially before any decision is made as to the type of obstetrical help to be provided. While in practice this will largely depend on palpation of the *ictus cordis* and testing of fetal motility and reflexes, the availability of very small and portable measuring devices for blood clinical chemistry, has made it feasible to analyse PO<sub>2</sub>, PCO<sub>2</sub>, pH, BE, lactate and glucose in blood samples also under farm conditions. Several veterinary obstetricians have explored research methods for monitoring the unborn calf during the birth process (for references see: 7,25,42). These include repeated blood sampling from the umbilical cord or fetal front legs for measurements of blood gases and acid-base balance analysis, pulse oximetry from within the calf's mouth, Doppler measurements of umbilical blood flow, and cardiotocography, i.e. the combined recording of FHR and intrauterine pressure changes. Development of asphyxia and acidosis, as established after birth, was found to be associated with characteristic changes in the dynamics of umbilical blood flow, FHR during the delivery process. The predictive values of such measurements remain to be established and their practicality will remain restricted to clinicians in university animal hospitals. But these approaches will enable to investigate the effects of a variety of obstetrical examinations and (pharmacological) treatments on the bovine fetus, to improve the quality of obstetrical interventions (or the refrain from further intervention).

#### **The first few days after calving**

Farmers usually notice whether the fetal placenta has been shed or not, but accurate records of the interval between delivery of

the calf and shedding of the placenta are not made and are also difficult to find in the literature. Yet, such information would be very useful in efforts to find possible links between (premature) placental separation and perinatal outcome. The same is true for inspection and histological investigation of the delivered fetal membranes. This may only take place when the occurrence of specific infections (such as *Neospora*) is presumed. But morphometry and gene expression studies on fetal cotyledons may provide crucial information as to specific aspects of the development and health of the belonging calf and therefore also the delivered placenta should more often become the object of investigation.

Hardly any accurate information is presently available on the rate and timing of the closing of the cervix after calving, despite the fact that a timely closure is of eminent importance for attaining of a subsequent conception (for example, see: 5). When a forceps was repeatedly inserted in the cervical canal from the moment of fetal delivery until 10 days after normal calvings (without retained placenta), a decrease in cervical diameter from 25 cm to 5.2 cm was found within the first 2 days (48). A rather different pattern of cervical closure was recently reported by Van Engelen and coworkers (44) after prostaglandin induced cows, in which ultrasonic crystals has been attached on the cervix for continuous measurements of cervical diameter. In these cows the diameter first increased during the first 15 hours, before it also reached some 5 cm at 48 hours after calving. It is not yet clear whether this difference is due to the presence of the retained placenta and/or to the use of a different methodology.

Postpartum uterine contractions are responsible for expulsion of uterine contents and reduction of uterine size. Intrauterine pressure recordings in puerperal cows have shown that the frequency of contractions decreases to almost zero within two days after uncomplicated calvings (3,31). But in cows with retained placenta, the frequency and amplitudes of uterine contractions remain elevated for at least another few days (31). Lack of contractility is thus very unlikely to be responsible for retention of the membranes.

There have been many efforts to pharmacologically influence uterine contractility during the postpartum period (for reviews see: 2,23). When given on the first day after delivery of the calf, stimulating effects either subsided within a few hours (oxytocin and longacting oxytocin analogues: 4) or were even completely absent (natural and synthetic analogues of prostaglandin F<sub>2</sub>α; Bajcsy, personal communication). Hirsbrunner (23) reported stimulating effects (both *in vivo* and *in vitro*) of different prostaglandin F and E formulations in nonpregnant cows. On the basis of these observations, her group evaluated possible positive effects of a single treatment with PGF<sub>2</sub>α, or a combination of PGF<sub>2</sub>α and PGE<sub>2</sub>, in dairy cows between 3-5 weeks postpartum. In a randomized, double-blind clinical trial they could not find beneficial effect on measured fertility variables, apart from a tendency for a shorter interval from calving to first insemination after the combined treatment. Postpartum uterine contractility during the first weeks after calving still requires much more investigation, especially in cases with a pathological puerperium.

#### **REFERENCES**

1. AALBERTS, M. – VAN DISSEL-EMILIANI, F. M. F. – VAN TOL, H. T. A. – TAVERNE, M. A. M. – BREEVELD-DWARKASING, V. N. A.: High iNOS mRNA and protein levels during early third trimester suggest a role for NO in prelabor cervical ripening in the bovine. *Mol. Reprod. and Develop.*, 2007. 74. 378-385.
2. BAJCSY, Á. Cs: Physiological and clinical aspects of uterine contractility during the postpartum period in cows. PhD-thesis, 2005. Utrecht University, The Netherlands.

3. BAJCSY, A. Cs. – SZENCI, O. – DOORNENBAL, A. – VAN DER WEIJDEN, G. C. – CSORBA, Cs. – KOCSISI, L. – SZÚCS, I. – OSTGARD, St. – TAVERNE, M. A. M.: Characteristics of bovine early puerperal uterine contractility recorded under farm conditions. *Theriogenology*, 2005. *64*. 99-111.
4. BAJCSY, A. Cs. – SZENCI, O. – VAN DER WEIJDEN, G. C. – DOORNENBAL, A. – MAASSEN, F. – BARTYIK, J. – TAVERNE, M. A. M.: The effect of a single oxytocin or carbetocin treatment on uterine contractility in early postpartum cows. *Theriogenology*, 2006. *65*. 400-414.
5. BEKANA, M. – JONSSON, P. – KINDAHL, H.: Bacterial isolates associated with retained fetal membranes and subsequent ovarian activity in cattle. *Vet. Rec.*, 1997. *140*. 232-234.
6. BERTOLINI, M. – MASON, J. B. – BEAM, S. W. – CARNEIR, G. F. – SWEEN, M. L. – KOMINEK, D. J. – MOYER, A. L. – FAMULA, T. R. – SAINZ, R. D. – ANDERSON, G. B.: Morphology and morphometry of in vivo- and in vitro-produced bovine concepti from early pregnancy to term and association with high birth weights. *Theriogenology*, 2002. *58*. 973-994.
7. BLEUL, U.: Peripartales Monitoring des Rinderfetus und Konsequenzen für die Therapie der Asphyxie. Habilitationsschrift, 2007. Veterinary Faculty, University of Zürich, Switzerland.
8. BLEUL, U. – SPIRIG, S. – HÄSSIG, M. – KÄHN, W.: Electrolytes in bovine prepartum mammary secretions and their usefulness for predicting parturition. *J. Dairy Sci.*, 2006. *89*. 3059-3065.
9. BREEVELD-DWARKASING, V. N. A.: The bovine cervix exposed: the cow as a model for studies on functional changes in the uterine cervix. PhD-thesis, 2002. Utrecht University, The Netherlands.
10. BREEVELD-DWARKASING, V. N. A. – TE KOPPELE, J. M. – BANK, R. A. – VAN DER WEIJDEN, G. C. – TAVERNE, M. A. M. – VAN DISSEL-EMILIANI, F. M. F.: Changes in water content, collagen degradation, collagen content and concentration in repeated biopsies of the cervix of pregnant cows. *Biol. Reprod.*, 2003a. *69*. 1608-1614.
11. BREEVELD-DWARKASING, V. N. A. – STRUIJK, P. C. – LOTGERING, F. K. – EIJSKOOT, F. – KINDAHL, H. – VAN DER WEIJDEN, G. C. – TAVERNE, M. A. M.: Cervical dilatation related to uterine EMG-activity and endocrinological changes during PGF-2 $\alpha$  induced parturition in cows. *Biol. Reprod.*, 2003b. *68*. 536-542.
12. BREUKELMAN, S.: Fetal and placental monitoring in cattle. PhD-thesis, 2005. Utrecht University, The Netherlands.
13. BREUKELMAN, S. P. – REINDERS, J. M. C. – JONKER, F. H. – DE RUIGH, L. – KAAL, L. M. T. E. – VAN WAGTENDONK-DE LEEUW, A. M. – VOS, P. L. A. M. – DIELEMAN, S. J. – BECKERS, J. F. – PERÉNYI, Zs. – TAVERNE, M. A. M.: Fetometry and fetal heart rates between days 35 and 108 in bovine pregnancies resulting from transfer of either MOET, IVP-coculture, or IVP-SOF embryos. *Theriogenology*, 2004. *61*. 867-882.
14. BREUKELMAN, S. P. – MULDER, E. J. H. – VAN OORD, H. A. – JONKER, F. H. – VAN DER WEIJDEN, G. C. – TAVERNE, M. A. M.: Continuous fetal heart rate monitoring during late gestation in cattle by means of Doppler ultrasonography: reference values obtained by computer-assisted analysis. *Theriogenology*, 2006. *65*. 486-498.
15. CHRISTENSON, L. K. – SHORT, R. E. – FORD, S. P.: Effects of ingestion of Ponderosa Pine needles by late pregnant cows on uterine blood flow and steroid secretion. *J. Anim. Sci.*, 1992. *70*. 531-537.
16. CONSTANT, F. – GUILLOMOT, M. – HEYMAN, Y. – VIGNON, X. – LAIGRE, P., SERVELY, J. et al.: Large offspring or large placenta syndrome? Morphometric analysis of late gestation placentomes from somatic nuclear transfer pregnancies complicated by hydroallantois. *Biol. Reprod.*, 2006. *75*. 122-130.
17. FERRELL, C. L. – REYNOLDS, L. P.: Uterine and umbilical blood flows and net nutrient-uptake by fetuses and uteroplacental tissues of cows gravid with either single or twin fetuses. *J. Anim. Sci.*, 1992. *70*. 426-433.
18. FRASER, A. F. – HERCHEN, H.: Terminal fetal postures observed in a bovine caesarean survey. *Appl. Anim. Ethol.* 1978. *4*. 315-322.
19. GINTHER, O. J.: Ultrasonic imaging and animal reproduction: Cattle (book 3). Equiservices Publishing, Wisconsin, 1989.
20. HARDING, R. – POORE, E. R.: The effects of myometrial activity on fetal thoracic dimensions and uterine blood flow during late gestation in the sheep. *Biol. Neonate*, 1984. *45*. 244-251.
21. HERZOG, K. – BOLLWEIN, H.: Application of Doppler ultrasonography in cattle reproduction. *Reprod. Dom. Anim.*, 2007. *42*. Supplement 2. 33-44.
22. HEYMAN, Y. – CHAVATTE-PALMER, P. – LEBOURHIS, D. – CAMOUS, S. – VIGNON, X. – RENARD, J. P.: Frequency and occurrence of late-gestation losses from cattle cloned embryos. *Biol. Reprod.* 2002. *66*. 6-13.
23. HIRSBRUNNER, G.: Bovine and equine uterine motility in vivo and ex vivo; a step forward in fathoming a mystery. Habilitationsschrift, 2007. Veterinary Faculty, University of Switzerland.
24. HOLLAND, M. D. – SPEER, N. C. – LEFEVER, D. G. – TAYLOR, R. E. – FILED, T. G. – ODDE, K. G.: Factors contributing to dystocia due to fetal malpresentation in beef cattle. *Theriogenology* 1993. *39*. 899-908.
25. JONKER, F.: Fetal death: comparative aspects in large domestic animals. *Anim. Reprod. Sci.*, 2004. *82-83*. 415-430.
26. KÄHN, W.: Sonographic fetometry in the bovine. *Theriogenology*, 1989. *31*. 1105-1121.
27. KINDAHL, H. – KORNMATITSUK, B. – KÖNIGSSON, K. – GUSTAFSSON, H.: Endocrine changes in late bovine pregnancy with special emphasis on fetal well-being. *Dom. Anim. Endocrinol.*, 2002. *23*. 321-328.
28. KINDAHL, H. – KORNMATITSUK, B. – GUSTAFFSON, H.: The cow in endocrine focus before and after calving. *Reprod. Dom. Anim.*, 2004. *39*. 217-221.
29. KOHAN-GHADR, H. R. – LEFEBVRE, R. C. – FECTEAU, G. – SMITH, L. C. – MURPHY, B. D. – SUZUKI JUNIOR, J. – GIRARD, C. – HÉLIE, P.: Ultrasonographic and histological characterization of the placenta of somatic nuclear transfer-derived pregnancies in dairy cattle. *Theriogenology*, 2008. *69*. 218-230.
30. KORNMATITSUK, B. – FRANZÉN, G. – GUSTAFSSON, H. – KINDAHL, H.: Endocrine measurements and calving performance of Swedish Red and White and Swedish Holstein dairy cattle with special respect to stillbirth. *Acta vet. Scand.*, 2003. *44*. 21-33.
31. MARTIN, L. R. – WILLIAMS, W. F. – RUSSEK, E. – GROSSE, T. S.: Postpartum uterine motility measurements in dairy cows retaining their fetal membranes. *Theriogenology*, 1981. *15*. 513-524.
32. MCEVOY, T. G.: Manipulation of domestic animal embryos and implications for development. *Reprod. Dom. Anim.*, 2003. *38*. 268-275.
33. MILES, J. R. – FARIN, CH. E. – RODRQUEZ, K. F. – ALEXANDER, J. E. – FARIN, P. W.: Angiogenesis and morphometry of bovine placentas in late gestation from embryos produced in vivo or in vitro. *Biol. Reprod.*, 2004. *71*. 1919-1926.
34. MURRAY, R. D. – WILLIAMS, A. J. – SHELDON, I. M.: Field investigation of perinatal mortality in Friesian cattle associated with myocardial degeneration and necrosis. *Reprod. Dom. Anim.*, 2008. *43*. 339-345.

35. NATHANIELSZ, P. W. – BAILEY, A. – POORE, E. R. – THORBURN, G. D. – HARDING, R.: The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation. *Am. J. Obstet. Gynec.*, 1980. *138*. 653-659.
36. SHINOZUKA, N. Y. A. – NATHANIELSZ, P. W.: Increased myometrial contracture frequency at 96-140 days accelerates fetal cardiovascular maturation. *Am. J. Physiol. Heart Circ. Physiol.*, 2000. *278*. H41-H49.
37. SLOSS, V. – JOHNSTON, D. E.: The causes and treatment of dystocia in beef cattle in western Victoria. II. Causes, methods of correction and maternal death rates. *Aust. Vet. J.* 1967. *43*. 13-21.
38. STABENFELDT, G. H. – OSBURN, B. J. – EWUNG, L. L.: Peripheral plasma progesterone levels in the cow during pregnancy and parturition. *Am. J. Physiol.*, 1970. *218*. 571-575.
39. STEVENSON, J. S. – CALL, E. P.: Reproductive disorders in the periparturient dairy cow. *J. Dairy Sci.*, 1988. *71*. 2572-2583.
40. TAVERNE, M. A. M. – BREEVELD-DWARKASING, V. N. A. – VAN DISSEL-EMILIANI, F.M.F. – BEVERS, M. M. – DE JONG, R. – VAN DER WEIJDEN, G. C.: Between prepartum luteolysis and onset of expulsion. *Dom. Anim. Endocr.*, 2002. *23*. 329-337.
41. TAVERNE, M. A. M. – VAN DER WEIJDEN, G. C.: Parturition in domestic animals: targets for future research. *Reprod. Dom Anim.*, 2008. In press.
42. TAVERNE, M. A. M.: The relation between the birth process and the condition of the newborn piglet and calf. *Vet. Res. Communic.*, 2008. In press.
43. VAN ENGELEN, E.: Smooth muscle cells in bovine cervical ripening and dilatation. PhD-thesis, 2008. Utrecht University, The Netherlands.
44. VAN ENGELEN, E. – TAVERNE, M. A. M. – EVERTS, M. E. – VAN DER WEIJDEN, G. C. – DOORNENBAL, A. – BREEVELD DWARKASING, V. N. A.: Cervical diameter in relation to uterine and cervical EMG-activity in early post partum dairy cows with retained placentas after PGF2alpha induced calving. *Theriogenology*, 2007. *68*. 213-222.
45. VAN ENGELEN, E. – BREEVELD-DWARKASING, V. N. A. – TAVERNE, M. A. M. – EVERTS, M. E. – VAN DER WEIJDEN, G. C. – RUTTEN, V. P. M. G.: MMP-2 expression precedes the final ripening process of the bovine cervix. *Mol. Reprod. Develop.*, 2008. In press
46. VOS, P. L. A. M. – PIETERSE, M. C. – VAN DER WEIJDEN, G. C. – TAVERNE, M. A. M.: Bovine fetal fluid collection: transvaginal, ultrasound-guided puncture technique. *Vet. Rec.*, 1990. *127*. 502-504.
47. WEHREND, A. – BOSTEDT, H.: Examinations on the incidence of cervical dystocia and disorders of cervical involution in the cow postpartum. *Dtsch. Tierärztl. Wschr.* 2003. *110*. 483-486.
48. WEHREND, A. – FAILING, K. – BOSTEDT, H.: Cervimetry and ultrasonographic observations of the cervix regression in dairy cows during the first 10 days post partum. *J. Vet. Med. A. Physiol. Pathol. Clin. Med.*, 2003. *50*. 470-473.
49. WU, G. – BAZER, F.W. – WALLACE, J. M. – SPENCER, T. E.: Intrauterine growth retardation: implications for the animal sciences. *J. Anim. Sci.*, 2006. *84*. 2316-2337.

## Infectious bovine rhinotracheitis and the epidemiological role of the other ruminant species

Julien Thiry, Benoît Muylkens, Etienne Thiry\*

*Virology and viral diseases, Department of infectious and parasitic diseases, Faculty of veterinary medicine, University of Liège, B-4000 Liège, Belgium*

\* Corresponding author: Dr E. Thiry, Virology and Viral Diseases, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Boulevard de Colonster, 20, B43b, B-4000 Liège, Belgium. Phone: (32) 4 366 42 50, fax: (32) 4 366 42 61, e-mail: etienne.thiry@ulg.ac.be

### ABSTRACT

Bovine herpesvirus 1 (BoHV-1) is responsible for infectious bovine rhinotracheitis (IBR), a disease inducing worldwide major economic losses in the cattle industry. In order to design eradication schemes, investigations involved the study of the prevalence of the infection in numerous countries affected by the disease but also the epidemiological importance of animals in terms of the transmission and maintenance of the infection. In this way, an extensive range of host species has been serologically identified as potentially infected with BoHV-1 and therefore susceptible to this infection. However, the specificity of the serological detection of IBR can be compromised because of possible infection with other viruses sharing antigenic and genetic properties with BoHV-1. These viruses were isolated from various ruminant species, especially water buffalo (*Bubalus bubalis*), goat, red deer (*Cervus elaphus*), reindeer (*Rangifer tarandus*) and elk (*Cervus canadensis*). Thus, the large spectrum of species susceptible to BoHV-1 can be explained by infection with either BoHV-1 or a related virus. To date, the evidence that a ruminant species is fully susceptible to BoHV-1 is provided by the viral isolation or experimental cross-infection studies. This article reviews the domestic and wildlife ruminant species that

could constitute potential reservoirs of BoHV-1. The evidence of the crossing of the host ruminant species barrier by BoHV-1 is reported as well as the serological suspicions reported in several animal species. The issues due to the existence of ruminant alphaherpesviruses related to BoHV-1 are taken into account by describing their serological relationship with BoHV-1. The factors responsible for BoHV-1 transmission between cattle and the other ruminant species are discussed. In addition, the current tools available to monitor and to prevent the disease or the related infections are also reviewed.

### INTRODUCTION

In 1841, Büchner described genital lesions observed in a venereal disease named "Bläschenausschlag". It was the first report of a disease caused by bovine herpesvirus 1 (BoHV-1) but its viral aetiology was only demonstrated in 1928 by Reisinger and Reimann. Until the sixties, the observed diseases in Europe were mainly infectious pustular vulvovaginitis (IPV) in cows and infectious pustular balanoposthitis (IPB) in bulls. In the same time, a respiratory pathology emerged in North American feedlots in 1954 and was called infectious bovine rhinotracheitis (IBR). The importation of dairy cattle to improve the milk production

enabled the spread of the infection in Europe (45). Since BoHV-1 is known as the agent responsible for IBR, the virus and its pathogenesis have been deeply studied because of the major economic losses occurring worldwide in the cattle industry. The consequences of IBR lead the World Organisation for Animal Health (OIE) to classify it in the list of diseases to be controlled. Several vaccination protocols associated with diagnostic methods have been developed in order to reduce clinical consequences of the disease but also restrictions imposed in the cattle market. A preliminary step towards the design of control/eradication schemes consisted in the investigation of the infection and its level in respective countries (45). In this way, serological methods as seroneutralisation and enzyme linked immunosorbent assay (ELISA) have been developed and further used in order to detect infected bovines. Quickly the same tools were used in other animal species with the aim to determine their epidemiological importance in terms of transmission and maintenance of infection. Anti-BoHV-1 antibodies have been then detected in several ruminant species. However, the further isolation of ruminant alphaherpesviruses related to, but distinct from BoHV-1 compromised the specificity of these serological methods (72). The current review focuses on the potential transmission of BoHV-1 between livestock and wildlife through the epidemiological role of ruminant species as BoHV-1 reservoir but also on the issue linked to the existence of closely related ruminant alphaherpesviruses.

#### **Cross-relationship between bovine herpesvirus 1 and other ruminant alphaherpesviruses**

Currently, six viruses are classified as antigenically and genetically closely related to BoHV-1 (72): bovine herpesvirus 5 (BoHV-5) responsible for severe meningo-encephalitis in calves, bubaline herpesvirus 1 (BuHV-1) inducing a subclinical genital infection in water buffalo (*Bubalus bubalis*), caprine herpesvirus 1 (CpHV-1) causing systemic disease in young kids and abortion in adult goats, cervid herpesvirus 1 (CvHV-1) responsible of severe ocular syndrome in red deer (*Cervus elaphus*), cervid herpesvirus 2 (CvHV-2) and elk herpesvirus 1 (ElkHV-1), which induce a subclinical genital infection respectively in reindeer (*Rangifer tarandus*) and elk (*Cervus canadensis*) (72).

The situation observed in Finland in 1982 illustrates the serological misinterpretation that can occur between BoHV-1 and its related viruses. An epidemiological survey in the Finnish reindeer population showed that 23% of animals were seropositive to BoHV-1, while all cattle were seronegative (16). These data strongly suggested a BoHV-1 infection of reindeer with an absence of transmission to cattle due to an apparent lack of contact between the two ruminant species. This hypothesis was rapidly rejected. From a BoHV-1 seropositive reindeer, a new virus was isolated from a reactivated animal and further characterised as CvHV-2. This infection provided a likely explanation of the presence of anti-CvHV-2 antibodies cross-reacting with BoHV-1 in reindeer (17). In spite of this epidemiological situation in reindeer, the Finnish cattle population maintained an IBR free status.

More recently, another situation has been observed in Belgium. During 2001 and 2002 hunting seasons, 28.9% of red deer were detected seropositive to BoHV-1 (Grégoire and Linden, unpublished data). In regards to the Finnish episode and the apparent lack of contact between cattle and red deer, it was suggested that a BoHV-1 related virus was spreading in the Belgian red deer population. A new strain of CvHV-1 has been isolated that is also the first ruminant alphaherpesvirus to be isolated in wild fauna (75). It is hypothesised that this virus is responsible of an endemic infection spreading in continental Europe that could explain BoHV-1 seroprevalence measured in the free-ranging red deer population in Belgium, Czech Republic, France, Germany and Hungary (30, 42, 51, 69, 75).

Another example revealing the importance of clarifying the cause of potential serological cross-relationships with BoHV-1 is the difficulty encountered in France to differentiate true and false BoHV-1 positive cattle in the framework of an IBR herd certification. In France, a herd gets a label "A" after repeated serological investigations leading to a so-called "IBR-free status". However, cattle purchased from these herds are sporadically identified as seropositive to BoHV-1 (49, 71). Among other hypotheses, one explanation of this "false" seropositivity would be the infection with a BoHV-1 related virus that crossed the species barrier. The only virus that could be responsible of IBR misdiagnosis in France is CpHV-1 because goats and cattle can be in close contact in some field situations. A recent epidemiological investigation using BoHV-1 blocking ELISA demonstrated that a BoHV-1 related alphaherpesvirus infection was either absent or at a very low prevalence in goats in continental France but a very high seroprevalence was measured in Corsica. This BoHV-1 related virus was further identified as CpHV-1 (74, 77). Therefore, cross-infection with a BoHV-1 related virus does not explain the apparent false seropositive cattle.

The same issue is also encountered in Italy where buffaloes represent an important part of the dairy industry mainly in central and southern regions. The analysis of 415 unvaccinated buffaloes revealed an apparent seroprevalence of 71.08% in BoHV-1 blocking ELISA (Cuteri, personal communication). The discrimination between BoHV-1 and BuHV-1 is very difficult to achieve because these viruses are the most closely related within ruminant alphaherpesviruses (72, 75). This situation is not restricted to Europe because BoHV-1 seropositive buffaloes are also detected in Argentina (Romera, personal communication).

#### **Virological evidence that bovine herpesvirus 1 crosses the host species barrier**

Several alphaherpesviruses are able to infect heterologous species leading to either various clinical signs or subclinical infections. For example, the development of a severe illness can occur with Aujeszky's disease, a fatal meningoencephalitis in cattle caused by suid herpesvirus 1, a pig alphaherpesvirus (89). In the same way, equine herpesvirus 1 causes nervous and ocular syndromes in alpacas and llamas in South America (53). In the last decades, several cross-infection studies have been performed to have a better knowledge about risks and consequences of infection of ruminant species with BoHV-1. These cross-infections can be associated not only with serological response, but also with viral latency, reactivation and re-excretion (70, 72).

Cross-infection of goats with BoHV-1 has been successfully performed several times under experimental conditions (1, 18, 50, 61, 73, 86). Goats excreted virus at high titres during acute phase of the infection and BoHV-1 was able to establish a latent infection. During the acute phase of the infection, clinical signs atypical of BoHV-1 infection were observed such as diarrhoea and adenitis (61, 73). After an induced reactivation, fever, anorexia and stupor were observed in association; viruses were re-excreted at high titres and PCR revealed that BoHV-1 established latency in the trigeminal ganglia of goats (61). BoHV-1 neutralising antibodies were also detected after primary infection (73) and serum titres remained stable for three months (61).

In the same way, the ovine species has been shown to be susceptible to BoHV-1 infection (24, 26, 36, 60, 70). Domestic sheep (*Ovis aries*) showed mild clinical signs such as nasal and ocular moderate sero-mucopurulent discharge associated to a mild conjunctivitis (70). The virus excretion was detected very early (5 hours) after the inoculation but titres remained low until the glucocorticoid treatment. All animals seroconverted. BoHV-1 is able to establish a latent infection in sheep. It is supported by the re-excretion of viruses at higher titres and the detection of BoHV-1 viral DNA in the trigeminal ganglia of sheep (26).

Red deer can be infected with BoHV-1 but needs a profound challenge. The susceptibility of red deer was supported by the rise of BoHV-1 neutralising antibodies and the isolation of excreted viruses at low titres for up to three days post-infection. However, there was no evidence that BoHV-1 established a latent infection which would have the potential to reactivate and act as a source of infection for cattle (41, 47, 54). Another deer species, the mule deer (*Odocoileus hemionus*), exhibited a greater susceptibility to experimental BoHV-1 infection by developing anorexia, depression and respiratory disease two days after infection. Virus was recovered for five days post-infection from nasal, ocular and rectal secretions. The seroconversion was similar than in cattle (10, 48). In reindeer, experimental BoHV-1 infection is asymptomatic and does not usually give rise to neutralising antibodies. The titres of nasally excreted viruses are very low and the virus cannot be reactivated experimentally (70).

In regards to these cross-infection experiments, it is therefore possible that these ruminant species can be infected naturally with BoHV-1 but the frequency of these infections must be low. Sheep (26) and red deer (41) do not play a major role in BoHV-1 transmission to calves. However, natural BoHV-1 infections have been reported in goats (40, 78, 88). BoHV-1 has been also isolated twice from lambs suffering of respiratory disorder in United States (79) as well as from one yearling and one adult free-ranging bighorn sheep (*Ovis canadensis*) in mountains of San Diego County (11). In the same way, vulvovaginitis has been described in captured blue wildebeest (*Connochaetes taurinus*) after corticosteroid treatment (32, 44). The experimental induction of vulvovaginitis in female and posthitis in bull with the blue wildebeest viral isolate confirmed the BoHV-1 aetiology (43). In an investigation performed in the Polish Bialowieza Primeval forest, a BoHV-1 strain was isolated from the spleen of a female calf European bison (*Bison bonasus*). However, none bison had significant antibody titres against BoHV-1 (7). To date, no natural BoHV-1 infection has been observed in deer species.

The hypothesis that BoHV-1 crosses the host species barrier is often suggested by serological analyses. Thus, the ruminant species demonstrated as susceptible to BoHV-1 were firstly described seropositive to BoHV-1 in numerous serological studies. However, these sero-epidemiological investigations have always to be taken cautiously due to the serological relationship between BoHV-1 and the closely related ruminant alphaherpesviruses, and more especially in buffaloes, goats and cervids. That could be illustrated by the reports of BoHV-1 isolation from water buffaloes in Australia and Malaysia. Viruses were isolated either from the prepuce of killed bulls (64) or from nasal and genital samples of a reactivated cow showing high BoHV-1 antibody titres (28). In both cases, no lesions were observed and viruses have not been genetically characterised. In regards to these data and especially the fact that viruses have been isolated from genital samples, it could be supposed that buffaloes were likely infected with BuHV-1 rather than BoHV-1. However, only the sequencing of the viral isolates is able to strongly identify BoHV-1 as the viral species infecting these animals.

### **A large number of ruminant species are seropositive to bovine herpesvirus 1**

A large spectrum of species is supposed to be susceptible to BoHV-1 (Table 1) as shown in the numerous serological investigations already performed. These results can be in part explained by the close antigenic relationship between BoHV-1 and its related alphaherpesviruses. Indeed, no diagnostic test is available to make a clear differentiation between these viruses. Therefore, most of the sero-epidemiologic investigations in the other ruminant species are performed with serological tools designed to detect BoHV-1 infection. To date, BoHV-1 has not been found to cause naturally occurring disease in wildlife.

Between 1960 and 1973, a serological investigation was performed in wild animals through Kenya, Tanzania, Uganda and

Zambia. The apparent seroprevalence of BoHV-1 seropositive animals was 64% in African buffalo (*Syncerus caffer*), 43% in common eland (*Taurotragus oryx*), 3% in impala (*Aepyceros melampus*), 23% in kob (*Kobus kob*), 40% in reedbeek (*Redunca redunca*), 20% in Thomson's gazelle (*Gazella thomsoni*), 11% in topi (*Damaliscus korrigum*) and 40% in Elypsan or Defassa waterbuck (*Kobus ellipsiprymnus*) (31, 52, 55, 56). Recently, an update of the IBR situation in African free-ranging animals was performed in Zimbabwe. The highest apparent seroprevalence was found in common eland and African buffalo (30%). In sable antelope (*Hyppotragus niger*), impala and kudu (*Tragelaphus strepsiceros*), the seroprevalence was around 12 to 14%. The lowest apparent seroprevalence was observed in giraffe (*Giraffa camelopardalis*), bushbuck (*Tragelaphus scriptus*), nyala (*Tragelaphus angasii*) and tsessebe (*Damaliscus lunatus*) (2). BoHV-1 was also serologically detected in South African animals: black wildebeest (*Connochaetes gnou*) (21%), red hartebeest (*Alcelaphus buselaphus*) (3%), blesbok (*Damaliscus pygargus*) (33%), gemsbok (*Oryx gazella*) (7%), African buffalo (44%), kudu (80%) and bushbuck (38%) (3). In regards to these data, the BoHV-1 apparent seroprevalence was determined in exotic ruminants living in United States zoos: 2.7% in addax (*Addax nasomaculatus*), 4.8% in springbok (*Antidorcas marsupialis*), 2% in blackbuck (*Antilope cervicapra*), 8.3% in black wildebeest, 17.9% in blesbok, 1.1% in Thomson's gazelle, 42.9% in Elypsan waterbuck, 33.3% in Defassa waterbuck and 7.7% in giraffe (15).

The American bison (*Bison bison*) seems also to be susceptible to BoHV-1. Indeed, 38% of free-ranging animals in Yellowstone national park and 43.8% of ranch-raised bison in North American states possess anti-BoHV-1 antibodies (59, 65). In free-ranging cervid species, the apparent BoHV-1 seroprevalence has been widely investigated. The BoHV-1 prevalence in white-tailed deer (*Odocoileus virginianus*) ranged between 15% in Minnesota (Ingebrigtsen et al., 1986) and 57% in Quebec (35, 58). In Hungary, PCR-positive results were observed in roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*). The BoHV-1 prevalence ranged between 12 to 47% (30). A similar situation was observed in the German national parks (19, 20). A lower prevalence detected in wild fallow deer, roe deer, chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) was observed in Central Italy (12, 23), France (5, 69), Norway (37) and Switzerland (22). The seropositivity of live-trapped pronghorns (*Antilocapra americana*) to BoHV-1 was reported in Idaho (Stauber et al., 1980), in Alberta and Saskatchewan (26%) (4) but these species were seronegative ten years later (34). Moose (*Alces alces*) were detected seropositive to BoHV-1 in Alberta (14%) (Zarnke and Yuill, 1981) while negative in Norway (37).

Although the high occurrence of antibodies in some ruminant species, the evidence that they are infected with BoHV-1 is questionable because experimental infection or reactivation failed to isolate the virus or reproduce the disease. As for example, the intrapreputial inoculation of BoHV-1 in the common eland was unable to develop balanoposthitis or to mount an immune response (39). A similar attempt to induce a respiratory tract disease in white-tailed deer by intranasal inoculation of BoHV-1 was unsuccessful (13). Nevertheless, these two species have been found seropositive to BoHV-1.

### **The factors responsible of bovine herpesvirus 1 transmission between cattle and the other ruminant species**

In absence of severe IBR like diseases, the BoHV-1 transmission between cattle and the other ruminant species has been very seldomly studied (26, 41). Contrarily, several studies aimed at identifying the risk factors of BoHV-1 transmission between cattle. These latter have to be taken into account in the BoHV-1 transmission either from or to the other ruminant species. Some of them are well characterised such as age, sex (males are more

**Table 1.** Ruminant species (27, 46) serologically positive to bovine herpesvirus 1 (BoHV-1)

Family	Subfamily	Species	BoHV-1 infection	Geographical distribution
<i>Antilocapridae</i>	<i>Antilocaprinae</i>	Pronghorn ( <i>Antilocapra americana</i> )	nd	America
<i>Bovidae</i>	<i>Aepycerotinae</i>	Impala ( <i>Aepyceros melampus</i> )	nd	Africa
		<i>Alcelaphinae</i>	Hartebeest ( <i>Alcelaphus buselaphus</i> )	nd
		Black wildebeest ( <i>Connochaetes gnou</i> )	nd	Africa
		Blue wildebeest ( <i>Connochaetes taurinus</i> )	Natural	Africa
		Topi ( <i>Damaliscus korrigum</i> )	nd	Africa
		Tsessebe ( <i>Damaliscus lunatus</i> )	nd	Africa
		Blesbok ( <i>Damaliscus pygargus</i> )	nd	Africa
	<i>Antilopinae</i>	Springbok ( <i>Antidorcas marsupialis</i> )	nd	Zoo (USA)
		Blackbuck ( <i>Antilope cervicapra</i> )	nd	Zoo (USA)
		Thomson's gazelle ( <i>Gazella thomsoni</i> )	nd	Africa
	<i>Bovinae</i>	American bison ( <i>Bison bison</i> )	nd	America
		European bison ( <i>Bison bonasus</i> )	Natural	Europe
		Domestic cattle ( <i>Bos taurus</i> ) *	Natural	Africa, America, Asia, Europe, Oceania
		Water buffalo ( <i>Bubalus bubalis</i> ) *	Natural	Asia, Europe, Oceania
		African buffalo ( <i>Syncerus caffer</i> )	nd	Africa
		Nyala ( <i>Tragelaphus angasii</i> )	nd	Africa
		Greater kudu ( <i>Tragelaphus strepsiceros</i> )	nd	Africa
		Bushbuck ( <i>Tragelaphus scriptus</i> )	nd	Africa
		Common eland ( <i>Taurotragus oryx</i> )	nd	Africa
	<i>Caprinae</i>	Domestic goat ( <i>Capra hircus</i> ) *	Natural	America, Europe, Oceania
		Ibex ( <i>Capra ibex</i> )	nd	Europe
		Domestic sheep ( <i>Ovis aries</i> )	Natural	America, Europe
		Bighorn sheep ( <i>Ovis canadensis</i> )	Natural	America
		Mouflon ( <i>Ovis musimon</i> )	nd	Europe
		Chamois ( <i>Rupicapra rupicapra</i> )	nd	Europe
	<i>Hippotraginae</i>	Addax ( <i>Addax nasomaculatus</i> )	nd	Zoo (USA)
		Roan antelope ( <i>Hippotragus equinus</i> )	nd	Africa
	Sable antelope ( <i>Hippotragus niger</i> )	nd	Africa	
	Gemsbok ( <i>Oryx gazella</i> )	nd	Africa	
<i>Reduncinae</i>	Waterbuck ( <i>Kobus ellipsiprymnus</i> )	nd	Africa	
	Kob ( <i>Kobus kob</i> )	nd	Africa	
	Lechwe ( <i>Kobus leche</i> )	nd	Africa	
	Southern reedbuck ( <i>Redunca arundinum</i> )	nd	Africa	
	Bohar reedbuck ( <i>Redunca redunca</i> )	nd	Africa	
<i>Cervidae</i>	<i>Capreolinae</i>	Moose ( <i>Alces alces</i> )	nd	America
	Roe deer ( <i>Capreolus capreolus</i> )	nd	Europe	
	White-tailed deer ( <i>Odocoileus virginianus</i> )	nd	America, Europe	
	Mule deer ( <i>Odocoileus hemionus</i> )	Experimental	Europe	
	Caribou ( <i>Rangifer tarandus caribou</i> )	nd	America	
	Reindeer ( <i>Rangifer tarandus tarandus</i> ) *	Experimental	Europe	
	<i>Cervinae</i>	Red deer ( <i>Cervus elaphus</i> ) *	Experimental	Europe, Oceania
		Elk ( <i>Cervus canadensis</i> ) *	nd	America
		Fallow deer ( <i>Dama dama</i> )	nd	Europe
<i>Giraffidae</i>	<i>Giraffa</i>	Giraffe ( <i>Giraffa camelopardalis</i> )	nd	Africa

\* Species also infected with a ruminant alphaherpesvirus related to BoHV-1 ; nd: not determined

frequently positive than females) and herd size (6, 62). Direct animal contacts, such as purchase of cattle, participation in cattle shows were also found to be important risk factors for the introduction of BoHV-1 (81, 82, 83). Other factors such as farm or cattle density may increase the risk of BoHV-1 introduction (85). The latency reactivation cycle has also a deep epidemiological impact since it is responsible for the maintenance of BoHV-1 into a cattle population. BoHV-1 infection of new generation cattle by latent carriers submitted to reactivation stimulus may occur at several occasions as for example at delivery (67), at mating, during transport (68) or following the introduction of heifers into the group of dairy cows (45).

The share of ecological niches with other ruminant species is the main factor that could contribute to BoHV-1 transmission between cattle and heterologous species. Direct or indirect

contact between BoHV-1 heterologous hosts and livestock is likely required and can occur in shared pastures, water holes and feeders. Moreover, the current development of breeding of ruminants taken from wild fauna has to be taken into account. Indeed, the increase of density in farmed animals could allow the emergence of epidemic BoHV-1 diseases whereas they should be sporadic in natural conditions and go unnoticed. The proximity with domestic animals enhances the risk of BoHV-1 transmission to recently introduced animals.

Once a heterologous species becomes infected, BoHV-1 can persist in a population until latent carrier animals died or have been taken out of that population. Recently, such transmission in red deer has been quantified under experimental conditions based on the basic reproduction ratio (R0) (41). This latter parameter describes infection dynamics in a population and is defined as the

average number of secondary cases generated by one primary case in a fully susceptible population of defined density (45). In a dairy cattle herd, R0 was estimated to be at least 7 (25). For red deer, the “worst case scenario” was discussed and the R0 was estimated to 1.2. The authors concluded that most likely red deer will be a satellite group for BoHV-1 because BoHV-1 will not survive longer than a few decades (several times the mean deer lifetime) in red deer populations (41).

### **The prevention and the monitoring of bovine herpesvirus 1 in the other ruminant species**

In the context of IBR control and eradication, some European countries have applied a strategy named “Differentiation of Infected from Vaccinated Animals” (84). BoHV-1 marker vaccines carrying a deletion of the gE gene were developed to prevent BoHV-1 infection. The use of these vaccines together with a serological detection of gE-specific antibodies in a BoHV-1 gE blocking ELISA allows the discrimination between infected and vaccinated animals (45). In regards to these developments and the close antigenic and genetic relationships between BoHV-1 and related viruses, these methods have been assessed in ruminant species against their specific viruses (73, 76, 77).

Thus, goats are protected against CpHV-1 infection by the intranasal administration of a live attenuated BoHV-1 gE-negative vaccine (73, 76). Such heterologous vaccination through the species barrier with an already licensed vaccine meets the “cascade” principle and the concept of “minor use - minor species”. A classical CpHV-1 inactivated vaccine confers also a good clinical protection (8, 66) but has very little chance to be marketed due to the poor interest from the veterinary pharmaceutical industry in the development of vaccines for minor species like goats. Similarly, buffaloes are widely vaccinated with BoHV-1 vaccine since it is demonstrated that BoHV-1 or a related virus is highly prevalent in buffalo livestock (Cuteri, personal communication). In the same way, it is possible to take advantage of a previous immunisation against BoHV-1 to afford a cross-protection against BoHV-5 (9, 14). However, such vaccination has to be only used when endemic situation is observed or when economical losses are induced. The efficacy of such method has to be assessed in red deer against CvHV-1 and in reindeer against CvHV-2 because they are both susceptible to BoHV-1. In another way, sheep could be also protected against: BoHV-1, a single injection of DNA plasmid encoding a truncated form of BoHV-1 gD alone or fused to bovine CD154 being able to induce protective immune responses (21, 38, 80).

Following a similar reasoning, BoHV-1 blocking ELISA have been tested in goats to discriminate BoHV-1 from a related alphaherpesvirus infection (77). The analysis of 2,548 serums in BoHV-1 gB blocking ELISA revealed that a ruminant alphaherpesvirus infection was spreading in Corsica while no goat was detected positive in continental France. Taking into account the results obtained in continental France, a specificity of 100% has been measured for the BoHV-1 gB blocking ELISA. By testing serums from goats experimentally infected with CpHV-1, the sensibility has been calculated to 93.5%. The analysis with a BoHV-1 gE blocking ELISA showed that 22.6% of gB-seropositive serums were also gE-seropositive. The BoHV-1 gE blocking ELISA was therefore not able to differentiate between BoHV-1 and CpHV-1 infections. However, the analysis of serums in a cross-seroneutralisation has strongly demonstrated that goat serum samples neutralised to a greater extent CpHV-1 than BoHV-1 (77). Such method was also successfully applied in cattle to detect and discriminate BoHV-1 from a BoHV-5 infection (87) but was validated only on a very few number of serums.

Besides that, few methods are available to distinguish BoHV-1 from its related ruminant alphaherpesviruses. A consensus PCR is able to detect bovine, caprine, red deer and reindeer alphaherpesviruses (57). The speed and the efficiency of this PCR on field samples were recently demonstrated by the

detection and the identification of the CvHV-1 Anlier strain in Belgium (75). Another diagnostic method that can be applied is an immunofluorescence assay using specific monoclonal antibodies to each ruminant alphaherpesvirus related to BoHV-1 (33). At a genomic level, a clear differentiation between each related virus can be afforded by restriction enzyme analysis (75). However, such methods are often time consuming and currently the use of BoHV-1 gB blocking ELISA should be the best serological tool to detect such infections in the other ruminant species. The full discrimination with BoHV-1 can be further afforded by cross-seroneutralisation.

### **CONCLUSIONS**

Until now, no severe epidemic resulting from a BoHV-1 infection was identified in ruminant species other than bovine. Nevertheless, several domestic and free ranging ruminants are susceptible to BoHV-1 which could be presumably responsible for sporadic diseases. Such infections, if they are present, have low clinical consequences but their epidemiologic role could be important. That could contribute to maintain BoHV-1 in a specific region and within a wild ruminant population that could become a virus reservoir for domestic cattle.

In this context, the risk of a BoHV-1 cross-infection in ruminant species has to be assessed. Thus, attention has to be paid regarding animal species proven susceptible to BoHV-1, ruminant alphaherpesviruses related to BoHV-1, prevalence of infections and rate of contacts between species. The species susceptibility assessment has to take into account excretion after primary infection, latent persistence, re-excretion after reactivation episode and serological response. That is of interest especially for buffaloes, bighorn sheep, blue wildebeest, goats, mule deer, sheep, red deer and reindeer that were demonstrated susceptible to BoHV-1. For the other ruminant species, the risk is evaluated through the prevalence rate of BoHV-1 infection because these animals are mainly free-ranging. Within factors responsible of BoHV-1 transmission between cattle and other ruminants, the direct or indirect contacts appear to be the most important. Goats and sheep usually share same pastures with cattle and contacts are likely to occur. The contact between farmed deer and cattle seems to be restricted but could emerge only if they are close to each other. The lowest rates concern free-ranging animals. However, the current development of breeding of ruminants taken from wild fauna could be a new issue. Indeed, the increase of farmed animal density allows the emergence of epidemic diseases whereas they should be sporadic in natural conditions and go unnoticed. Moreover, the proximity with domestic animals enhances the risk of transmission of these viruses to recently introduced animals.

The presence of / or freedom from related alphaherpesvirus infection in a given ruminant species has also to be taken into account because it could be responsible for IBR misdiagnosis. In this context, the distribution of ruminant alphaherpesviruses has to be studied in more details especially in wild ruminant populations. The use of BoHV-1 gB blocking ELISA is currently the best serological tool to detect such infections. Nevertheless, it is still of interest to study the antigenic relationship between BoHV-1 and the related ruminant alphaherpesviruses in order to develop new serological methods able to clearly discriminate these infections. Besides the monitoring, the prevention of infection by control measures and vaccination in these minor ruminant species is an important animal health concern and could contribute to an efficient control of BoHV-1 infections.

### **REFERENCES**

1. ACKERMANN, M. – METZLER, A. E. – McDONOUGH, H. – BRUCKNER, L. – MULLER, H. K. – KIHM, U.: Stellen nicht-bovine paarhufer ein IBR-Virus-reservoir dar? I. BHV-1 und CapHV-1 infektions und reaktivierungsversuche an ziegen, virustyp-spezifitat der humoralen antikörper und

- charakterisierung der viralen antigene. Schweiz. Arch. Tierheilkd., 1986. 128. 557-573.
2. ANDERSON, E. C. – ROWE, L. W.: The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, rift valley fever, ephemeral fever and bluetongue and to *Leptospira* sp in free-ranging wildlife in Zimbabwe. Epidemiol. Infect., 1998. 121. 441-449.
  3. BARNARD, B. J.: Antibodies against some viruses of domestic animals in southern African wild animals. Onderstepoort J. Vet. Res., 1997. 64. 95-110.
  4. BARRETT, M. W. – CHALMERS, G. A.: A serologic survey of pronghorns in Alberta and Saskatchewan, 1970-1972. J. Wildl. Dis., 1975. 11. 157-163.
  5. BLANCOU, J.: Serologic testing of wild roe deer (*Capreolus capreolus* L.) from the Trois Fontaines forest region of eastern France. J. Wildl. Dis., 1983. 19. 271-273.
  6. BOELAERT, F. – SPEYBROECK, N. – DE KRUIF, A. – AERTS, M. – BURZYKOWSKI, T. – MOLENBERGHS, G. – BERKVEN, D. L.: Risk factors for bovine herpesvirus-1 seropositivity. Prev. Vet. Med., 2005. 69. 285-295.
  7. BORCHERS, K. – BRACKMANN, J. – WOLF, O. – RUDOLPH, M. – GLATZEL, P. – KRASINSKA, M. – KRASINSKI, Z. A. – FRÖLICH, K.: Virologic investigations of free-living European bison (*Bison bonasus*) from the Bialowieza Primeval Forest, Poland. J. Wildl. Dis., 2002. 38. 533-538.
  8. CAMERO, M. – BELLACICCO, A. L. – TARSIANO, E. – DECARO, N. – MARTELLA, V. – TEMPESTA, M. – BUONAVOGLIA, C.: Intravaginal administration of an inactivated vaccine prevents lesions induced by caprine herpesvirus-1 in goats. Vaccine, 2007. 25. 1658-1661.
  9. CASCIO, K. E. – BELKNAP, E. B. – SCHULTHEISS, P. C. – AMES, A. D. – COLLINS, J. K.: Encephalitis induced by bovine herpesvirus 5 and protection by prior vaccination or infection with bovine herpesvirus 1. J. Vet. Diagn. Invest., 1999. 11. 134-139.
  10. CHOW, T. L. – DAVIS, R. W.: The susceptibility of mule deer to infectious bovine rhinotracheitis. Am. J. Vet. Res., 1964. 25. 518-519.
  11. CLARK, R. K. – WHETSTONE, C. A. – CASTRO, A. E. – JORGENSEN, M. M. – JENSEN, J. F. – JESSUP, D. A.: Restriction endonuclease analysis of herpesviruses isolated from two peninsular bighorn sheep (*Ovis canadensis cremnobates*). J. Wildl. Dis., 1993. 29. 50-56.
  12. CUTERI, V. – DIVERIO, S. – CARNIELETTO, P. – TURILLI, C. – VALENTE, C.: Serological survey for antibodies against selected infectious agents among fallow deer (*Dama dama*) in central Italy. Zentralbl. Veterinarmed. B., 1999. 46. 545-549.
  13. DAVIS, J. W. – KARSTAD, L. H. – TRAINER, D. O.: Infectious diseases of wild mammals. Iowa State University Press. Ames, 1973. 167-168.
  14. DEL MÉDICO ZAJAC, M. P. – PUNTEL, M. – ZAMORANO, P. I. – SADIR, A. M. – ROMERA, S. A.: BHV-1 vaccine induces cross-protection against BHV-5 disease in cattle. Res. Vet. Sci., 2006. 81. 327-334.
  15. DOYLE, L. G. – HEUSCHELE, W. P.: Prevalence of antibody to bovine herpesvirus 1 in wild ruminants captive in United States zoos. J. Am. Vet. Med. Assoc., 1983. 183. 1255-1256.
  16. EK-KOMMONEN, C. – VEIJALAINEN, P. – RANTALA, M. – NEUVONEN, E.: Neutralizing antibodies to bovine herpesvirus 1 in reindeer. Acta Vet. Scand., 1982. 23. 565-569.
  17. EK-KOMMONEN, C. – PELKONEN, S. – NETTLETON, P. F.: Isolation of a herpesvirus serologically related to bovine herpesvirus 1 from a reindeer (*Rangifer tarandus*). Acta Vet. Scand., 1986. 27. 299-301.
  18. ENGELS, M. – PALATINI, M. – METZLER, A. E. – PROBST, U. – KIHM, U. – ACKERMANN, M.: Interactions of bovine and caprine herpesviruses with the natural and the foreign hosts. Vet. Microbiol., 1992. 33. 69-78.
  19. FRÖLICH, K.: Seroepizootiologic investigations of herpesviruses in free-ranging and captive deer (cervidae) in Germany. J. Zoo. Wildl. Med., 1996. 27. 241-247.
  20. FRÖLICH, K. – HAMBLIN, C. – PARIDA, S. – TUPPURAINEN, E. – SCHETTLER, E.: Serological survey for potential disease agents of free-ranging cervids in six selected national parks from Germany. J. Wildl. Dis., 2006. 42. 836-843.
  21. GERDTS, V. – SNIDER, M. – BROWNLIE, R. – BABIUK, L. A. – GRIEBEL, P. J.: Oral DNA vaccination in utero induces mucosal immunity and immune memory in the neonate. J. Immunol., 2002. 168. 1877-1885.
  22. GIACOMETTI, M. – TOLARI, F. – MANNELLI, A. – LANFRANCHI, P.: Seroepidemiologic investigations in the Alpine ibex (*Capra i. ibex*) of Piz Albris in the canton of Grigioni (Switzerland). Schweiz Arch. Tierheilkd., 1995. 137. 537-542.
  23. GIOVANNINI, A. – CANCELLOTTI, F. M. – TURILLI, C. – RANDI, E.: Serological investigations for some bacterial and viral pathogens in fallow deer (*Cervus dama*) and wild boar (*Sus scrofa*) of the San Rossore Preserve, Tuscany, Italy. J. Wildl. Dis., 1988. 24. 127-132.
  24. GIULIANI, S. – SHARMA, R.: Experimental infection of lambs with bovine herpesvirus type-1 (BHV-1). Int. J. Anim. Sci., 1995. 10. 73-75.
  25. HAGE, J. J. – SCHUKKEN, Y. H. – BARKEMA, H. W. – BENEDICTUS, G. – RIJSEWIJK, F. A. – WENTINK, G. H.: Population dynamics of bovine herpesvirus 1 infection in a dairy herd. Vet. Microbiol., 1996. 53. 169-180.
  26. HAGE, J. J. – VELLEMA, P. – SCHUKKEN, Y. H. – BARKEMA, H. W. – RIJSEWIJK, F. A. – VAN OIRSCHOT, J. T. – WENTINK, G. H.: Sheep do not have a major role in bovine herpesvirus 1 transmission. Vet. Microbiol., 1997. 57. 41-54.
  27. HERNÁNDEZ FERNÁNDEZ, M. – VRBA, E. S.: A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. Biol. Rev. Camb. Philos. Soc., 2005. 80. 269-302.
  28. IBRAHIM, A. – SAW, S. P. – FATIMAH, I. – SAHAREE, A. A.: Isolation of infectious bovine rhinotracheitis virus from buffalo in Malaysia. Vet. Rec., 1983. 112. 303-304.
  29. INGEBRIGTSEN, D.K. – LUDWIG, J. R. – MCCLURKIN, A. W.: Occurrence of antibodies to the etiologic agents of infectious bovine rhinotracheitis, parainfluenza 3, leptospirosis, and brucellosis in white-tailed deer in Minnesota. J. Wildl. Dis., 1986. 22. 83-86.
  30. KALMAN, D. – EGYED, L.: PCR detection of bovine herpesviruses from nonbovine ruminants in Hungary. J. Wildl. Dis., 2005. 41. 482-488.
  31. KAMINJOLO, J. S. – PAULSEN, J.: The occurrence of virus-neutralizing antibodies to infectious bovine rhinotracheitis virus in sera from hippopotami and buffaloes. Zentralbl. Veterinarmed. B., 1970. 17. 864-868.
  32. KARSTAD, L. – JESSETT, D. M. – OTEMA, J. C. – DREVEMO, S.: Vulvovaginitis in wildebeest caused by the virus of infectious bovine rhinotracheitis. J. Wildl. Dis., 1974. 10. 392-396.
  33. KEUSER, V. – SCHYNTS, F. – DETRY, B. – COLLARD, A. – ROBERT, B. – VANDERPLASSCHEN, A. – PASTORET, P. P. – THIRY, E.: Improved antigenic methods for differential diagnosis of bovine, caprine, and cervine alphaherpesviruses related to bovine herpesvirus 1. J. Clin. Microbiol., 2004. 42. 1228-1235.
  34. KINGSCOTE, B. F. – BOHAC, J. G.: Antibodies to bovine bacterial and viral pathogens in pronghorns in Alberta, 1983. J. Wildl. Dis., 1986. 22. 511-514.
  35. LAMONTAGNE, L. – SADI, L. – JOYAL, R.: Serological evidence of bovine herpesvirus 1-related virus infection in the white-tailed deer population on Anticosti Island, Quebec. J. Wildl. Dis., 1989. 25. 202-205.
  36. LEHMKUHL, H. D. – CUTLIP, R. C.: Protection from parainfluenza-3 virus and persistence of infectious bovine

- rhinotracheitis virus in sheep vaccinated with a modified live IBR-PI-3 vaccine. *Can. J. Comp. Med.*, 1985. *49*. 58-62.
37. LILLEHAUG, A. – VIKØREN, T. – LARSEN, I. L. – AKERSTEDT, J. – THARALDSEN, J. – HANDELAND, K.: Antibodies to ruminant alpha-herpesviruses and pestiviruses in Norwegian cervids. *J. Wildl. Dis.*, 2003. *39*. 779-786.
  38. MANOJ, S. – GRIEBEL, P. J. – BABIUK, L. A. – VAN DRUNEN LITTEL-VAN DEN HURK, S.: Targeting with bovine CD154 enhances humoral immune responses induced by a DNA vaccine in sheep. *J. Immunol.*, 2003. *170*. 989-996.
  39. MARÉ, C. J.: Susceptibility of the common eland to infectious bovine rhinotracheitis virus. *J. Am. Vet. Med. Assoc.*, 1971. *159*. 614-616.
  40. MOHANTY, S. B. – LILLIE, M. G. – CORSELIUS, N. P. – BECK, J. D.: Natural infection with infectious bovine rhinotracheitis virus in goats. *J. Am. Vet. Med. Assoc.*, 1972. *160*. 879-880.
  41. MOLLEMA, L. – RIJSEWIJK, F. A. M. – NODELIJK, G. – DE JONG, M. C. M.: Quantification of the transmission of bovine herpesvirus 1 among red deer (*Cervus elaphus*) under experimental conditions. *Vet. Microbiol.*, 2005. *111*. 25-34.
  42. MULLER, T. – KRAMER, M. – BEIER, D.: Untersuchungen zum Vorkommen von Antikörpern gegen ausgewählte bovine und ovine Viruserkrankungen bei Reh- (*Capreolus capreolus*), Rot- (*Cervus elaphus*), Dam- (*Dama dama*) und Muffelwild (*Ovis musimon*) im Bundesland Brandenburg. *Zeitschrift Für Jagdwissenschaft*, 1997. *43*. 166-175.
  43. MUSHI, E. Z. – KARSTAD, L.: Experimental infection of wildebeest with the herpesvirus of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis. *J. Wildl. Dis.*, 1979. *15*. 579-583.
  44. MUSHI, E. Z. – KARSTAD, L. – JESSETT, D. M. – ROSSITER, P. B.: Observations on the epidemiology of the herpesvirus of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis in wildebeest. *J. Wildl. Dis.*, 1979. *15*. 481-487.
  45. MUYLKENS, B. – THIRY, J. – KIRTEN, P. – SCHYNTS, F. – THIRY, E.: Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. *Vet. Res.*, 2007. *38*. 181-209.
  46. MYERS, P. – ESPINOSA, R. – PARR, C. S. – JONES, T. – HAMMOND, G. S. – DEWEY, T. A.: The Animal Diversity Web. Accessed February 15, 2008, at <http://animaldiversity.org>.
  47. NETTLETON, P. F. – THIRY, E. – REID, H. W. – PASTORET, P. P.: Herpesvirus infections in cervidae. *Rev. Sci. Tech. Off. Int. Epiz.*, 1988. *7*. 977-988.
  48. NETTLETON, P. F. – EK-KOMMONEN, C. – TANSKANEN, R. – REID, H. W. – SINCLAIR, J. A. – HERRING, J. A.: Studies in the epidemiology and pathogenesis of alphaherpesviruses from red deer (*cervus elaphus*) and reindeer (*rangifer tarandus*). In: REID, H. W. (ed): The management and Health of Farmed deer. Kluwer academic publishers. Dordrecht, 1988. 143-147.
  49. PETIT, E.: Etude sur la procédure « résultats aberrants » I.B.R. proposée par l'A.C.E.R.S.A. *Epidémiol. Santé Anim.*, 2002. *42*. 133-150.
  50. PIRAK, M. – THIRY, E. – BROCHIER, B. – PASTORET, P. P.: Infection expérimentale de la chèvre par le virus de la rhinotrachéite infectieuse bovine (Bovine herpesvirus type 1) et tentative de réactivation virale. *Rec. Méd. Vét.*, 1983. *1103-1106*.
  51. POSPISIL, Z. – VYVLECKA, R. – CIHAL, P. – LANY, P. – ZENDULKOVA, D.: Detection of herpesvirus serum antibodies in red deer (*Cervus elaphus*) imported into the Czech Republic. *Vet. Med.*, 1996. *41*. 279-282.
  52. RAMPTON, C. S. – JESSETT, D. M.: The prevalence of antibody to infectious bovine rhinotracheitis virus in some game animals of East Africa. *J. Wildl. Dis.*, 1976. *12*. 2-6.
  53. REBHUN, W. C. – JENKINS, D. H. – RIIS, R. C. – DILL, S. G. – DUBOVI, E. J. – TORRES, A.: An epizootic of blindness and encephalitis associated with a herpesvirus indistinguishable from equine herpesvirus I in a herd of alpacas and llamas. *J. Am. Vet. Med. Assoc.*, 1988. *192*. 953-956.
  54. REID, H. W. – NETTLETON, P. F. – POW, I. – SINCLAIR, J. A.: Experimental infection of red deer (*Cervus elaphus*) and cattle with a herpesvirus isolated from red deer. *Vet. Rec.*, 1986. *119*. 156-158.
  55. RWEYEMAMU, M. M.: Probable occurrence of infectious bovine rhinotracheitis virus in Tanzania in wildlife and cattle. *Nature*, 1970. *225*. 738-739.
  56. RWEYEMAMU, M. M.: The incidence of infectious bovine rhinotracheitis antibody in Tanzanian game animals and cattle. *Bull. Epizoot. Dis. Afr.*, 1974. *22*. 19-22.
  57. ROS, C. – BELAK, S.: Studies of genetic relationships between bovine, caprine, cervine, and rangiferine alphaherpesviruses and improved molecular methods for virus detection and identification. *J. Clin. Microbiol.*, 1999. *37*. 1247-1253.
  58. SADI, L. – JOYAL, R. – ST-GEORGES, M. – LAMONTAGNE, L.: Serologic survey of white-tailed deer on Anticosti Island, Quebec for bovine herpesvirus 1, bovine viral diarrhoea, and parainfluenza 3. *J. Wildl. Dis.*, 1991. *27*. 569-577.
  59. SAUSKER, E. A. – DYER, N. W.: Seroprevalence of OHV-2, BVDV, BHV-1, and BRSV in ranch-raised bison (Bison bison). *J. Vet. Diagn. Invest.*, 2002. *14*. 68-70.
  60. SHANKAR, H. – YADAV, P.: Experimental infection of sheep with BHV-1 (IBR/IPV virus) and its possible role epizootiology. *Indian Vet. Med. J.*, 1987. *11*. 71-76.
  61. SIX, A. – BANKS, M. – ENGELS, M. – BASCUNANA, C.R. – ACKERMANN, M.: Latency and reactivation of bovine herpesvirus 1 (BHV-1) in goats and of caprine herpesvirus 1 (CapHV-1) in calves. *Arch. Virol.*, 2001. *146*. 1325-1335.
  62. SOLIS-CALDERON, J. J. – SEGURA-CORREA, V. M. – SEGURA-CORREA, J. C. – ALVARADO-ISLAS, A.: Seroprevalence of and risk factors for infectious bovine rhinotracheitis in beef cattle herds of Yucatan Mexico. *Prev. Vet. Med.*, 2003. *57*. 199-208.
  63. STAUBER, E. H. – AUTENRIETH, R. – MARKHAM, O. D. – WHITBECK, V.: A seroepidemiologic survey of three pronghorn (*Antilocapra americana*) populations in southeastern Idaho, 1975-1977. *J. Wildl. Dis.*, 1980. *16*. 109-115.
  64. ST GEORGE, T.D. – PHILPOTT, M.: Isolation of infectious bovine rhinotracheitis virus from the prepuce of water buffalo bulls in Australia. *Aust. Vet. J.*, 1972. *48*. 126.
  65. TAYLOR, S. K. – LANE, V. M. – HUNTER, D. L. – EYRE, K.G. – KAUFMAN, S. – FRYE, S. – JOHNSON, M. R.: Serologic survey for infectious pathogens in free-ranging American bison. *J. Wildl. Dis.*, 1997. *33*. 308-311.
  66. TEMPESTA, M. – CAMERO, M. – GRECO, G. – PRATELLI, A. – MARTELLA, V. – BUONAVOGLIA, C.: A classical inactivated vaccine induces protection against caprine herpesvirus 1 infection in goats. *Vaccine*, 2001. *19*. 3860-3864.
  67. THIRY, E. – SALIKI, J. – SCHWERS, A. – PASTORET, P. P.: Parturition as a stimulus of IBR virus reactivation. *Vet. Rec.*, 1985. *116*. 599-600.
  68. THIRY, E. – SALIKI, J. – BUBLLOT, M. – PASTORET, P. P.: Reactivation of infectious bovine rhinotracheitis virus by transport. *Comp. Immunol. Microbiol. Infect. Dis.*, 1987. *10*. 59-63.
  69. THIRY, E. – VERCOUTER, M. – DUBUISSON, J. – BARRAT, J. – SEPULCHRE, C. – GERARDY, C. – MEERSSCHAERT, C. – COLLIN, B. – BLANCOU, J. – PASTORET, P. P.: Serological survey of herpesvirus infections in wild ruminants of France and Belgium. *J. Wildl. Dis.*, 1988. *24*. 268-273.
  70. THIRY, E. – ACKERMANN, M. – BANKS, M. – BELAK, K. – BELAK, S. – CAMPBELL, I. – EK-KOMMONEN, C. – ENGELS,

- M. – MEYER, G. – REID, H. – ROS, C. – SIX, A.: Risk evaluation of cross-infection of cattle with ruminant alphaherpesviruses related to bovine herpesvirus type 1. 3. Internationales Symposium zur BHV-1-/BVD-Bekämpfung, Stendal, Germany. 2001. 99-104.
71. THIRY, E. – LEMAIRE, M. – THIRY, J. – MUYLKENS, B.: Certification: comment s'assurer de l'introduction d'un animal indemne dans un troupeau? Exemple de l'IBR. In: Brard, C. – Camuset, P. (eds): Pathologie infectieuse. Journées nationales des GTV, Nantes, France. 2007. 925-930.
  72. THIRY, J. – KEUSER, V. – MUYLKENS, B. – MEURENS, F. – GOGEV, S. – VANDERPLASSCHEN, A. – THIRY, E.: Ruminant alphaherpesviruses related to bovine herpesvirus 1. *Vet. Res.*, 2006. *37*. 169-190.
  73. THIRY, J. – TEMPESTA, M. – CAMERO, M. – TARSITANO, E. – BELLACICCO, A. L. – THIRY, E. – BUONAVOGLIA, C.: A live attenuated glycoprotein E negative bovine herpesvirus 1 vaccine induces a partial cross-protection against caprine herpesvirus 1 infection in goats. *Vet. Microbiol.*, 2006. *113*. 303-308.
  74. THIRY, J. – KEUSER, V. – SCHYNTS, F. – CHARTIER, C. – TEMPESTA, M. – ESPEJO-SERRANO, J. – SAEGERMAN, C. – THIRY, E.: Evaluation de la prévalence sérologique de l'infection à herpesvirus caprin 1 dans le sud-ouest de l'Europe. *Epidémiol. Santé Anim.*, 2006. *49*. 55-58.
  75. THIRY, J. – WIDÉN, F. – GRÉGOIRE, F. – LINDEN, A. – BELÁK, S. – THIRY, E.: Isolation and characterisation of a ruminant alphaherpesvirus closely related to bovine herpesvirus 1 in a free-ranging red deer. *BMC. Vet. Res.*, 2007. *3*. 26.
  76. THIRY, J. – TEMPESTA, M. – CAMERO, M. – TARSITANO, E. – MUYLKENS, B. – MEURENS, F. – THIRY, E. – BUONAVOGLIA, C.: Clinical protection against caprine herpesvirus 1 genital infection by intranasal administration of a live attenuated glycoprotein E negative bovine herpesvirus 1 vaccine. *BMC. Vet. Res.*, 2007. *3*. 33.
  77. THIRY, J. – SAEGERMAN, C. – CHARTIER, C. – MERCIER, P. – KEUSER, V. – THIRY, E.: Serological evidence of caprine herpesvirus 1 infection in Mediterranean France. *Vet. Microbiol.*, 2008. *128*. 261-268.
  78. TOLARI, F. – WHITE, H. – NIXON, P.: Isolation and reactivation of bovid herpesvirus 1 in goats. *Microbiologica*, 1990. *13*. 67-71.
  79. TRUEBLOOD, M. S. – SWIFT, B. L. – MCHOLLAND-RAYMOND, L.: A bovine herpesvirus isolated from sheep. *Can. J. Comp. Med.*, 1978. *42*. 97-99.
  80. VAN DRUNEN LITTEL – VAN DEN HURK, S.: Rationale and perspectives on the success of vaccination against bovine herpesvirus-1. *Vet. Microbiol.*, 2006. *113*. 275-282.
  81. VAN SCHAİK, G.: Risk and economics of disease introduction to dairy farms. *Tijdschr. Diergeneesk.*, 2001. *126*. 414-418.
  82. VAN SCHAİK, G. – SCHUKKEN, Y. H. – NIELEN, M. – DIJKHUIZEN, A. A. – BENEDICTUS, G.: Risk factors for introduction of BHV1 into BHV1-free Dutch dairy farms: a case-control study. *Vet. Q.*, 2001. **23**. 71-76.
  83. VAN SCHAİK, G. – SCHUKKEN, Y. H. – NIELEN, M. – DIJKHUIZEN, A. A. – BARKEMA, H. W. – BENEDICTUS, G.: Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. *Prev. Vet. Med.*, 2002. *54*. 279-289.
  84. VANNIER, P. – CAPUA, I. – LE POTIER, M. F. – MACKAY, D. K. – MUYLKENS, B. – PARIDA, S. – PATON, D. J. – THIRY, E.: Marker vaccines and the impact of their use on diagnosis and prophylactic measures. *Rev. Sci. Tech.*, 2007. *26*. 351-372.
  85. VONK NOORDEGRAAF, A. – LABROVIC, A. – FRANKENA, K. – PFEIFFER, D. U. – NIELEN, M.: Simulated hazards of losing infection-free status in a Dutch BHV1 model. *Prev. Vet. Med.*, 2004. *62*. 51-58.
  86. WAFULA, J. S. – MUSHI, E. Z. – WAMWAYI, H.: Reaction of goats to infection with infectious bovine rhinotracheitis virus. *Res. Vet. Sci.*, 1985. *39*. 84-86.
  87. WELLENBERG, G. J. – MARS, M. H. – VAN OIRSCHOT, J. T.: Antibodies against bovine herpesvirus (BHV) 5 may be differentiated from antibodies against BHV1 in a BHV1 glycoprotein E blocking ELISA. *Vet. Microbiol.*, 2001. *78*. 79-84.
  88. WHETSTONE, C. A. – EVERMANN, J. F.: Characterization of bovine herpesviruses isolated from six sheep and four goats by restriction endonuclease analysis and radioimmunoprecipitation. *Am. J. Vet. Res.*, 1988. *49*. 781-785.
  89. WITTMANN, G.: Aujeszky's disease in ruminants. In: WITTMANN G. (ed.): Herpesvirus diseases of cattle, horses, and pigs. Developments in veterinary virology. Kluwer academic publishers. Boston, 1989. 163-175.
  90. ZARNKE, R. L. – YUILL, T. M.: Serologic survey for selected microbial agents in mammals from Alberta, 1976. *J. Wildl. Dis.*, 1981. *17*. 453-461.

## Neospora caninum – new insights into the new parasite

Alexander J. Trees

*Veterinary Parasitology, Liverpool School of Tropical Medicine and Faculty of Veterinary Science, University of Liverpool, Pembroke Place, Liverpool, L3 5QA*  
 Email: trees@liverpool.ac.uk

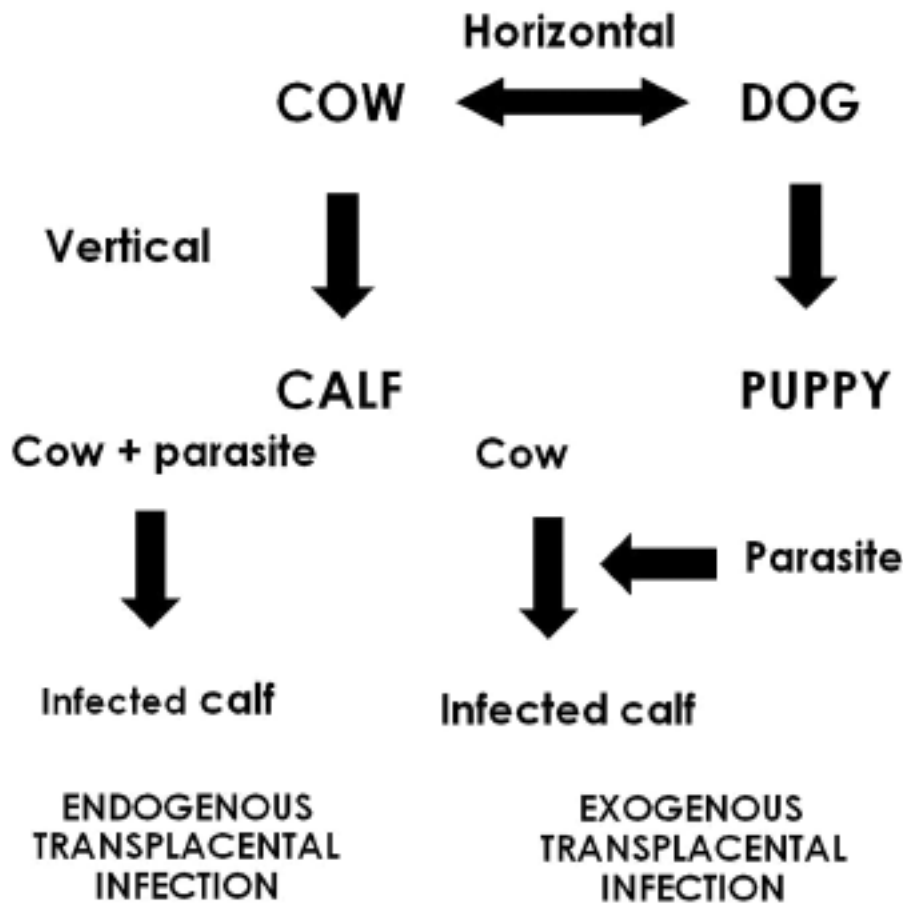
### ABSTRACT

This review highlights some of the recent advances in the understanding of bovine neosporosis which have particular relevance to its epidemiology and control. With respect to transmission, the distinction between endogenous and exogenous transplacental infection and its implications is discussed; the clinical consequences of oocyst infection revealed by experimentation are described; and other possible

routes of infection are considered. Recent experiments looking at immunological events at the materno-foetal interface and their implications with respect to the pathogenesis of abortion are described. Finally, the prospects for vaccination and the relative importance of various control options are considered.

### KEY WORDS

Neospora caninum; bovine; transmission; control.



**Figure 1.** Schematic life cycle of *Neospora caninum*

**Figure 2.** Vertical transmission of *N. caninum* in cattle can occur in two ways. These have very different implications for control

## INTRODUCTION

There has been a substantial amount of research on *Neospora caninum* in the last 15 or so years since it was first recognised as a cause of abortion in cattle. Many of the key facts around its biology and transmission were elucidated in the early studies, especially by scientists working at the University of California, Davis, and subsequent research has confirmed and extended those findings which included experimental and epidemiological evidence that *N. caninum* causes abortion, estimates of risk factors and economic effects, and the seminal demonstration that vertical transmission is extremely common and efficient and can occur generation to generation. A further and later key discovery was the demonstration that the dog is a definitive host and sheds oocysts of *N. caninum* in its faeces after ingestion of infected tissue (25). An extremely comprehensive and useful review has recently covered all aspects of the epidemiology and control of neosporosis (8). This paper, aims to consider some of the recent insights which have developed or are developing as a result of research and which influence our understanding of the epidemiology, immunology and biology of *N. caninum* in cattle and which particularly inform practical approaches to control.

### Transmission and all that

A schematic representation of the life-cycle of *N. caninum* is shown in *Figure 1*. However, the vertical transplacental transmission which is such a feature of bovine infection occurs in two ways, which have been called endogenous and exogenous transplacental infection (TPI) to respectively describe foetal infection due to recrudescence of a persistent maternal infection or de novo infection of the dam in pregnancy *Figure 2* (42). This distinction is helpful because the control strategies are very different in each case and there are also

significant implications for the design and application of future vaccines.

### Endogenous transplacental infection

It is not known what provokes persistent infection to recrudescence in pregnant cows. It is tempting to ascribe some external stressor or exposure to infection or toxin as a cause, but in cows housed together under good experimental conditions, recrudescence (as indicated by an anamnestic surge in antibody level) occurs at different times through almost the entire second half of pregnancy (14). This observation argues for a subject-specific, spontaneous shift in the competence of immune surveillance associated with the physiological state in the last half of pregnancy. It is thought that this may only occur in pregnant females which were themselves infected *in utero* because post-natal experimental infection outwith pregnancy, either with tachyzoites or oocysts, does not lead to endogenous TPI in the subsequent pregnancy (27, 45). However, there is epidemiological evidence that post-natal infection may lead to chronicity and subsequent endogenous TPI (29). A key research question is to resolve this issue because it has major implications with respect to the significance of oocyst infections (see below). If recrudescence in pregnancy proves to occur mainly or only in animals which were themselves transplacentally infected, it denotes a particular type of immunological tolerance in which a full spectrum of immune responses are manifest by the subject but its competence to control a persistent infection is temporarily modulated by physiological events. This paradigm has obvious and far-reaching implications in many infections. From a more practical point of view, it underpins a primary control strategy in bovine neosporosis, that of selective breeding described below.

- **Recrudescence of persistent maternal infection (endogenous TPI)**
- **Oocysts from dogs**
  - **pre-natal exposure via dam (exogenous TPI)**
  - **post-natal**

**Figure 3.** Sources of *N. caninum* infection for cattle. In some parts of the world other canid hosts e.g. coyote may be sources of oocysts

### Sources of infection

#### *Oocysts and dogs*

A number of experiments have now confirmed that the dog is a definitive host of *N. caninum* and oocysts are shed when infected bovine tissue is fed to dogs (8). Furthermore, it has been shown experimentally that oocyst infection of pregnant cattle can result in abortion or congenital infection of healthy, full term calves (12, 27). This crucially supports the epidemiological evidence that epidemics of *Neospora*-associated abortions are due to point-source infection presumed to derive from oocysts (24, 26 and others). The experimental infections have indicated that the timing of this exogenous TPI influences the outcome. Experimental oocyst infection around mid-gestation may provoke abortion. Infection late in pregnancy leads to infected healthy calves. If these are females of dairy breeds, there is risk associated with their retention for replacements because they may then be sources of endogenous transplacentally infected calves which in turn can lead to the population of the herd with infected females.

Oral infections of dogs are difficult experiments to conduct, but enough have now been done to produce a consistent picture (8). The patent period is short (a few days between 1 and 3 weeks post-infection) and the total number of oocysts produced is relatively low in comparison with related protozoa such as *Toxoplasma gondii*, and is usually less than one million over the duration of patency. This is consistent with cross-sectional field surveys looking at oocyst prevalence in dog faeces which have found very low prevalences with very low faecal concentrations of oocysts. The largest such study in over 24,000 German dogs found *N. caninum* oocysts in just seven (38). Another feature of experimental dog infections is that not all dogs which shed oocysts seroconvert (8). Collectively these results from dog infections indicate that when investigating bovine abortions *post-hoc* examination of dogs, either serological or faecal, is unlikely to provide any meaningful information.

#### *Other definitive hosts and sylvatic cycles*

In addition to the dog, the coyote has been shown to be a definitive host of *N. caninum* (13) and it is likely that there are sylvatic cycles involving other canid definitive hosts and their prey (11) which may contribute to environmental contamination. From knowledge of the definitive host range of related protozoa, it is likely that oocyst shedding will be restricted to the genus *Canis*, which would include wolf but exclude the red fox *Vulpes vulpes*. Whether the latter is a definitive host is an important question in Britain and Europe and is unresolved. *N. caninum* infected tissue provoked oocyst shedding when fed to dogs but not when fed to foxes (37). On the other hand oocysts identified as *N. caninum* have been found in faeces of wild foxes (44).

#### *Is venereal transmission a potential post-natal source of infection?*

The possibility of venereal infection has been thoroughly researched. Whilst parasite DNA can be found in semen from seropositive bulls, and heifers can be infected by artificially

infected semen, quantitative studies indicate that successful venereal infection is unlikely (4, 5, 9, 30, 39, 40). Moreover, there is no epidemiological evidence that venereal spread occurs.

All of the above findings taken together with the weight of experimental and epidemiological investigations of other potential post-natal sources of infection, indicate that, in most environments, there are a very limited number of sources of infection to cattle as summarised in *Figure 3*.

### The pathogenesis of abortion

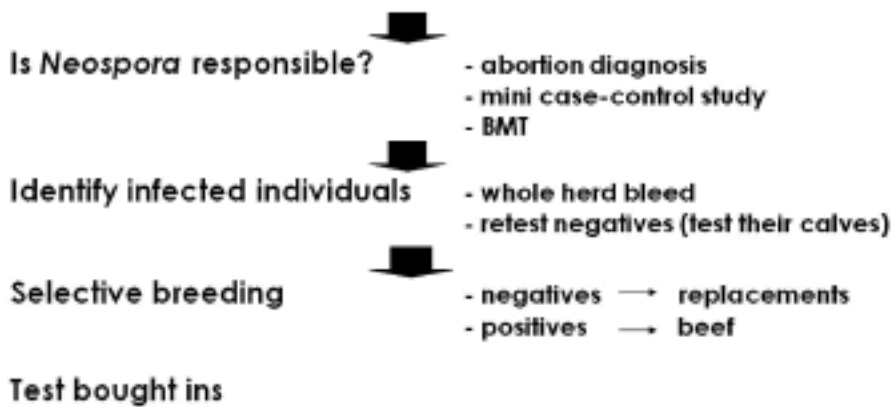
As with many infectious agents and most parasites, infection with *N. caninum* is much more common than disease. Indeed it is the fact that most cows infected with *N. caninum* do not abort (but they may produce healthy congenitally infected calves) which, if undetected by serological investigation, allows infection within the closed or semi-closed population of a dairy herd to increase in prevalence. Why then do some infected animals abort?

There is clear evidence from experimental studies that the time in gestation of exposure of the foetus to infection is a major determinant of clinical outcome (12, 20, 27, 45) and epidemiological evidence supports this (31). This influence of gestation age on the outcome of infection is the same whether the source is oocyst infection or endogenous infection. In neither case has TPI in early pregnancy been observed experimentally and this accords with many (but not all) epidemiological studies that early foetal loss reflected as infertility is not a feature of natural bovine neosporosis (23, 34). There is also some evidence that there may be strain variation in virulence in cattle in that experimental challenge of pregnant cows with Nc(Nowra) was less pathogenic than Nc(Liverpool) (46)

In terms of the molecular and cellular mechanisms which may influence clinical outcome, there have been two hypotheses; either abortion is primarily provoked by the direct deleterious effects on placental trophoblasts of IFN-g which is produced in response to *N. caninum* infection, or parasite – induced pathology in the placenta and/or foetus itself induces foetal death (19). In other words, is it the parasite, or the host's immune response to it, which trigger disease? Recent experimental and field studies are both suggesting that it is poorly controlled parasite multiplication and the resultant tissue necrosis which causes abortion and that IFN-g responses are protective rather than harmful at the local level. Thus in naturally occurring *Neospora*-associated abortions parasite load assayed by real-time PCR decreased significantly with foetal age, as did the severity of lesions (6). The authors concluded that foetal age influences pathogenesis. In experimental infections, challenge at 70 days of gestation was associated with extensive necrosis of foetal and placental tissues and foetal death, whereas challenge at 210 days which did not cause foetal death was accompanied by only occasional and mild lesions (10). In the same experiment, there was a marked increase in local IFN- $\gamma$  production in both groups. This was much greater at 70 days challenge, but was still substantial at the 210 days gestation

# BOVINE NEOSPOROSIS

## WORKED EXAMPLE – PROBLEM HERD CONTROL PROGRAMME



**Figure 4.** Flow chart showing possible approach to control in a herd with a higher than normal incidence of abortion. BMT means Bulk Milk Tank sample antibody test to estimate herd prevalence

challenge suggesting that the placental IFN- $\gamma$  response *per se* is not foetopathic (33).

It is thus possible to create the following scenarios. Where endogenous TPI occurs, spontaneous immunomodulation provoked by pregnancy may transiently reduce Th1 control of latent infection in the dam (20) such that there is recrudescence of infection which, if it occurs when foetal immune ontogeny is poorly developed results in an overwhelming infection and foetopathy. This is usually around mid-pregnancy because spontaneous recrudescences appear to be rare in early pregnancy and if they occur later in pregnancy foetal competence can contain parasite multiplication. This is consistent with an intervention study in naturally infected cattle in which progesterone administration (which is reported to reduce Th1 responses in pregnancy) at 120 days did not protect against abortion but instead significantly increased abortion (3). Where exogenous TPI exposes the foetus to infection this leads either to abortion if challenge is around mid-pregnancy or congenital infection if in later pregnancy – which can again be related to foetal incompetence. Whilst the foetus is vulnerable to infection in early pregnancy (intravenous challenge at 70 days gestation with  $10^7$  parasites is consistently lethal, (45 and others), experimental infection of pregnant cows with oocysts early in pregnancy did not lead to foetal infection, for reasons not yet understood (12, 27, 41). Thus natural exogenous infection in early pregnancy may only rarely cause foetal loss – not because the foetus isn't vulnerable but because infection doesn't reach it. In these scenarios the overarching determinant of the fate of the foetus is its immunocompetence, except that foetopathy is uncommon in early pregnancy because the foetus, whilst vulnerable, is not often exposed to infection of either endogenous or exogenous origin at that stage.

### Prospects for vaccination

It has proved impossible to provoke good protective immunity against parasitic infections with non-living vaccines except in one or two instances; thus the successful protozoal vaccines in commercial veterinary use mainly include live organisms attenuated in some way. A killed vaccine for *N. caninum* has been licensed in the US but evidence of its efficacy is variable and it is not licensed in Europe (1, 2, 18, 32). However, there is good evidence from both field and experimental studies that protective immunity to *N. caninum* in cattle occurs naturally and can be induced. Notably, infection of cattle prior to pregnancy whether naturally acquired, or experimentally induced with live unattenuated parasites, protects against experimental challenge in pregnancy (20, 46, 47). These

experiments provide encouraging proof of principle for vaccination. However, there are many problems yet to overcome. The encouraging results above relate to exogenous challenge and it is likely to be much more complex immunologically to protect against endogenous TPI (42, 48); the use of live vaccines has drawbacks; and the induction of protective, enduring immunity with non-living preparations is challenging. The ideal requirements for a vaccine have been summarised in a workshop of the World Association for the Advancement of Veterinary Parasitology, in 2005 (7).

### Practical control options

In spite of the lack of a vaccine or a drug treatment, the large body of research on bovine neosporosis in the last twenty years has yielded a good (although still incomplete) understanding of the biology and transmission of this infection, so that evidence-based control strategies can be advocated. Key facts are an understanding of the efficiency and importance of endogenous TPI; the lack of horizontal cow to cow transmission; the development of good serological assays to identify infected cattle and an understanding of the dynamics of antibody levels in persistently infected cattle; and an appreciation of the role of dogs in infection and disease. Furthermore, there have been a number of thorough cost-benefit studies examining the value of different control strategies (16, 17, 22)

Thus the generally favoured approach to endemic problems in a dairy herd is to identify infected females and selectively breed them to beef breeds and not to use them to breed replacement dairy heifers. In this way, the prevalence of persistently infected females in the herd is decreased without drastic and costly recourse to culling (which usually means selling the infection to other unsuspecting farmers). Infected cows, which in all other respects may be healthy and productive, can be retained in the herd – there is no clear consensus from various field studies that milk-yield in non-aborting cattle is adversely affected (8) and they are not a risk to other cows. Nor is there evidence that *N. caninum* is zoonotic (8). Whilst this will be the best option in most circumstances, it does not control exogenous infection. This centres on dogs, but beyond good hygienic calving practices (proper disposal of all placentae and foetuses) and preventing dogs access to stored feed, there is little one can do. As stated above serological or faecal investigation of farm dogs is likely to be meaningless. Young dogs are more likely to be a source of oocysts so keeping old dogs or spayed females on the farm is preferable to having breeding females or young dogs. The influence of non-farm dogs will depend on the type of feed they are likely to receive.

Most pet-dogs should provide a low-risk although in an extensive German study dog densities in urban as well as rural areas were risk factors for herd positivity or predictors of herd prevalence (35, 36, 43). On the other hand, hunt packs fed raw bovine carcasses may be important in disseminating oocysts over farmland (28). A possible plan for control is shown in *Figure 4*.

### The future

There are still important and outstanding research questions about bovine neosporosis. These include reconciling the low productivity of oocysts by dogs with their apparent role in causing epidemic abortion outbreaks; understanding what provokes parasite recrudescence in persistently infected female cattle; determining the efficacy of chemotherapy in eliminating neonatal infections (15, 21); clarifying the role of foxes in the epidemiology of infection; and, of course, developing an efficacious vaccine. Notwithstanding these unresolved questions, soundly based control measures based on surveillance and management can now be recommended with some confidence.

### ACKNOWLEDGMENTS

Many thanks to my long term collaborator and colleague, Diana Williams and to other colleagues at the University of Liverpool, who contributed to this work (co-authors in cited papers) and to Debbie Kelleher and Jayne Roberts. Grateful thanks also for collaboration with Milt McAllister (University of Illinois, USA) and for helping us get started with *N. caninum* research to J. P. Dubey. Finally many thanks to Franz Conraths and all participants in the EU COST854 programme for stimulating discussions. For funding we are grateful to BBSRC, DEFRA, MDC, Wellcome Trust, RCVS Trust, Pfizer, Novartis and the EU.

### REFERENCES

- ANDRIANARIVO, A. G. – ANDERSON, M. L. – PACKHAM, – ROWE, J.D. – GARDNER, I. A. – REYNOLDS, J. P. – CHOROMANSKI, L. – CONRAD, P. A.: Immune responses during pregnancy in heifers naturally infected with *Neospora caninum* with and without immunisation. *Parasitol. Res.*, 2005. 96. 24-31
- ANDRIANARIVO, A. G. – ROWE, J. D. – BARR, B. C. – ANDERSON, M. L. – PACKHAM, A. E. – SYERLOW, K. W. – CHOROMANSKI, L. – LOUI, C. – GRACE, A. – CONRAD, P. A.: A POLYGEN-adjuvanted killed *Neospora caninum* tachyzoite preparation failed to prevent foetal infection in pregnant cattle following i.v/i.m. experimental tachyzoite challenge. *Int. J. Parasitol.*, 2000. 30. 985-990
- BECH-SABAT, G. – LOPEZ-GATIUS, F. – SANTOLARIA, P. – GARCIA-ISPIERTO, I. – PABON, M. – NOGAREDA, C. – YANIZ, J.L. – ALMERIA, S.: Progesterone supplementation during mid-gestation increases the risk of abortion in *Neospora*-infected dairy cows with high antibody titres. *Vet. Parasitol.*, 2007. 145. 164-167
- CAETANO-DA-SILVA, A. – FERRE, I. – COLLANTES-FERNANDEZ, E. – NAVARRO, V. – ADURIZ, G. – UGARTE-GARAGALZA, C. – ORTEGA-MORA, L. M.: Occasional detection of *Neospora caninum* DNA in frozen extended semen from naturally infected bulls. *Theriogenology*, 2004. 62. 1329-1336
- CANADA, N. – MEIRELES, C. S. – FERREIRA, P. – DA COSTA, J. M. C. – ROCHA, A.: Artificial insemination of cows with semen *in vitro* contaminated with *Neospora caninum* tachyzoites failed to induce neosporosis. *Vet. Parasitol.*, 2006. 139. 109-114
- COLLANTES-FERNANDEZ, E. – RODRIGUEZ-BERTOS, E.A. – ARNAIZ-SECO, I. – MORENO, B. – ADURIZ, G. – ORTEGA-MORA, L. M.: Influence of the stage of pregnancy on *Neospora caninum* distribution, parasite loads and lesions in aborted fetuses. *Theriogenology*, 2006. 65. 629-641
- CONRATHS, F. J. – ORTEGA-MORA, L. M.: Options for control of protozoal abortion in ruminants practical experience. Conclusions, p. 229. Workshop Session T. 20<sup>th</sup> Int. Conf. World. Assoc. Adv. Vet. Parasitol. Christchurch, New Zealand, 16<sup>th</sup> – 20<sup>th</sup> October 2005
- DUBEY, J. P. – SCHARES, G. – ORTEGA-MORA, L. M.: Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.*, 2007. 20. 323-367
- FERRE, I. – ADURIZ, G. – DEL-POZO, I. – REGIDOR-CERRILLO, J. – ATXAERANDIO, R. – COLLANTES-FERNANDEZ, E. – HURTADO, A. – UGARTE-GARAGALZA, C. – ORTEGA-MORA, L. M.: Detection of *Neospora caninum* in the semen and blood of naturally infected bulls. *Theriogenology*, 2005. 63. 1504-1518
- GIBNEY, E. H. – KIPAR, A. – ROSBOTTOM, A. – GUY, C. S. – SMITH, R. F. – HETZEL, U. – TREES, A. J. – WILLIAMS, D. J. L.: The extent of parasite associated necrosis in the placenta and foetal tissues of cattle following *Neospora caninum* infection in early and late gestation correlates with foetal death. *Int. J. Parasitol.*, 2008. 38. 579-588.
- GONDIM, L. F. P.: *Neospora caninum* in wildlife. *Trends in Parasitol.*, 2006. 22. 247-252
- GONDIM, L. F. P. – MCALLISTER, M. M. – ANDERSON-SPRECHER, R. C. – BJORKMAN, C. – LOCK, T. F. – FIRKINS, L. D. – GAO, L. – FISCHER, W. R.: Transplacental transmission and abortion in cows administered *Neospora caninum* oocysts. *J. Parasitol.*, 2004. 90. 1394-1400
- GONDIM, L. F. P. – MCALLISTER, M. M. – PITT, W. C. – ZEMLOCKA, D. E.: Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int. J. Parasitol.*, 2004. 34. 159-161
- GUY, C. S. – WILLIAMS, D. J. L. – KELLY, D. F. – MCGARRY, J. W. – GUY, F. – BJORKMAN, C. – SMITH, R. F. – TREES, A. J.: *Neospora caninum* in persistently infected, pregnant cows: spontaneous transplacental infection is associated with an acute increase in maternal antibody. *Vet. Rec.*, 2001. 149. 443-449
- HAERDI, C. – HAESSIG, M. – SAGAR, H. – GREIF, G. – STAUBLI, D. – GOTTSSTEIN, B.: Humoral immune reaction of newborn calves congenitally infected with *Neospora caninum* and experimentally treated with toltrazuril. *Parasitol. Res.*, 2006. 99. 534-540
- HASLER, B. – REGULA, G. – STARK, K.D.C. – SAGER, H. – GOTTSSTEIN, B. – REIST, M.: Financial analysis of various strategies for the control of *Neospora caninum* in dairy cattle in Switzerland. *Prev. Vet. Med.*, 2006. 77. 230-253
- HASLER, B. – STARK, K. D. C. – SAGER, H. – GOTTSSTEIN, B. – REIST, M.: Simulating the impact of four control strategies on the population dynamics of *Neospora caninum* infection in Swiss dairy cattle. *Prev. Vet. Med.*, 2006. 77. 254-283
- HEUER, C. – NICHOLSON, C. – RUSSEL, D. – WESTON, J.: Field study in dairy cattle from New Zealand. *Vet Parasitol.*, 2004. 125. 137-146
- INNES, E. A. – AURELIE, G. – ANDRIANARIVO, A. G. – BJORKMAN, C. – WILLIAMS, D. J. L. – CONRAD, P. A.: Immune responses to *Neospora caninum* and prospects for vaccination. *Trends Parasitol.*, 2002. 18. 497-504
- INNES, E. A. – WRIGHT, S. E. – MALEY, S. – RAE, A. – SCHOCK, A. – KIRVAR, E. – BARTLEY, P. – HAMILTON, C. – CAREY, I. M. – BUXTON, D.: Protection against vertical transmission in bovine neosporosis. *Int. J. Parasitol.*, 2001. 31. 1523-1534
- KRITZNER, S. – SAGER, H. – BLUM, J. – KREBBER, R. – GREIF, G. – GOTTSSTEIN, B.: An explorative study to assess the efficacy of toltrazuril-sulfone (Ponazuril) in calves experimentally infected with *Neospora caninum*. *Ann. Clin. Microbiol. Antimicrob.*, 2002. 1. 4
- LARSON, R. L. – HARDIN, D. K. – PIERCE, V. L.: Economic considerations for diagnostic and control options for

- Neospora caninum*-induced abortions in endemically infected herds of beef cattle. J. Am. Vet. Med. Assoc., 2004. 224. 1597-1604
23. LOPEZ-GATIUS, F. – PABON, M. – ALMERIA, S.: *Neospora caninum* infection does not affect early pregnancy in dairy cattle. Theriogenology, 2004. 62. 606-613
  24. McALLISTER, M. M. – BJORKMAN, C. – ANDERSON-SPRECHER, R. – ROGERS, D. G.: Evidence of point-source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. J. Am. Vet. Med. Assoc., 2000. 217. 881-887
  25. McALLISTER, M. M. – DUBEY, J. P. – LINDSAY, D. S. – JOLLEY, W. R. – WILLS, R. A. – MCGUIRE, A. M.: Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol., 1998. 28. 1473-1478
  26. McALLISTER, M. M. – WALLACE, R. L. – BJORKMAN, C. – GAO, L. – FIRKINS, L. D.: A probable source of *Neospora caninum* infection in an abortion outbreak in dairy cows. Bovine Pract., 2005. 39. 69-74
  27. McCANN, C. M. – McALLISTER, M. M. – GONDIM, L. F. P. – SMITH, R. F. – CRIPPS, P. J. – KIPAR, A. – WILLIAMS, D. J. L. – TREES, A. J.: *Neospora caninum* in cattle: experimental infection with oocysts can result in exogenous transplacental infection, but not endogenous transplacental infection in the subsequent pregnancy. Int. J. Parasitol., 2007. 37. 1631-1639
  28. MCGARRY, J. W. – STOCKTON, C. M. – WILLIAMS, D. J. L. – TREES, A. J.: Protracted shedding of oocysts of *Neospora caninum* by a naturally infected foxhound. Int. J. Parasitol., 2003. 89. 628-630
  29. MOEN, A. R. – WOUDE, W. – MUL, M.F. – GRAAT, E. A. – VAN WERVEN, T.: Increased risk of abortion following *Neospora caninum* abortion outbreaks: a retrospective and prospective cohort study in four dairy herds. Theriogenology, 1998. 49. 1301-1309
  30. ORTEGA-MORA, L. M. – FERRE, I. – DEL POZO, I. – CAETANO DA SILVA, A. – COLLANTES-FERNANDEZ, E. – REGIDOR-CERRILLO, J. – UGARTE-GARAGALZA, C. – ADURIZ, G.: Detection of *Neospora caninum* in semen of bulls. Vet. Parasitol., 2003. 117. 301-308
  31. PEREIRA-BUENO, J. – QUINTANILLA-GOZALOL, A. – SEIJAS-CARBALLEDO, A. – COSTAS, E. – ORTEGA-MORA, L. M.: Observational studies in *Neospora caninum* infected dairy cattle: pattern of transmission and age-related antibody fluctuations. Int. J. Parasitol., 2000. 30. 906-909
  32. ROMERO, J. J. – PEREZ, E. – FRANKENA, K.: Effect of a killed whole *Neospora caninum* tachyzoite vaccine on the crude abortion rate of Costa Rican dairy cows under field conditions. Vet. Parasitol., 2004. 123. 149-159
  33. ROSBOTTOM, A. – GIBNEY, E. H. – GUY, C. S. – KIPAR, A. – SMITH, R. F. – KAISER, P. – TREES, A. J. – WILLIAMS, D. J. L.: Upregulation of cytokines is detected in the placenta of cattle infected with *Neospora caninum* and is more marked early in gestation when fetal death is observed. Infect. Immun., 2008. 76. 2352-2361.
  34. SAGER, H. – FISCHER, I. – FURRER, K. – STRASSER, M. – WALDVOGEL, A. – BOERLIN, P. – AUDIGE, L. – GOTTSCHALK, B.: A Swiss case-control study to assess *Neospora caninum*-associated bovine abortions by PCR, histopathology and serology. Vet. Parasitol., 2001. 102. 1-15
  35. SCHARES, G. – BARWALD, A. – STAUBACH, C. – ZILLER, M. – KLOSS, D. – SCHROEDER, R. – LABOHM, R. – DRAGER, K. – FASEN, W. – HESS, R. G. – CONRATHS, F. J.: Potential risk factors for bovine *Neospora caninum* infection in Germany are not under the control of farmers. Parasitology, 2004. 129. 301-309
  36. SCHARES, G. – BARWALD, A. – STAUBACH, C. – ZILLER, M. – KLOSS, D. – WURM, R. – RAUSER, M. – LABOHM, R. – DRAGER, K. – FASEN, W. – HESS, R. G. – CONRATHS, F. J.: Regional distribution of bovine *Neospora caninum* infection in the German state of Rhineland-Palatinate modelled by logistic regression. Int. J. Parasitol., 2003. 33. 1631-1640
  37. SCHARES, G. – HEYDORN, A.O. – CUPPERS, A. – MEHLHORN, H. – GEUE, L. – PETERS, M. – CONRATHS, F. J.: In contrast to dogs, red foxes (*Vulpes vulpes*) did not shed *Neospora caninum* upon feeding of intermediate host tissues. Parasitol. Res., 2002. 88. 44-52
  38. SCHARES, G. – PANTCHEV, N. – BARUTZKI, D. – HEYDORN, A. O. – BAUER, C. – CONRATHS, F. J.: Oocysts of *Neospora caninum*, *Hammondia heydorni*, *Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. Int. J. Parasitol., 2005. 35. 1525-1537
  39. SERRANO, E. – FERRE, I. – OSORO, K. – ADURIZ, G. – MATEOS-SANZ, A. – MARTINEZ, A. – ATXAERANDIO, R. – HIDALGO, C. O. – ORTEGA-MORA, L. M.: Intrauterine *Neospora caninum* inoculation of heifers. Vet. Parasitol., 2006. 135. 197-203
  40. SERRANO, E. – FERRE, I. – OSORO, K. – ADURIZ, G. – MOTA, R. A. – MARTINEZ, A. – DEL-POZO, I. – HIDALGO, C. O. – ORTEGA-MORA, L. M.: Intrauterine *Neospora caninum* inoculation of heifers and cows using contaminated semen with different numbers of tachyzoites. Theriogenology, 2007. 67. 729-737
  41. TREES, A. J. – McALLISTER, M. M. – GUUY, C. S. – MCGARRY, J. W. – SMITH, R. F. – WILLIAMS, D. J. L.: *Neospora caninum* oocyst challenge of pregnant cows. Vet. Parasitol., 2002. 109. 147-154
  42. TREES, A. J. – WILLIAMS, D. J. L.: Endogenous and exogenous transplacental infection in *Neospora caninum* and *Toxoplasma gondii*. Trends Parasitol., 2005. 21. 558-561
  43. VON BLUMRODER, D. – STAMBUSCH, R. – LABOHM, R. – KLAWONN, W. – DRAGER, K. – FASEN, W. – CONRATHS, F. J. – SCHARES, G.: Potential risk factors for the serological detection of *Neospora caninum*-infections in cattle in Rhineland-Palatinate (Germany). Tierartzl. Prax. G., 2006. 36. 141-147
  44. WAPENAAR, W. – JENKINS, M. C. – O'HANDLEY, R. M. – BARKEMA, H. W.: *Neospora caninum*-like oocysts in feces of free ranging red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*) based on microscopic examination, PCR and DNA-sequencing. J. Parasitol., 2006. 92. 1270-1274
  45. WILLIAMS, D. J. L. – GUY, C. S. – MCGARRY, J. W. – GUY, F. – TASKER, L. – SMITH, R. F. – MACEachern, K. – CRIPPS, P. J. – KELLY, D. F. – TREES, A. J.: *Neospora caninum* associated abortion in cattle: the time of experimentally induced parasitaemia during gestation determines foetal survival. Parasitol., 2000. 121. 347-358
  46. WILLIAMS, D. J. L. – GUY, C. S. – SMITH, R. F. – ELLIS, J. – BJORKMAN, C. – REICHEL, M. P. – TREES, A. J.: Immunization of cattle with live tachyzoites of *Neospora caninum* confers protection against fetal death. Infect. Immun., 2007. 175. 1343-1348
  47. WILLIAMS, D. J. L. – GUY, C. S. – SMITH, R. F. – GUY, F. – MCGARRY, J. W. – MCKAY, J. S. – TREES, A. J.: First demonstration of protective immunity against foetopathy in cattle with latent *Neospora caninum* infection. Int. J. Parasitol., 2003. 34. 1059-1065
  48. WILLIAMS, D. J. L. – TREES, A. J.: protecting babies: vaccine strategies to prevent foetopathy in *Neospora caninum*-infected cattle. Parasite Immunol., 2006. 28. 61-67

# Incidence of subclinical metabolic disorders in Hungarian dairy herds during the last decade

Endre Brydl, László Könyves, Lászlóné Tegzes, Viktor Jurkovich, Attila Tirián

Szent István University Faculty of Veterinary Science. Department of Animal Hygiene, Herd Health and Veterinary Ethology. Budapest 1400 PO Box 2., Hungary, e-mail: Brydl.Endre@aotk.szie.hu

## INTRODUCTION

In the early years of the 1970's more than 30 thousand Holstein Friesian heifers and in calf cows were imported from USA and Canada and simultaneously the local Hungarian Fleckvieh populations were subjected to crossbreeding with Holstein Friesians. These two major actions in the Hungarian dairy industry have increased the milk yield of herds substantially (Table 1.).

**Table 1.** Average milk production in standard lactation in Hungary 1970-2007 (34)

Year	Number of standard lactation	Average milk yield in lactation	Butter fat, %	Milk protein, %
1970	98 000	3 458	3.88	
1980	278 000	4 138	3.75	
1985	293 000	4 875	3.71	
1990	288 000	5 534	3.66	3.24
1995	200 000	5 856	3.87	3.23
1996	200 700	5 909	3.78	3.21
1997	199 922	5 966	3.72	3.23
1998	202 225	6 399	3.73	3.36
1999	206 148	6 523	3.77	3.33
2000	200 221	6 773	3.78	3.28
2001	192 398	7 195	3.75	3.27
2002	184 679	7 449	3.76	3.29
2003	176 411	7 618	3.82	3.27
2004	161 582	7 753	3.74	3.20
2005	158 305	7 983	3.58	3.18
2006	151 978	8 122	3.52	3.17
2007	149 177	8 362	3.51	3.16

In many cases the annual milk yield of cows exceeds 9000 kg/animal at large-scale dairy farms, where the population size varies between 500-2000 head of cows (Figure 1.).

The considerably genetic progress has increased the nutritional requirements and made the cow populations more sensitive to even minor feeding, housing and management failures. Erroneous feeding often induces subclinical/clinical metabolic disorders some days/weeks prior to and especially after parturition with increased perinatal mortality (42), decreased production and evolving reproduction failures in terms e.g. of calving intervals (Figure 2.).

Majority of the losses are caused by subclinical metabolic disorders. For early detection of subclinical cases metabolic profile tests (MPT) have been developed and applied all over the world since the late sixties (35, 36, 37).

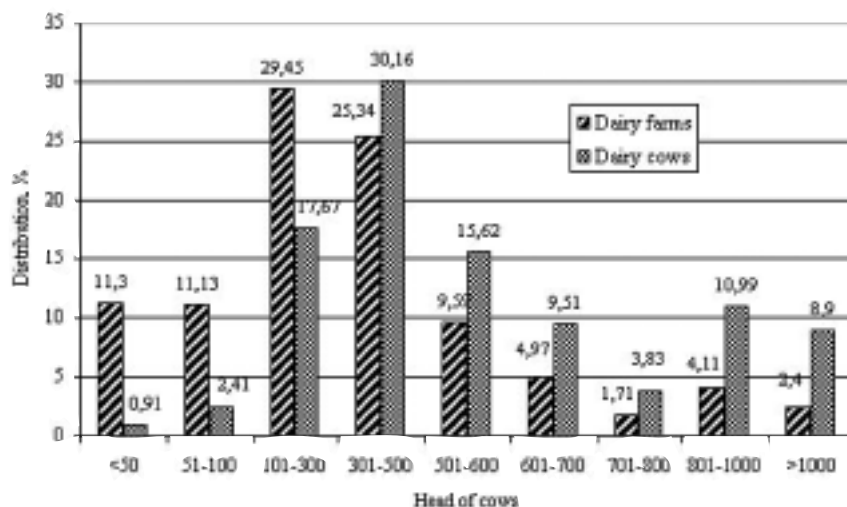
The use of metabolic profile test in cattle was pioneered by Payne et al. (35) at the Compton Institute of Animal Health. The method has been adapted and modified since then and is now widely used in many countries (2, 5, 26, 27, 28, 40, 41).

The system contributes information for decision-making about nutrition in a more precise and detailed way, and more quickly than most of the conventional approaches. The test compares biochemical data of selected cows of problem herds with those assumed normal values (37). Metabolic profile tests provide useful information concerning metabolic, nutritional and reproductive status of dairy herds (39).

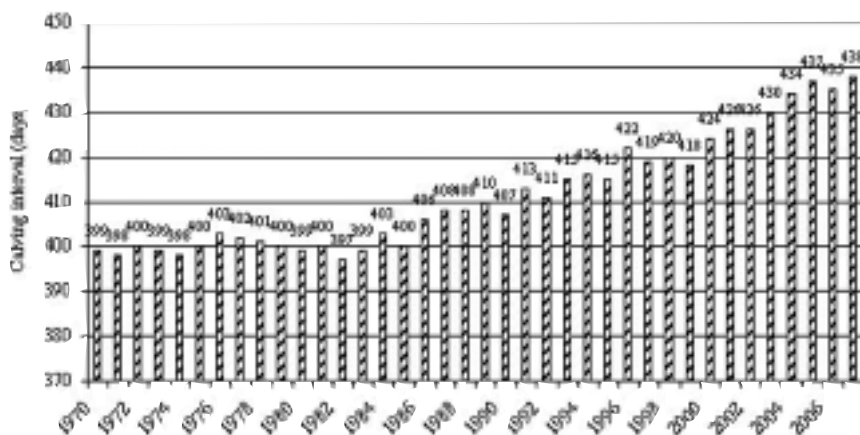
The metabolic profile approach to dairy herds should be used as a means of 'asking the cows what they think of their diet' (3).

Proper planning is needed to get as correct result as possible. It is also important to emphasise that metabolic profile test is an examination of the herd and not the individual cow. The proper use of metabolic profiles depends much on timing of blood sampling, the selection of cows to be sampled and use of background information about the farm, feeding and feeding system and physical state as well as performance of the cows.

As changes in the diet of ruminants require changes in the character of rumen activity, blood samples for metabolic profiles should not be taken until two weeks after a major change in the feeding had taken place. Minor dietary changes



**Figure 1.** Distribution of dairy farms according to size and proportion of cows kept in farms of respective size (34)



**Figure 2.** Calving interval of dairy cows in Hungary between 1970 and 2007 (34)

such as increasing/decreasing quantity of ingredient(s) in the ration that had been fed to cows for long do not need much time for adaptation, therefore the test may give unbiased results 7-10 days after the dietary change had manifested. Changes in the forage type, such as feeding corn silage or alfalfa haylage from new depot, housing or the introduction of CGF, require the full two weeks, as does the introduction of a new type of concentrates.

Feeding can change most biochemical parameters of the blood; therefore the time between the actual end of a feeding session and taking biological samples from the cow should be standardised. In most profile tests 2 hours gap is let elapsed between conclusion of feeding and sampling. Cows should not be separated at milking time and confined for hours without access to food when waiting for blood sampling as it can also affect the result.

In circumstances where the major part of the concentrate input is mixed with the forages, TMR is offered to the cows three-four times a day and in this case is available for most of 24 hours, the timing of the test in relation to feeding is less critical.

The cow in the early lactation is the most important because what happens to her around calving especially in the first few weeks after parturition has major influence on her milk production and the reactivation of ovaries after calving, so her fertility performance in the subsequent lactation. In generally blood sampling for metabolic profiles should be done in every quarter of the year.

Of equal importance is the need to test as soon as possible after the introduction of a new ratio, so the judgment of the cow's biochemistry can be made available as quick as possible (see above). Therefore planning of the metabolic profile test needs to be done in advance and should take into account both expected calving pattern and the feed changes. Without planning along these lines, time may be lost and productivity along with it (2).

It is assumed that dietary energy and protein are the major factors that influence most likely the reproduction of primiparous and multiparous cows.

The energy metabolism is evaluated by glucose, non-esterified fatty acid (NEFA) and beta-hydroxy-butyrate (BHB) content of the blood. Glucose is not as sensitive to changes in energy balance as BHB and NEFA because of homeostatic control. NEFA is more sensitive and more direct measure of fat mobilization than BHB. It is of especial value in dry cows (3).

Blood glucose concentrations are usually lower if the blood samples are stored under high temperature condition (e.g. in summer months if the sampling takes hours), that is why blood samples should be cooled down immediately after they were taken.

NEFA is more sensitive than blood glucose as an indicator of energy status of the dairy cow. NEFA begins to increase several weeks prior to parturition, peaking at the time of

calving and decreases gradually to normal level several weeks after parturition. Blood glucose follows similar pattern, however there may be a period in early lactation, when blood metabolite levels, particularly free fatty acids, are not entirely responsive to energy intake, but are perhaps under additional hormonal regulation (38).

Concerning the protein metabolism there are a direct relations between protein intake and the concentration of blood urea nitrogen (38). Blood urea reflects the rate of arrival in the rumen of effective rumen degradable protein (RDP) and the balance with fermentable metabolizable energy (FME) (2).

According to Blowey (4) protein digestion in the ruminants is basically a two-step process. Part of the protein entering the rumen (the rumen degradable protein, RDP) is degraded into ammonia by microbial action; the ammonia produced being re-assimilated into microbial protein by a further group of rumen micro-organism. This is eventually digested by the cow when microbial protein passes into the small intestine. The remainder of the protein (undegradable protein, UDP) passes through the rumen unchanged to undergo primary digestion in the small intestine.

Urea levels in the plasma are primarily derived from rumen ammonia although certain amount also originates from the hepatic deamination of amino acid. One part of the circulating urea is excreted by the kidneys; another part is recycled into rumen, particularly at low plasma urea level, with saliva and directly across the rumen wall. The excretion of urea is energy demanding process proven by the fact that excretion of 1000 g nitrogen as urea requires 22.9 MJ energy (32).

Good number of factors might increase the blood concentration of urea (4).

- Increased protein intake.
- Increased proportion of RDP in the ration, which can result in a higher proportion of dietary protein being converted to ammonia.
- Decreased energy intake, leading to depressed rumen microbial ammonia assimilation and an increased 'leakage' of ammonia from the rumen. The microbial protein synthesis is highly energy demanding process.
- Increased rumen pH allows greater 'leakage' of ammonia from the rumen, because free ammonia (NH<sub>3</sub>) diffuses across the rumen wall more rapidly than the ionized ammonium (NH<sub>4</sub><sup>+</sup>) radical.

- Increased body tissue catabolism and/or renal failure which in turn lead to increased hepatic deamination of amino acids. This is unlikely to occur on herd basis (1).

Some macro minerals are also measured in metabolic profile test such as Mg, inorganic phosphorus, calcium, sodium and potassium. The firm homeostasis for calcium is well recognized, rendering it poor indicator for the metabolic profile test. Low plasma magnesium indicates dietary deficiency, but at very high intakes blood magnesium raises only to an upper limit and excess is excreted with urine. Copper, zinc,

manganese and selenium status are assessed by both, concentration of blood and pigmented hair samples. The most reliable results are expected by sampling of pregnant animals receiving no supplementary concentrations, since such products normally contain quite high level of trace elements. Blood sample can also be analysed for iodine and cobalt status, but interpretation is difficult (1).

#### OWN EXAMINATION

At the Faculty of Veterinary Science Budapest a comprehensive and complex metabolic profile test (MPT) was developed and has been used since 1985 (5, 9).

The results of the metabolic profile test has been done during the last 10 years were summarised annually (5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 29, 30, 31) and attempts were made to draw conclusions and detect trends of changes the incidence of subclinical metabolic disorders. The results of the ten years survey are summarised in this paper.

#### MATERIALS AND METHODS

Metabolic profile test (MPT) has been performed for the last ten years at more than 100 large scale dairy farms that housed approximately 45.000 head of Holstein-Friesian cows, aged 5-6 years on average. The MPT is based not only on laboratory examinations of blood, urine, rumen fluid, pigmented hair and feed samples, but on field examinations (farm visits) as well. During farm visits data were collected on the feed quality, the method and strategy of feeding, the milk production and milk composition, the reproductive performance of the cows, health status of the herd as well as body condition of the animals were scored in every case.

The biological samples were taken from clinically healthy cows, randomly assigned from various groups of cows, 3-5 hours after the morning feeding. The groups differed from each other in respect of daily milk yield, stage of lactation and

gestation as well. During the last ten years 20938 cows were sampled.

In the present study the following groups were tested in a number of herds:

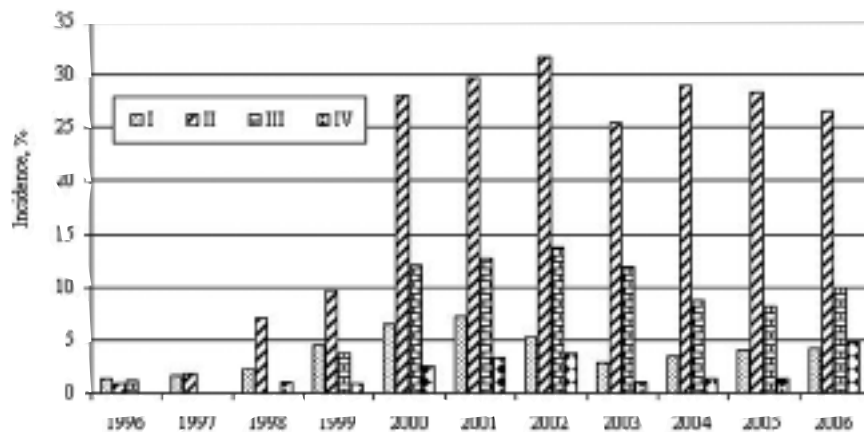
- Group I.: dry cows, 1-2 weeks before the expected parturition;
- Group II.: cows 1-2 weeks after calving;
- Group III.: cows at the peak of lactation;
- Group IV.: cows 1 week after drying off.

The number of sampled cows according to the different stage of lactation and gestation are summarised in *Table 2*.

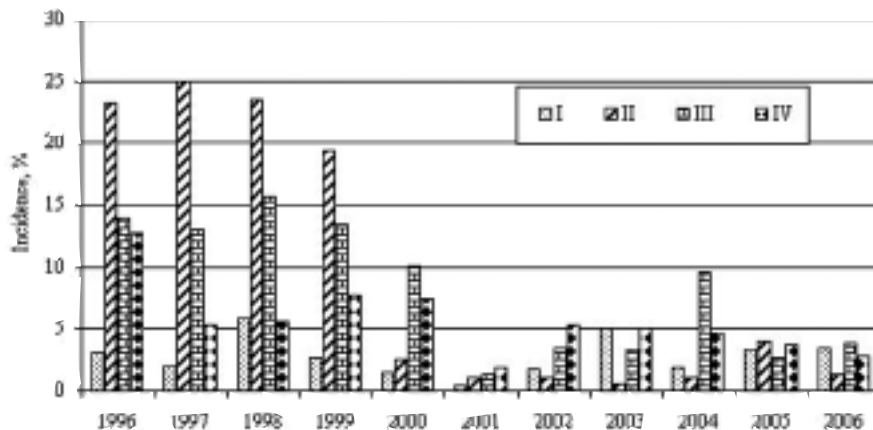
**Table 2.** Number of sampled cows in the different the stage of lactation and gestation during the last ten years.

Years/Groups	I	II	III	IV	All
1996	228	220	316	260	1024
1997	307	343	426	512	1588
1998	463	468	620	521	2072
1999	541	458	515	571	2085
2000	642	509	661	754	2566
2001	454	360	580	485	1879
2002	490	394	618	564	2066
2003	447	388	565	540	1940
2004	511	393	638	548	2090
2005	426	377	546	534	1883
2006	422	329	516	478	1745
All	4931	4239	6001	5767	20938

The samples were cooled down to 4 °C and transported immediately after sampling to the laboratory. The determinations from whole blood started immediately upon arrival of the samples or at least the next day morning, depending the distance between the university and the herd tested.



**Figure 3.** Incidence rate of subclinical fat mobilisation syndrome in dairy cows in Hungary in 1996–2006



**Figure 4.** Incidence of hyperketonaemia in dairy cows in Hungary between 1996–2006

The aim of the study was:

1. To survey the occurrence and tendency of the subclinical metabolic disorders occurring some weeks prior to expected parturition till the peak of lactation in large-scale dairy herds;
2. To contribute to the better understanding of the pathogenesis of the most frequent metabolic diseases of high yielding dairy cows;
3. To elaborate an expert advice for eliminating and preventing the metabolic disorders.

## RESULTS AND DISCUSSION

The percentile distributions of the most important subclinical metabolic disorders diagnosed in the period of study are summarised in *Figure 3-5*.

The occurrence of management related production diseases, such as fat mobilisation syndrome, ketosis, disturbances of acid-base balance were the highest in group II., due to energy imbalance and no gradually increased ration of concentrates at the beginning of lactation.

The energy imbalance were caused on the one hand by the too fatty body condition in the last part of lactation and in the dry period, neglecting the body scores of cows at grouping of the animals and on the other hand by the decreased appetite of cows at calving and during a few days after parturition. Over condition in the last part of lactation and in the dry period increase the risk of enhanced fat mobilisation because of the higher body weight loss of fat cows in comparison with that of cows of normal body condition.

Provision of concentrated feed beyond requirement and/or disproportional incorporation of grain concentrates in the daily ration before the expected parturition can equally lead to decreased appetite. This is explained by the fact that the concentrate contains great quantity of easily degradable

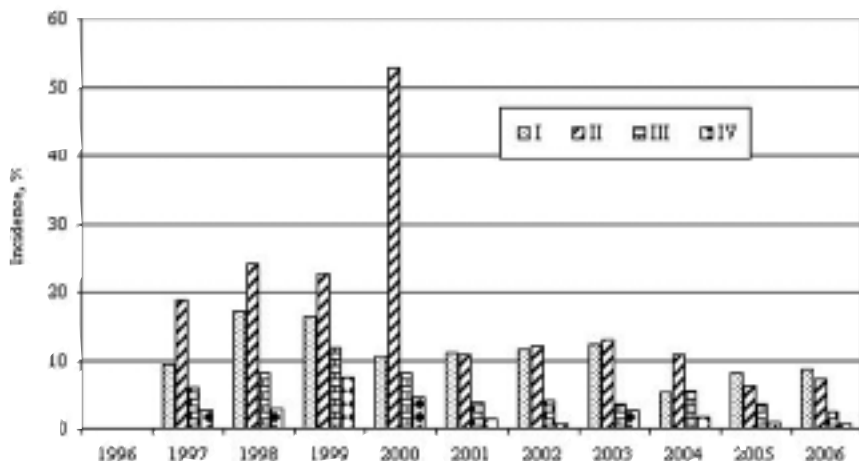
carbohydrates, which in turn helps the formation of propionate, butyrate and lactate in the rumen.

The effect of the change of the proportion of hay and concentrate in the ration on the pH of ruminal fluid and quantity and proportion of volatile fatty acids are illustrated by *Figure 7*.

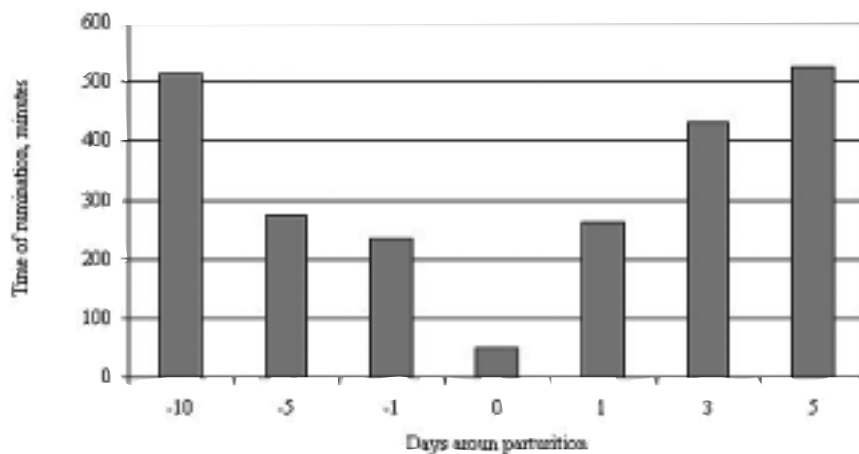
Under normal feeding conditions the energy requirement of dairy cows is met in 60–70% by volatile fatty acids fermented in the rumen. The volatile fatty acids (acetate, propionate and butyrate) are metabolites of the volatile fatty acid fermenters in the rumen. Life functions of these bacteria are pH dependent, viz. they are only able to multiply at pH 6.2–7.0, consequently the pH value of the ruminal fluid is one of the most important factor in the energy metabolism of dairy cow.

As it known salivation is decreased or reduced to nil during calving due to break of chewing or to reduced period and intensity of chewing (*Figure 6.*, 10). On this reason the ration of concentrate should be limited a few days around calving in order to prevent rumen acidosis, otherwise the multiplication of volatile fatty acid fermenters will be decreased or almost stopped below pH 6.2 consequently the concentration of volatile fatty acids decrease (*Figure 7.*, 25.). On the other hand this pH creates favourable conditions for the butyrate and lactate fermenters. Consequently the butyrate and lactate fermentation increases, which in turn leads acid load or imminent metabolic acidosis (*Figure 5.*) due to latent or clinical rumen acidosis especially because the saliva is not enough to make buffer effect (33).

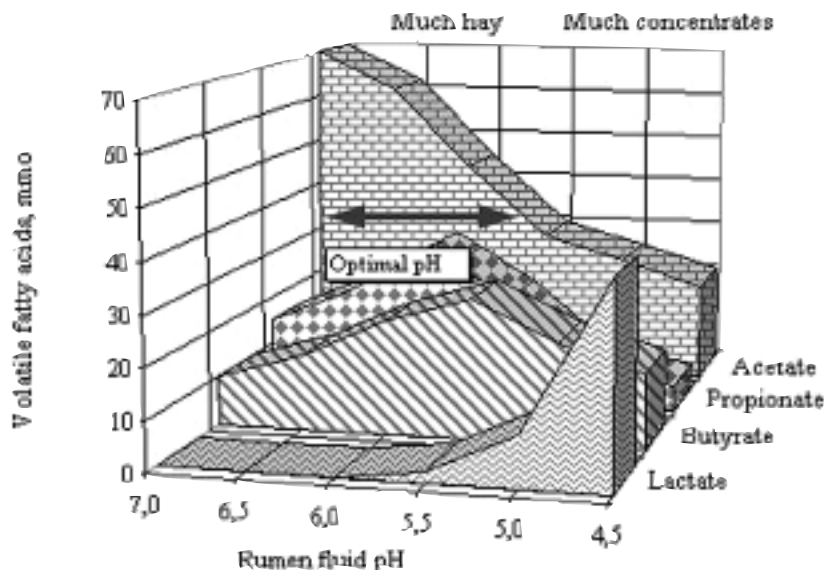
Due to rumen acidosis the rumen motility and the appetite of the cow will decrease and this may result in energy deficiency. If the energy deficiency is greater than 15-20 MJ NEI/day/cow the energy demand of the dairy cow will be met by increased fat mobilisation. It means that 60-85% of the energy requirement of the cow will be met by fat mobilisation, which in turn



**Figure 5.** Incidence of imminent metabolic acidosis in dairy cows under field condition in Hungary between 1996–2006



**Figure 6.** Time of rumination around parturition in dairy cows (10)



**Figure 7.** The effect of different mode of feeding on the rumen fluid pH and the concentration of Volatile Acids (25)

increases the risk of manifestation of subclinical or clinical fat mobilisation syndrome (Figure 3.) and hyperketonaemia (Figure 4).

In order to eliminate or prevent the occurrence of energy imbalance and other subclinical metabolic disorders in dairy herds, multi-phase periparturient feeding strategy should be introduced.

Multi-phase feeding strategy of periparturient dairy cows:

1. To realise the energy balance:

1.1. Body condition scoring has to be applied (0-5 scores) regularly. The required scores are  $\approx 3.5$ .

1.2. At the calculation of the ration and at the grouping of the cows not only the body weight, the daily milk production and the stage of pregnancy have to be taken into consideration, but the body condition score too!

1.3. During the dry period the feed intake should have to be  $\approx 56$  MJ NEL in 10-12 kg of dry matter. Concentrate should have not to be offered to the cows in this period at all.

1.4. During the close-up period the ration of concentrate does not exceed 3 kg daily.

1.5. The feeding of concentrate should have to be started with 1 kg of ration on the exact day of calving and it has to be increased by 2 kg of concentrate every second day until the 14<sup>th</sup> day after parturition. In case that the feed intake does not meet the requirement of cows the ration of concentrate should be increased to reach 12 kg of concentrate/day/cow in the same way mentioned above.

2. To avoid the high urea concentration in the blood amino acid balancing should be introduced in the feeding.

3. The elimination and prevention of ruminal acidosis (acid load) can be promoted by offering viable *Saccharomyces cerevisiae* to dairy cows.

#### REFERENCES

- ANDREWS, A. H. – BLOWEY, R. W. et al.: Bovine medicine, 1st edition Ed, G. London etc.: Blackwell, 1992.
- ANDREWS, A. H. – BLOWEY, R. W. et al.: Bovine medicine, diseases and Husbandry of Cattle, 2nd edition, G. London etc., Blackwell Science, 2004. 1193p.
- ANDREWS, A. H.: The Health of Dairy Cattle. 1st edition, London etc, Blackwell, 2000. 359p.
- BLOWEY et al.: Blood urea nitrogen is generally accepted as a good index of ration crude protein, Franzmann and Thorne 1970, Franzmann 1972.
- BRYDL E. – GÖNYE S. – SÁLYI G.: A nagyüzemi szarvasmarha-állományok átfogó, komplex takarmányozási és

állategészségügyi értékelési rendszere (Complex and comprehensive metabolic profile test performed at large scale dairy farms), MÉM Értesítő, 1987. 15. 584.

- BRYDL E. – KOVÁCS F.: Prognosis and prevention of metabolic diseases in cow herds of high dairy performance, Proceedings of the 6th International Congress of Animal Hygiene, Skara, Sweden, 1988. 203.
- BRYDL E. – KOVÁCS F. et al.: Occurrence of production diseases in large dairy herds in Hungary, Proceedings of the seventh International Congress on Production Disease in Farm Animals, Cornell University, Ithaca, USA, 1989. 266.
- BRYDL, E.: A nagyüzemi szarvasmarha-állományok átfogó, komplex takarmányozási és állategészségügyi értékelési rendszere (Complex and comprehensive metabolic profile test performed at large scale dairy farms), Magyar Állatorv. Lapja, 1989. 44. 121.
- BRYDL, E.: Komplex anyagforgalmi vizsgálatok nagyüzemi tehenészetekben (Complex metabolic investigations in large-scale dairy farms), Magyar Állatorv. Lapja, 1990. 45. 719.
- BRYDL E.: A tejhasznú tehenek ellés körüli anyagforgalmi zavarainak megelőzése többfázisú előkészítéssel (A Nemzetközi Állathigiéniai Társaság 8. "kongresszusközi" szimpóziuma), Magyar Állatorv. Lapja, 1995. 50. 600-607.
- BRYDL E.: Állathigiénia és állomány-egészségügy a tejtermelő tehenészetekben, Magyar Állatorv. Lapja, 1997. 119. 89-93.
- BRYDL E. – MRS TEGZES L. et al.: Occurrence of metabolic disorders in large-scale dairy farms (experiences of a five year study), Proceedings of the 9th International Congress in Animal Hygiene, 88-91, 17-21 August, 1997, Helsinki, Finland,
- BRYDL E.: Szubklinikai anyagforgalmi zavarok tejhasznú tehenészetekben 1991-1997 közötti időszakban, Proceeding of the Xth Conference of the Hungarian Association for Buiatrics, Middle-European Buiatrics Congress, Siófok, 1998. május 21-23. p. 19-22.
- BRYDL E. – KÖNYVES L. et al.: Occurrence of metabolic disorders in large-scale dairy farms (Results of a 7 year study), Proceedings of the 10th ICPD, Utrecht, The Netherlands, August 24-28. 1998.
- BRYDL E.: Szubklinikai anyagforgalmi zavarok tejhasznú tehenészetekben az 1991-1997 közötti időszakban, Magyar Állatorv. Lapja, 1999. 121. 82-84.

16. BRYDL, E. – KÖNYVES, L. – MRS TEGZES, L.: Occurrence of subclinical metabolic disorders in dairy farms in Hungary in 1997 and 1998, Proceedings of The 1st Middle-European Buiatrics Congress, Balatonfüred, 1999. május 27-29. p. 295-299.
17. BRYDL E. – KÖNYVES L. et al.: Occurrence of metabolic disorders in large scale dairy farms in Hungary (Results of a 3 year study), Proceedings of the Xth International Congress on Animal Hygiene, Maastricht, The Netherlands, 2-6 July, 2000., p. 415-418.
18. BRYDL E. – RAFAI P. et al.: Subclinical metabolic disorders in Holstein Friesian dairy herds in Hungary (poster) Proceedings of the 3rd Middle-European Congress for Buiatrics, Milovy, Czech Republic, 24-25 May, 2001., p. 307.
19. BRYDL E. – JURKOVICH V., et al.: Szubklinikai anyagforgalmi betegségek előfordulása tejtermelő tehenészetekben Magyarországon 2001-ben, Magyar Állatorv. Lapja, 2003. 125. 393-400.
20. BRYDL E. – JURKOVICH V. et al.: Occurrence of subclinical metabolic disorders in large-scale dairy farms in Hungary in 2002, Proceedings of the IVth Central European Congress for Buiatrics, Lovran, Croatia, April 23-27. 2003, p. 167.
21. BRYDL E. – KÖNYVES L. et al.: Occurrence of subclinical metabolic disorders in large-scale dairy herds in Hungary in 2001, Proceedings of the XIth Congress on Animal Hygiene, Mexico, February 2003. p. 193-197.
22. BRYDL E.: A szarvasmarha-tenyésztés fejlesztésének szükségessége és szakmai lehetőségei, Magyar Állatorv. Lapja, 2004. 126. 722-731.
23. BRYDL E. – KÖNYVES L. et al.: Subclinical metabolic disorders in peripartal dairy cows in Hungary in 2003. Proceedings of BUIATRISIMA, 1st Swiss Buiatrics Congress, Bern, Switzerland, 19-21. October 2005. p. 94.
24. BRYDL E. – KÖNYVES L. et al.: Subclinical metabolic disorders in peripartal dairy cows in Hungary in 2004. 24th World Buiatrics Congress, Nice, 2006. Ref.: 0831-1
25. DIRKSEN, G.: Acidosis, In Philipson, A. T. (Ed): Physiology of Digestion and Metabolism in the Ruminant, Oriel Press Ltd., 1970. 612-625.
26. GAÁL T.: Bőtejelő tehenek zsírmobilizációs betegségének (zsírmáj-szind-rómájának) oktana, kórfejlődése és megelőzésének lehetőségei, kandidá-tusi értekezés, Budapest, 1983.
27. HARASZTI, J. – SZENCI, O. et al.: Peripartal blood profile studies in high production dairy cows with special regard to reproductive reactivation, Acta Vet. Acad. Sci. Hung., 1980. 28. 197-207.
28. HARASZTI J. –HUSZENICZA GY. –MOLNAR L.: A szárazonállás alatti takarmányozás hatása az ellés körüli idő metabolikus folyamataira, különös tekintettel annak szaporodásbiológiai következményeire. Magyar Állatorv. Lapja. 1984. 39. 144-151.
29. JURKOVICH V. – BRYDL E. et al.: Szubklinikai anyagforgalmi zavarok előfordulása tejhasznú tehenekben, 2001-ben. 13. Magyar Buiatrikus Konferencia, 2002. október 10-12., Hajdúszoboszló
30. KÖNYVES L. – BRYDL E. et al.: Occurrence of subclinical metabolic disorders in large-scale dairy farms in Hungary in 2001. In Book of Abstracts of the 53rd Annual Meeting of the European Association for Animal Production, Cairo, Egypt, September 1-4. 2002.
31. KÖNYVES L. – JURKOVICH V. et al.: Anyagforgalmi zavarok előfordulása húshasznú tehenekben az ellés körüli időszakban és a tenyészszezonban. 18. Magyar Buiatrikus Kongresszus, Siófok, 2007. október 10-13. Proceedings, p. 55-65.
32. KUTAS F.: A közti anyagcsere, In: Brydl E. (szerk): A szarvasmarha anyagforgalmi betegségei és mérgezései, Mezőgazdasági Kiadó, Budapest, 1987. 13-85.
33. KUTAS F.: A kérődzők sav-bázis anyagcseréje és annak zavarai, In: Brydl E. (szerk): A szarvasmarha anyagforgalmi betegségei és mérgezései, Mezőgazdasági Kiadó, Budapest, 1987. 100-112.
34. MÉSZÁROS GY.: Personal information, 2008
35. PAYNE, J. M. – DEW, S. M. et al.: The use of the metabolic profile test in dairy herds, The Veterinary Record, 1970. 87. 150-158.
36. PAYNE, J. M. – HIBBIT, K. G. – SANSOM, B. F.: Production Disease in Farm Animals, Bailliere Tindall. London, 1972.
37. PAYNE, J. M. – ROWLANDS, G. J. et al.: A statistical appraisal of the results of metabolic profile tests on 75
38. RADOSTITS, O. M. – GAY, C. C, et al.: Veterinary medicine, 9th edition. London etc.: W.B Saunders, 2000. 1877p
39. SEIFI, H. A. – BAZARGANI, T. T.: Diagnosis of production diseases: the use of metabolic profile tests. Indian-Journal-of-Animal-Science. 2003. 73. 763-764.
40. SOMMER, H.: Preventive medicine in dairy cows, Veterinary medicine review, 1975. 1/2. 42-50.
41. SOMMER, H.: The role of metabolic profile test in the control of cattle feeding, Magyar Állatorvosok Lapja, 1995, vol.50.no. 714-717p.
42. TIRIÁN A. – SZENCI O. et al.: A holtellések gyakoriságának és a szubklinikai anyagforgalmi zavarok előfordulásának vizsgálata tejhasznú tehenészetben – előzetes közlemény. 18. Magyar Buiatrikus Kongresszus, Siófok, 2007. október 10-13. Proceedings, p. 94-9



# Notices