瑞士與台灣乳業科技論壇
Dairy Technology Application - Switzerland and Taiwan
2017年9月12日
行政院農業委員會畜產試驗所技術服務館

乳牛酮症及白血病檢測科技
Testing for Bovine Ketosis and Leukosis

蕭振文、涂柏安、賈玉祥
Jen-Wen Shiau, Po-An Tu and Yu-Shine Jea
畜產試驗所新竹分所
Hsin-chu Branch
Livestock Research Institute(LRI)

Tainan, Taiwan
September 12, 2017

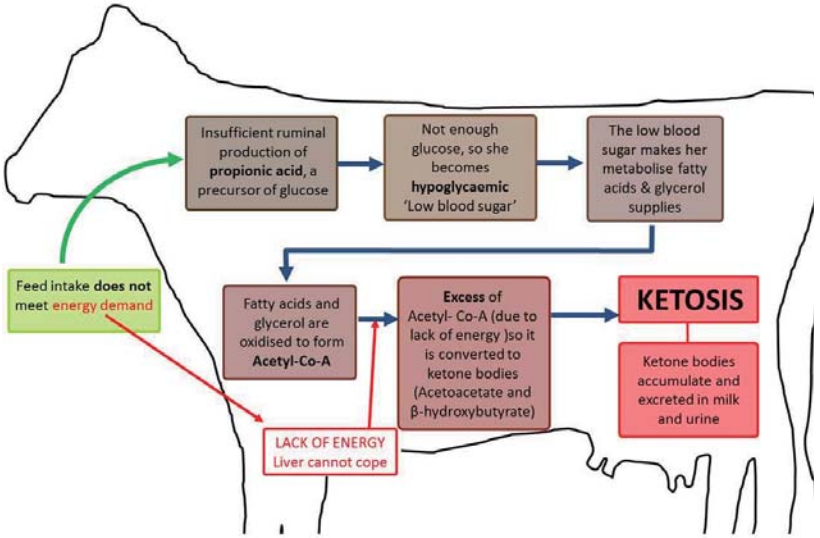
綱要

- 乳牛酮症
- 牛白血病
- 乳牛群性能改良計畫(DHI)

乳牛酮症(Ketosis)

- 乳牛酮症是最普遍的代謝性疾病。
- 主要因碳水化合物及揮發性脂肪酸代謝紊亂所引起之全身性代謝性疾病。
- 潛在性酮症主要發生在產後2-7週，發病率可高達45% 。
- 高產牛的酮症發病率較高，常發生在3～5胎的乳牛。
- 近年來，隨著對乳牛產乳量提高，發生酮症的機率也提升。初產30天淘汰率多3倍。
- 酮症不僅降低乳牛產乳量(3-7%)和乳品質，也影響乳牛繁殖性能(低1.2-1.7倍的初次配種受胎率)等。
- 如何預防乳牛酮症發生，將成為乳牛泌乳期間是否獲利的重要關鍵。

Insufficient feed intake causes Ketosis



http://www.farmhealthonline.com/disease-management/cattle-diseases/ketosis/

酮症檢測

乳牛是否罹患酮症，可經由檢測血、尿和牛乳中酮體而判定。酮體主要由BHBA、乙醯乙酸(AcAc, acetoacetate)和丙酮組成，其中BHBA占總酮體的8成以上，組成穩定，容易定量測定。

- 聞氣味，敏感度低於50%。
- 尿中酮體(Urine Ketone)測定， 用Ketostix(Bayer)試片測定。
- 乳中酮體測定，用Keto Test(敏感性83%,特異性82%)、PortaBHB 試片(敏感性89%,特異性80%)測定。
- 測血酮儀：Abbott Precision Xtra (敏感性＞ 90%,特異性＞ 95%) 、Nova Biomedical Meters。
- 檢測血液中的BHBA準確性最高(中等敏感＞80%，特異性95%)。

Urine Ketones

- Ketostix® test strip
  - about $0.20 each
  - have to stimulate urination
  - dip and read within 10 seconds

Other Cowside Ketone Tests - Milk

- PortaBHB™ test strip
  - ~$2.50 per test
  - dip and read 60 seconds later
  - use 100 umol/l cut-point
  - 89% sensitive
  - 80% specific

Other Cowside Ketone Tests - Blood

- Abbott Precision Xtra™ human hand-held system
- Consider ≥1.2 mmol/L as ketosis
  - >90% sensitive
  - >95% specific

Nova Biomedical Meters

- Nova Vet®
  - bovine calibration
  - $3.20 per strip
- Nova Max® Plus
  - human version
  - $2.00 per strip

ease-management/cattle-diseases/ketosis/

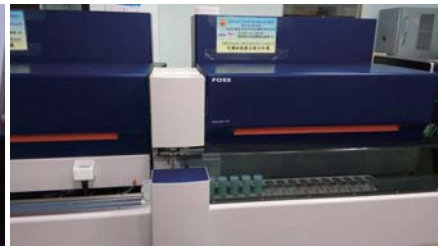
乳牛酮症之檢測

- 臨床症狀
- BHBA大於3.0 mmol/L，應出現乳量下降、減少瘤胃活動、呆滯憂鬱、正常直腸溫度、對乾草的食慾高於青貯或穀類等。
- 依BHBA分類酮症
  - 1.2-2.9 mmol/L 潛在性酮症
  - ≧3.0 mmol/L 臨床型酮症
  - ≧1.2 mmol/L 酮症(hyperketonemia)

酮症檢測

DHI實驗室分析 BHBA 是 Gold standard

- DHI乳樣檢測BHBA，脂肪：蛋白質比，脂肪增加，蛋白質下降≧1.4，即為酮症指標。
- 結合測試日牛乳分析結果資訊，應用所有可用資訊，包括牛隻基本資料、泌乳期、胎次、丙酮、MUN等，改善血中BHBA的預測。



酮症之預防

- 防止乳牛產前過胖
- 避免劣質飼料
- 使用添加劑：膽鹼、鈷、丙二醇等。
- 乳牛分娩前2週至產後4週，加強補充與脂肪代謝相關之營養成分，如甲硫胺酸、膽鹼和鈷，以避免酮症發生。
- 在乳牛酮症高峰期（產後1週至7週），每週進行血酮監測，及時發現罹患酮症的乳牛並適度進行治療。
- 轉換期是一個關鍵的階段，牛隻照顧好，將能為場主帶來最佳的利潤。

牛白血病Bovine leukosis

- **牛白血病病毒（BLV）**

會影響乳牛的生產。加拿大的研究顯示，40%的乳牛群及11%肉牛群感染BLV。其中5%感染牛隻呈現病症，在牛的淋巴節、子宮、第四胃、心、脾、腎及腦發展出腫瘤。使乳產量下降並造成潛在死亡。

- **牛白血病**

由一種反轉錄病毒引起，病毒寄居於血液淋巴細胞內，在臨床階段，腫瘤位於肩部、乳房下皮下淋巴顯而易見，位於子宮及心臟的內在腫瘤則無法檢視。
動物一旦感染BLV會終生帶毒。BLV不但感染牛，綿羊和山羊均易感染，但不會感染人。

牛白血病

- **傳染方式**

水平感染：含病毒之極少量血液、乳汁、唾液、鼻液或其他含淋巴球之媒介物經由接觸、吸血昆虫、醫療注射器，由感染牛傳給其他牛。污染的釘耳標夾、修蹄器具、針頭等均有潛在感染病毒的可能。
垂直感染：病毒可經胎盤、產道或初乳由母牛傳給出生前仔牛，感染母牛之重直感染率約10-20%。

牛白血病

- **經濟衝擊**
  - BLV引起淋巴肉瘤，導致為未成年牛的淘汰或者死亡，帶來巨大的經濟損失。
  - 感染母牛減少乳產量、失重或成為倒臥牛。一般感染牛不易被查覺，大多在屠宰時才發現。
  - 販賣種畜及牛胚的業者會因為失去市場而受到較大的財務衝擊，因為已採取牛白血病控制方案的國家要求出示無BLV的證明。
- **管制**
  - 目前無法治療，終生感染，由於高度感染性，一旦動物確診，應與獸醫擬出行動方案，淘汰感染牛隻達到清淨場的目標。
  - 生產者應採管理措施以減少病毒的潛在散布。

牛白血病管理措施

- 仔牛不吸吮陽性母牛的初乳。
- 使用滅菌消毒的針頭且不重複使用。
- 外科手套必需更換。
- 可能被血液污染的設備應加強清洗並消毒。
- 仔牛應在個別牛欄內飼養並有適度空間以防經由唾液或鼻液傳播。
- 仔牛使用非外科去角。
- 待產牛飼於個別待產牛欄，消毒並更換墊料。

牛白血病檢測

病毒分離及鑑定

- 電子顯微鏡檢測：取血液離心後、收集Buffy coat 、取淋巴球層洗後培養、兩天後將培養液離心、取沉澱顆粒層固定、製成超薄切片，以電子顯微鏡檢查 BLV 微粒。
- BLV 抗原或粒子檢出：取血液，離心後取淋巴球接種細胞內，約培養一週後，由細胞融合 (Syncy-tia) 的形成判斷有無 BLV 的感染。另外六月齡以下牛隻，因可能有移行抗體，亦不能檢出 BLV 的感染，故抗體陽性者必需是六個月於以上的檢體，使用此法才有意義。

牛白血病之檢測

- **血清學診斷**：病毒由蛋白質、核酸，脂肪等所構成。蛋白質含有病毒封套上兩種醣蛋白質 (gp30、gp51) 及病毒內部的數種核心蛋白質 (P10、p12、p15、p24、p32、p38、p70RT)。
- BLV 感染後病毒含有gp及p兩種抗原，在患牛血中會出現抗 gp 及 p 兩種抗體，其中抗 gp抗體會持續在感染牛體內產生，故gp抗體的檢出最具有價值診斷。
- **實驗室診斷**：包括瓊脂凝膠免疫擴散試驗（agar gel immunodiffusion）、聚合酶鏈反應（PCR）及ELISA等。
- 牛白血病無法治療，移除感染牛隻，可清除本病。
- 由感染母牛生下的小牛應隔離飼養至6個月大，並經由血清學檢查陰性。

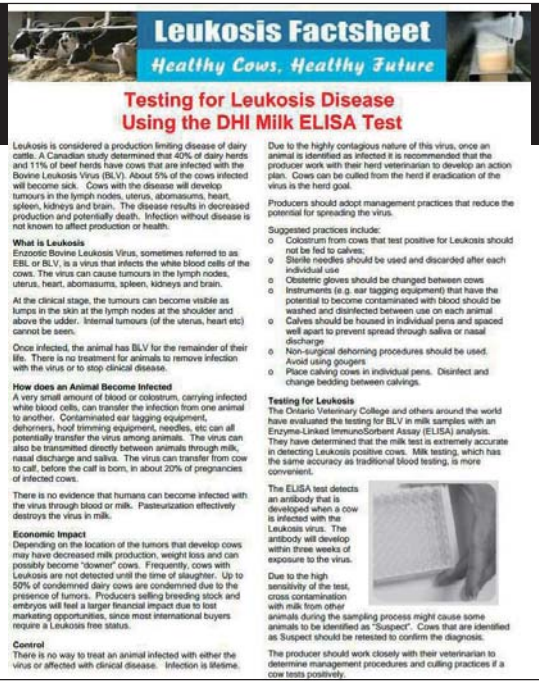
牛白血病之檢測

- **病理學診斷**：在解剖上可發現各內臟器官有明顯的彌漫性和結節性病變外，另配合臨床血液病理學檢驗，特別是血液中淋巴球數的消長增減情形是牛白血病珍斷上的重要指標。
- **臨床血液病理學檢驗**：白血病牛的血液變化有淋巴球增多症 (Lymphocytosis) 和未成熟淋巴球、異型淋巴球、異常淋巴球之出現。淋巴球增多症之診斷，主要是依牛品種和年齡別檢查血液中之淋巴球數而定。

牛白血病檢測

乳汁檢測之ELISA分析法

- 測定陽性牛準確度極高，測牛乳更方便。
- 測定感染病毒牛隻產生的抗體，感染後3週內即產生抗體。
- 因為測試敏感度高，採樣時可能造成樣品污染，可疑牛有必要再次確診。
- 生產者應與獸醫密切合作擬定管理措施，並進行陽性牛的淘汰作業。



Leukosis Factsheet

Healthy Cows, Healthy Future

Testing for Leukosis Disease Using the DHI Milk ELISA Test

Leukosis is considered a production limiting disease of dairy cattle. A Canadian study determined that 40% of dairy herds and 11% of beef herds have cows that are infected with the Bovine Leukosis Virus (BLV). About 5% of the cows infected will become sick. Cows with the disease will develop tumours in the lymph nodes, uterus, abomasums, heart, spleen, kidneys and brain. The disease results in decreased production and potentially death. Infection without disease is not known to affect production or health.

What is Leukosis

Enzootic Bovine Leukosis Virus, sometimes referred to as EBL or BLV, is a virus that infects the white blood cells of the cows. The virus can cause tumours in the lymph nodes, uterus, heart, abomasums, spleen, kidneys and brain.

At the clinical stage, the tumours can become visible as lumps in the skin at the lymph nodes at the shoulder and above the udder. Internal tumours (of the uterus, heart etc) cannot be seen.

Once infected, the animal has BLV for the remainder of their life. There is no treatment for animals to remove infection with the virus or to stop clinical disease.

How does an Animal Become Infected

A very small amount of blood or colostrum, carrying infected white blood cells, can transfer the infection from one animal to another. Contaminated ear tagging equipment, dehorners, hoof trimming equipment, needles, etc can all potentially transfer the virus among animals. The virus can also be transmitted directly between animals through milk, nasal discharge and saliva. The virus can transfer from cow to calf, before the calf is born, in about 20% of pregnancies of infected cows.

There is no evidence that humans can become infected with the virus through blood or milk. Pasteurization effectively destroys the virus in milk.

Economic Impact

Depending on the location of the tumors that develop cows may have decreased milk production, weight loss and can possibly become "downer" cows. Frequently, cows with Leukosis are not detected until the time of slaughter. Up to 50% of condemned dairy cows are condemned due to the presence of tumors. Producers selling breeding stock and embryos will feel a larger financial impact due to lost marketing opportunities, since most international buyers require a Leukosis free status.

Control

There is no way to treat an animal infected with either the virus or affected with clinical disease. Infection is lifetime.

Due to the highly contagious nature of this virus, once an animal is identified as infected it is recommended that the producer work with their herd veterinarian to develop an action plan. Cows can be culled from the herd if eradication of the virus is the herd goal.

Producers should adopt management practices that reduce the potential for spreading the virus.

Suggested practices include:

- Colostrum from cows that test positive for Leukosis should not be fed to calves;
- Sterile needles should be used and discarded after each individual use
- Obstetric gloves should be changed between cows
- Instruments (e.g. ear tagging equipment) that have the potential to become contaminated with blood should be washed and disinfected between use on each animal
- Calves should be housed in individual pens and spaced well apart to prevent spread through saliva or nasal discharge
- Non-surgical dehorning procedures should be used. Avoid using gougers
- Place calving cows in individual pens. Disinfect and change bedding between calvings.

Testing for Leukosis

The Ontario Veterinary College and others around the world have evaluated the testing for BLV in milk samples with an Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis. They have determined that the milk test is extremely accurate in detecting Leukosis positive cows. Milk testing, which has the same accuracy as traditional blood testing, is more convenient.

The ELISA test detects an antibody that is developed when a cow is infected with the Leukosis virus. The antibody will develop within three weeks of exposure to the virus.

Due to the high sensitivity of the test, cross contamination with milk from other animals during the sampling process might cause some animals to be identified as "Suspect". Cows that are identified as Suspect should be retested to confirm the diagnosis.

The producer should work closely with their veterinarian to determine management procedures and culling practices if a cow tests positively.

IDEXX Leukosis Serum X2 Ab Test

- Detects antibodies against BLV in **individual or pools** of up to 10 individual serum or plasma samples.
- More countries are recognizing the need for **eradication of enzootic bovine leukosis** in herds. **With no available vaccine or treatment, testing and separating or culling infected cattle is the only effective control.**
- For enzootic bovine leukosis, OIE prescribes the ELISA as a test to be used for international trade.
- High diagnostic specificity of 99.4%–100%

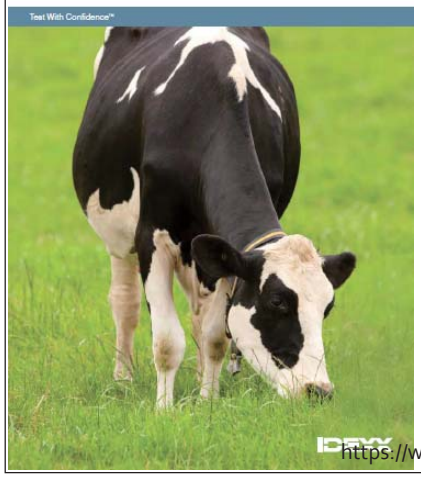
https://www.idexx.com/livestock-poultry/ruminant/blv.html

IDEXX Leukosis Serum Screening Ab Test

- detects all types of antibodies against the virus (e.g., envelope proteins, capsid proteins) in individual or pool sera.
- The test are based on the use of an ultrapurified virus lysate and are standardized to detect the European standard serum for (E5) diluted to 1:100 in negative bovine serum, according to the requirements of the Council Directive 64/432/EEC (modified on December 11, 1984, June 26, 1991 and March 2001).

https://www.idexx.com/livestock-poultry/ruminant/blv.html

IDEXX Leukosis Milk Verification Ab test



- Detects all types of antibodies against the BLV in bovine tank milk.
- Milk provides a simple, accurate medium for regular disease screening.
- Bulk tank or individual cow milk can be used as the sample matrix.
- ELISA testing capabilities gives you a seamless, cost-effective and profitable disease management solution that's easy for customers and veterinarians.
- Disease testing with milk samples simplifies collection and transport logistics.

https://www.idexx.com/livestock-poultry/ruminant/blv.html

DHI乳牛群性能改良計畫

年	乳牛場(戶)	DHI乳牛場(戶)	荷蘭牛頭數(頭)	泌乳牛頭數(頭)	參加DHI測乳牛頭數	牛乳產量(公噸)
2005	638	287	122,457	53,151	21,949	303,496
2006	636	260	123,587	52,269	20,770	323,165
2007	619	241	126,689	53,107	19,057	322,220
2008	591	219	123,115	52,566	18,781	315,559
2009	572	189	121,967	53,170	17,100	321,781
2010	571	178	122,983	55,296	15,292	336,036
2011	556	178	127,586	57,196	15,282	350,894
2012	560	187	129,445	59,145	26,227	348,489
2013	554	170	130,409	60,500	24,870	358,146
2014	550	167	128,608	60,103	25,706	363,145
2015	546	176	132,009	61,859	26,902	375,499
2016	545	175	129,071	59,601	27,099	378,488
2017		173			24,262	

牛乳樣驗室

- 新竹分所牛乳檢驗室於通過TAF評鑑認可，認證檢測項目包括**乳脂肪、蛋白質、乳糖、總固形物、檸檬酸、尿素氮、體細胞數、乳酸度、比重、生菌數、無脂固形物**等，累計認證共11個檢測項目。
- 通過授權使用「**TAF及ilac-MRA實驗室組合標記**」，將牛乳檢驗室提升至國際水準。
- 通過國際畜政聯盟(The International Committee for Animal Recording, **ICAR**)基準實驗室國際網成員之「乳質分析委員會」會員。

牛乳樣驗室

- 牛乳檢驗室秉持「**公信、準確、精確**」品質政策，爲乳業界提供檢驗服務，提升檢測品質及技術能力。
- 牛乳檢驗室已經過公正單位認證，做為**國內**乳質檢測之**標竿**，同時與**國外**相互認證，提高實驗室檢測之公信力及競爭力。