

The Case of the Cow Leandra and Her Daughter Jamaicana

Igor Ajuriaguerra, Alfonso del Río, Álvaro Lagüera, Damián Canales, Luis Dubarbie, Manuel Pérez, and Manuel Sainz de Aja (VETERINARIOS ASOCIADOS, S.C.)

The case starts with a call from a dairy farmer requesting a reproductive control service, specified on the day of the visit. We arrived at a farm with 80 adult cows, animals that had given birth at least once, 16 heifers under one year old and 22 heifers over one year but under 32 months. The farmer hired us to perform a reproductive control on the herd. We started by taking data, case history... It seemed that the number of young animals was small for the number of adult cows. After asking the farmer about the young animals, he told us that many calves were dying in the first two months of life due to problems of diarrhoea and pneumonia. Breeding management was not ideal, but it did not appear so bad as to have such a high mortality rate. The age of first insemination is 16 months, as the heifers do not reach the desired size and weight at an earlier age. **“They do not become pregnant well and have irregular cycles”**, the farmer told us. He has heifers that were inseminated and then entered oestrous two or three months later. He did not use a pregnancy test, so he is not sure if the cause is really that they did not become pregnant or if he has a problem of embryonic death or abortions. As for the cows, he tells us a similar story. They do not become pregnant and they do not cycle normally, oestrous that does not coincide with three-week cycles and many cows that are believed to be pregnant re-enter oestrus two or three months later.

With all the animals identified and their reproductive status updated, we started with a periodic control: one visit every 14 days. After four visits, it was clear that the cows became pregnant better than the farmer had told us. We began to perform a pregnancy test 24 days after insemination and we reconfirmed these cows after 14 days, at the following visit. We subsequently reviewed these pregnant cows at 57 days of gestation, with two objectives: learn the sex of the foetus or foetuses and observe their vitality or viability. The result was that the cows become pregnant, but when we went to reconfirm the pregnant cows, 37% of the cows had suffered a loss of gestation or embryonic death. We learned that we had cows that abort.

During this period, we detected that heifers that had given birth and entered the yard for lactating cows all suffered from a serious infection during their first week in this yard,

with a significant increase in heart and respiratory rate, dyspnoea and a significant increase in body temperature. After a four-day treatment with NSAIDs and antibiotics, their vital signs began to normalise but they did not fully recover. Their normal feed intake and corresponding production did not recover until at least 20 days since the start of lactation. The cows that had been dry outside the lactating cow yard also suffered a similar infection when they gave birth and were introduced to the lactation yard. However, their symptoms were less spectacular and they recovered before the first-time heifers.

All this led me to suspect the presence of the BVD virus in the herd, and I returned to ask the farmer if he had ever performed any tests for diseases related to reproductive changes. The farmer's response was unique: **“I have never looked, but there is one thing that I didn't tell you: I stopped vaccinating more than a year ago because that vaccine that they say works for abortion doesn't do anything, they continue aborting or getting pregnant just as poorly”**. Based on a friend's referral, he vaccinated the herd with a polyvalent vaccine against BVD, IBR, parainfluenza and syncytial virus, re-vaccinating every 6 months for two years. This had the farmer convinced that his problem was not BVD, because if his problem was BVD the vaccine would have had to have solved it. It was not effective and he stopped using the vaccine.

To confirm my suspicions and taking into account that the farmer did not want to spend much money on a study of abortion causes, blood was drawn from 17 heifers aged between 6 and 19 months who had never been vaccinated against BVD. The blood was sent to the laboratory and a serology was ordered with the aim of ruling out other causes. The results were clearly positive for the BVD antibodies in 70% of the animals. The hypothesis began to be confirmed, there was a high circulation of the BVD virus in the animals on the farm (IMAGE 1).

We took a milk sample from the tank, where there was milk from all the lactating cows, to perform PCR and look for the presence of BVD antigens. The milk PCR indicated the presence of the BVD antigen in the lactating cows, which gave us two possibilities regarding the lactating cows: either there were cows in the viraemia stage

Erreg. Zk./Nº Reg. 0.14.04816
Sarrera/Entrada 09/10/2014
Irteera/Salida 16/10/2014

VETERINARIOS ASOCIADOS, S.C.
Paseo La Ventilla, 13 - SOLARES
39710 MEDIO CUDEYO (CANTABRIA)

Informe emitido por NEIKER (Instituto Vasco de Investigación y Desarrollo Agrario)

IRTEERAKO TXOSTENA / INFORME DE SALIDA

JABEA/PROPIETARIO-ERREFERENTZIA/REFERENCIA:
MOTA/ESPECIE: BOVINO - ABELGORRIA
LAGINKETA/MUESTREO: Cliente/Bezeroak
LAGINAK/MUESTRAS:
17 Suero sanguíneo / Odol serumo

MICROBIOLOGIA/INMUNOLOGIA

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | ELISAb BVD Bp80 * | ELISAb IBp8E * | ELISAI NEOS * | FC CLAH * |
|-----------|------------|-----------------|-------------------|----------------|---------------|-----------|
| 001.00 | 7391 | Suero sanguíneo | POS | Neg | Neg | --- |
| 002.00 | 9396 | Suero sanguíneo | POS | Neg | Neg | --- |
| 003.00 | 4490 | Suero sanguíneo | POS | POS | Neg | --- |
| 004.00 | 3739 | Suero sanguíneo | POS | Neg | POS | --- |
| 005.00 | 6390 | Suero sanguíneo | POS | Neg | Neg | --- |
| 006.00 | 5271 | Suero sanguíneo | POS | Neg | --- | Neg |
| 007.00 | 3515 | Suero sanguíneo | POS | Neg | --- | Neg |
| 008.00 | 3514 | Suero sanguíneo | POS | Neg | --- | Neg |
| 009.00 | 3516 | Suero sanguíneo | POS | Neg | --- | Neg |
| 010.00 | 8772 | Suero sanguíneo | ODU | Neg | --- | Neg |
| 011.00 | 8773 | Suero sanguíneo | Neg | Neg | --- | Neg |
| 012.00 | 8771 | Suero sanguíneo | Neg | Neg | --- | Neg |
| 013.00 | 155 | Suero sanguíneo | Neg | Neg | --- | Neg |
| 014.00 | 151 | Suero sanguíneo | POS | Neg | --- | Neg |
| 015.00 | 5270 | Suero sanguíneo | POS | Neg | --- | Neg |
| 016.00 | 1857 | Suero sanguíneo | POS | Neg | --- | Neg |
| 017.00 | 1856 | Suero sanguíneo | Neg | Neg | --- | Neg |

ELISAb BVD Bp80: ELISAb anticuerpos BVD/Bp80 * ELISAb IBp8E: ELISAb IBp8E * ELISAI NEOS: ELISAI anticuerpos NEOSPORIS *
FC CLAH: Fijación del Complemento CLASIFICACIONES *
POS: Positivo Neg: Negativo Neg+: Negativo cercano al punto de corte. Remitir nueva muestra.

IMAGE 1

Erreg. Zk./Nº Reg. 0.14.05234
Sarrera/Entrada 24/10/2014
Irteera/Salida 29/10/2014

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IRTEERAKO TXOSTENA / INFORME DE SALIDA

JABEA/PROPIETARIO-ERREFERENTZIA/REFERENCIA:
MOTA/ESPECIE: BOVINO - ABELGORRIA
LAGINKETA/MUESTREO: Cliente/Bezeroak
LAGINAK/MUESTRAS:
1 Leche tanque / Tangako esnea

MICROBIOLOGIA/INMUNOLOGIA

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | rRT-PCR Pesti-H * |
|-----------|------------|--------------|-------------------|
| 001.00 | MAZON | Leche tanque | POS |

rRT-PCR Pesti-H: rRT-PCR Pesti-H *
POS: Positivo Neg: Negativo Neg+: Negativo cercano al punto de corte. Remitir nueva muestra.

IMAGE 2

who were excreting the BVD antigen in milk or we had a BVD persistently infected cow among the lactating cows (IMAGE 2).

We took blood samples from all the lactating cows and we ordered a study for the presence of P80 antibodies and we added a comment: perform ELISA to determine

Erreg. Zk./Nº Reg. 0.14.05767_p1
Entrada/Sarrera 11/11/2014
Salida/Irteera 14/11/2014

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AURRETIAZKO IRTEERAKO TXOSTENA / INFORME DE SALIDA PRELIMINAR

JABEA/PROPIETARIO-ERREFERENTZIA/REFERENCIA:
ESPECIE/MOTA: BOVINO - ABELGORRIA
MUESTREO/LAGINKETA: Cliente/Bezeroak
MUESTRAS/LAGINAK:
80 Sangre entera / Odol osoa

MICROBIOLOGIA/INMUNOLOGIA

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | ELISAag BVD | ELISAb BVD Bp80 * |
|-----------|------------|---------------|-------------|-------------------|
| 001.00 | 0166 | Sangre entera | --- | POS |
| 002.00 | 1475 | Sangre entera | --- | POS |
| 003.00 | 9423 | Sangre entera | --- | POS |
| 004.00 | 8511 | Sangre entera | --- | POS |
| 005.00 | 9680 | Sangre entera | --- | POS |
| 006.00 | 4985 | Sangre entera | --- | POS |
| 007.00 | 3885 | Sangre entera | --- | POS |
| 008.00 | 1477 | Sangre entera | --- | POS |
| 009.00 | 7391 | Sangre entera | --- | POS |
| 010.00 | 4984 | Sangre entera | --- | POS |
| 011.00 | 8580 | Sangre entera | --- | POS |
| 012.00 | 0165 | Sangre entera | --- | POS |
| 013.00 | 9050 | Sangre entera | --- | POS |
| 014.00 | 1618 | Sangre entera | --- | POS |
| 015.00 | 7680 | Sangre entera | --- | POS |
| 016.00 | 4742 | Sangre entera | --- | POS |
| 017.00 | 3886 | Sangre entera | --- | POS |
| 018.00 | 0422 | Sangre entera | --- | POS |
| 019.00 | 9396 | Sangre entera | --- | POS |
| 020.00 | 7426 | Sangre entera | --- | POS |
| 021.00 | 3172 | Sangre entera | --- | POS |
| 022.00 | 9392 | Sangre entera | --- | POS |
| 023.00 | 2088 | Sangre entera | --- | POS |
| 024.00 | 8227 | Sangre entera | --- | POS |
| 025.00 | 0168 | Sangre entera | --- | POS |
| 026.00 | 8006 | Sangre entera | --- | POS |
| 027.00 | 4304 | Sangre entera | Neg | POS |
| 028.00 | 0446 | Sangre entera | --- | POS |
| 029.00 | 4491 | Sangre entera | --- | POS |
| 030.00 | 5974 | Sangre entera | --- | POS |
| 031.00 | 8150 | Sangre entera | --- | POS |
| 032.00 | 7392 | Sangre entera | --- | POS |
| 033.00 | 2345 | Sangre entera | --- | POS |
| 034.00 | 7482 | Sangre entera | --- | POS |
| 035.00 | 4943 | Sangre entera | --- | POS |

ELISAag BVD: ELISA para la detección de antígenos del Virus BVD (FC/ELIS-117)
POS: Positivo Neg: Negativo Neg+: Negativo cercano al punto de corte. Remitir nueva muestra.

IMAGE 3

| | | | | |
|--------|------|---------------|-----|-----|
| 036.00 | 9693 | Sangre entera | --- | POS |
| 037.00 | 7325 | Sangre entera | --- | POS |
| 038.00 | 6939 | Sangre entera | Neg | Neg |
| 039.00 | 4555 | Sangre entera | --- | POS |
| 040.00 | 7711 | Sangre entera | --- | POS |
| 041.00 | 2914 | Sangre entera | --- | POS |
| 042.00 | 4490 | Sangre entera | --- | POS |
| 043.00 | 6564 | Sangre entera | --- | POS |
| 044.00 | 8208 | Sangre entera | --- | POS |
| 045.00 | 1445 | Sangre entera | --- | POS |
| 046.00 | 4804 | Sangre entera | --- | POS |
| 047.00 | 5479 | Sangre entera | --- | POS |
| 048.00 | 1475 | Sangre entera | --- | POS |
| 049.00 | 6938 | Sangre entera | --- | POS |
| 050.00 | 9172 | Sangre entera | --- | POS |
| 051.00 | 1264 | Sangre entera | --- | POS |
| 052.00 | 6390 | Sangre entera | --- | POS |
| 053.00 | 0764 | Sangre entera | --- | POS |
| 054.00 | 4419 | Sangre entera | --- | POS |
| 055.00 | 3739 | Sangre entera | --- | POS |
| 056.00 | 7580 | Sangre entera | POS | Neg |
| 057.00 | 2716 | Sangre entera | --- | POS |
| 058.00 | 2726 | Sangre entera | --- | POS |
| 059.00 | 2169 | Sangre entera | --- | POS |
| 060.00 | 3631 | Sangre entera | --- | POS |
| 061.00 | 9296 | Sangre entera | --- | POS |

Erreg. Zk./Nº Reg. 0.14.05767_p1
Entrada/Sarrera 11/11/2014
Salida/Irteera 14/11/2014

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | ELISAag BVD | ELISAb BVD Bp80 * |
|-----------|------------|---------------|-------------|-------------------|
| 062.00 | 0447 | Sangre entera | --- | POS |
| 063.00 | 3361 | Sangre entera | --- | POS |
| 064.00 | 8329 | Sangre entera | --- | POS |
| 065.00 | 2343 | Sangre entera | --- | POS |
| 066.00 | 7463 | Sangre entera | --- | POS |
| 067.00 | 4201 | Sangre entera | --- | POS |
| 068.00 | 6635 | Sangre entera | --- | POS |
| 069.00 | 8148 | Sangre entera | --- | POS |
| 070.00 | 2701 | Sangre entera | --- | POS |
| 071.00 | 2702 | Sangre entera | --- | POS |
| 072.00 | 1756 | Sangre entera | --- | POS |
| 073.00 | 1760 | Sangre entera | --- | POS |
| 074.00 | 1758 | Sangre entera | --- | POS |
| 075.00 | 1757 | Sangre entera | --- | POS |
| 076.00 | 2035 | Sangre entera | --- | POS |
| 077.00 | 7141 | Sangre entera | --- | POS |
| 078.00 | 4268 | Sangre entera | --- | POS |
| 079.00 | 7044 | Sangre entera | --- | POS |
| 080.00 | 4267 | Sangre entera | --- | POS |

ELISAag BVD: ELISA para la detección de antígenos del Virus BVD (FC/ELIS-117)
POS: Positivo Neg: Negativo Neg+: Negativo cercano al punto de corte. Remitir nueva muestra.

the BVD antigen in the cows that were negative. Of 80 cows, 77 were positive for P80 antibodies in blood and 3 negative. One of the negative cows was positive for the BVD antigen in blood. This led us to think that the cow Leandra 7580, a 49-month old cow, might possibly be persistently infected with BVD (IMAGE 3).

She was apparently a normal cow. She did not show any alterations that would make one think that she could be a persistently infected cow, but the farmer considered her the bad one of the herd. He thought that it was due to the fact that she was sick many times as a calf and that caused a delay in her growth, which is why she was much smaller than the others. She is a cow who suffered an abortion as a heifer, this delayed her first birth to the age of 28 months. When we detected her she had given birth 96 days previously and was producing her second lactation. The calf from her second birth died in its second month of life, due to repeated infections that dogged her since she was born. The farmer treated the calf with antibiotics and NSAIDs, but in the end it died. However, the calf from the first birth was alive. She was among the 17 heifers whose blood had been tested, number 0151 (Jamaicana). She became pregnant at 15 months of age and had the best body condition and size of the heifers in her same batch. Her laboratory tests were positive for P80 antibodies.

In all, there were several things that were not normal: an animal resulting from a persistently infected cow is another persistently infected cow and should not present P80 antibodies in her blood. It was also not normal that she was the best heifer of the batch or that she had exemplary reproductive behaviour.

Ten days after taking the first samples, another blood sample was drawn from both mother and daughter and sent to the laboratory. A P80 antibody and BVD antigen test was ordered for both (IMAGE 4).

The result was the same. The mother presented the BVD antigen with high levels similar to the first sample drawn, and the daughter continued to be positive for P80 antibodies and negative for the presence of the antigen in her blood. It seemed that we had found a daughter resulting from a persistently infected mother who did not present

the antigen in her blood and had seroconverted, as she presented P80 antibodies.

Twenty-three days after the first extraction, another blood sample was taken from the cow and genotyping was ordered to learn which serotype it was and to confirm the presence of the BVD antigen. The result regarding the presence of the antigen and antibodies was the same. It was BVD type 1 (IMAGE 5).

In all three tests performed, with a 23-day interval between the first and the last, the cow showed high levels of the BVD antigen, which confirmed that this was not a transitory viraemia since the cow had similar titres in all three tests. If this was a transitory viraemia, they would not be similar. They would have been falling and in the last sample extracted normally there would be no BVD antigen in the cow's blood. The cow was persistently infected, only we had one thing that in theory was impossible: the serology of her daughter.

We decided to draw one last blood sample from the mother, before putting her down, and another from the daughter to send to the laboratory for a maternity test, which would confirm whether they were truly mother and daughter. The result was negative, she was not her daughter. The farmer mixed them up when identifying the animals. Two calves were born that day and they were very similar to each other. The partner calf to the false daughter, the true daughter, died in her first months of life due to infection, the daughter from the persistently infected cow's first birth. This maternity test is what definitively demonstrated that the cow was persistently infected, since the resulting calves had to be persistently infected (IMAGES 6 AND 7).

We ended up testing all the animals on the farm to be sure that there were no other persistently infected animals in the herd. We did not find any other persistently infected cows. Blood was drawn from the calves between the ages of 6 and 9 months to see if they had P80 antibodies in their blood. This would indicate to us whether or not the virus was circulating. The calf born 26 days after putting down the persistently infected cow was the first to not have P80 antibodies in her blood. The calves who were born later continued to be negative (IMAGE 8).

Erreg. Zk./Nº Reg. 0.14.06015
 Sarrera/Entrada 20/11/2014
 Irteera/Salida 04/12/2014

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 39710 MEDIO CUDEYO (CANTABRIA)

Informe emitido por: NEIKER (Instituto Vasco de Investigación y Desarrollo Agrario)

IRTEERAKO TXOSTENA / INFORME DE SALIDA

JABEA/PROPIETARIO-ERREFERENTZIA/REFERENCIA:
 MOTA/ESPECIE: BOVINO - ABELGORRIA
 LAGINKETA/MUESTREO: Cliente/Bezeroak
 LAGINAK/MUESTRAS:
 3 Sangre entera / Odol osoa

MICROBIOLOGIA/INMUNOLOGIA

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | ELISAag BVD | ELISAg BVD BDP80 * | rRT-PCR Pestil-H * |
|-----------|--------------|---------------|-------------|--------------------|--------------------|
| 002.06 | 0151 (HISA) | Sangre entera | Reg | POS | Reg |
| 002.06 | 7580 (MADRE) | Sangre entera | POS | POS | POS |

ELISAag BVD: ELISA para la detección de anticuerpos del Virus BVD BVD BDP80. ELISAag BVD BDP80: ELISA para la detección de anticuerpos BVD BDP80. rRT-PCR Pestil-H: rRT-PCR Pestil-H para la detección de BVD BDP80. * POS: Positivo. Reg: Negativo. Reg*: Negativo cuando el punto de corte muestra muestra.

IMAGE 4

Erreg. Zk./Nº Reg. 0.14.06290
 Sarrera/Entrada 03/12/2014
 Irteera/Salida 16/12/2014

VETERINARIOS ASOCIADOS, S.C.
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 39710 MEDIO CUDEYO (CANTABRIA)

Informe emitido por: NEIKER (Instituto Vasco de Investigación y Desarrollo Agrario)

IRTEERAKO TXOSTENA / INFORME DE SALIDA

JABEA/PROPIETARIO-ERREFERENTZIA/REFERENCIA:
 MOTA/ESPECIE: BOVINO - ABELGORRIA
 LAGINKETA/MUESTREO: Cliente/Bezeroak
 LAGINAK/MUESTRAS:
 1 Sangre entera / Odol osoa

MICROBIOLOGIA/INMUNOLOGIA

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | ELISAag BVD | ELISAag BVD BDP80 * | rRT-PCR BVDv1. 2 * | rRT-PCR Pestil-H * |
|-----------|------------|---------------|-------------|---------------------|--------------------|--------------------|
| 002.06 | 7580 | Sangre entera | POS | Reg | BVDv1. 2 | POS |

ELISAag BVD: ELISA para la detección de anticuerpos del Virus BVD BVD BDP80. ELISAag BVD BDP80: ELISA para la detección de anticuerpos BVD BDP80. rRT-PCR BVDv1. 2: rRT-PCR BVDv1. 2 para la detección de BVD BDP80. rRT-PCR Pestil-H: rRT-PCR Pestil-H para la detección de BVD BDP80. * POS: Positivo. Reg: Negativo. Reg*: Negativo cuando el punto de corte muestra muestra.

IMAGE 5

ANÁLISIS DE FILIACIÓN⁽¹⁾
(Informe emitido por Xenética Fontao, S.A.)

Remitente: VETERINARIOS ASOCIADOS S.C. Entrada: 30/01/2015
 Dirección: PASEO DE LA VENTILLA, 13 - 39710 SOLARES CANTABRIA
 Propietario: [REDACTED] Cód.: [REDACTED] C.E.A.: [REDACTED]

Nº Muestra: 15M01534 I.M.R.: 3 Especie: VACUNO Raza: FRISONA
 Nombre: JAMAICANA Nº Registro: [REDACTED] C.I.B.: [REDACTED] 151
 Sexo: HEMBRA Fecha Nac.: 26/05/2013 Tipo de Muestra: SANGRE ENTERA

I.M.B. Identificación de Muestra Recibida

| LOCÍ | MUESTRA | PADRE | MADRE |
|------|----------|---------|---------|
| 1 | BM1818 | 262 262 | 260 266 |
| 2 | BM1824 | 182 188 | 178 188 |
| 3 | BM2113 | 135 135 | 125 139 |
| 4 | CSRM60 | 96 102 | 98 102 |
| 5 | ETH10 | 219 219 | 219 225 |
| 6 | ETH185 | 228 232 | 228 233 |
| 7 | ETH225 | 140 150 | 148 150 |
| 8 | ETH3 | 117 117 | 117 127 |
| 9 | ILSTS006 | 288 292 | 288 294 |
| 10 | INRA005 | 137 139 | 137 137 |
| 11 | INRA023 | 206 206 | 210 210 |
| 12 | INRA063 | 172 172 | 170 172 |
| 13 | SPS115 | 254 256 | 248 252 |
| 14 | TGLA122 | 151 163 | 149 163 |
| 15 | TGLA126 | 115 115 | 117 117 |
| 16 | TGLA227 | 83 87 | 97 97 |
| 17 | TGLA53 | 162 162 | 156 168 |

Cód. Análisis: F1501064

PADRE 08M19908
FRAM5694028588
JOCKO BESN

NO COMPATIBLE

MADRE 15M01535
ESPH3903363143
LEANDRA

NO COMPATIBLE

Lugo, 13 de Febrero de 2015

IMAGE 6

ANÁLISIS DE FILIACIÓN⁽¹⁾
(Informe emitido por Xenética Fontao, S.A.)

Remitente: VETERINARIOS ASOCIADOS S.C. Entrada: 30/01/2015
 Dirección: PASEO DE LA VENTILLA, 13 - 39710 SOLARES CANTABRIA
 Propietario: [REDACTED] Cód.: [REDACTED] C.E.A.: [REDACTED]

Nº Muestra: 15M01535 I.M.R.: 4 Especie: VACUNO Raza: FRISONA
 Nombre: LEANDRA Nº Registro: [REDACTED] C.I.B.: [REDACTED] 7580
 Sexo: HEMBRA Fecha Nac.: 03/10/2010 Tipo de Muestra: SANGRE ENTERA

I.M.B. Identificación de Muestra Recibida

| LOCÍ | MUESTRA | PADRE | MADRE |
|------|----------|---------|---------|
| 1 | BM1818 | 260 266 | 260 266 |
| 2 | BM1824 | 178 180 | 178 188 |
| 3 | BM2113 | 125 137 | 125 139 |
| 4 | CSRM60 | 92 102 | 98 102 |
| 5 | ETH10 | 213 225 | 219 225 |
| 6 | ETH185 | 228 238 | 228 233 |
| 7 | ETH225 | 148 148 | 148 150 |
| 8 | ETH3 | 117 129 | 117 127 |
| 9 | ILSTS006 | 294 294 | 288 294 |
| 10 | INRA005 | 137 139 | 137 137 |
| 11 | INRA023 | 210 210 | 210 210 |
| 12 | INRA063 | 170 170 | 170 172 |
| 13 | SPS115 | 248 248 | 248 252 |
| 14 | TGLA122 | 143 163 | 149 163 |
| 15 | TGLA126 | 117 117 | 117 117 |
| 16 | TGLA227 | 81 97 | 97 97 |

Cód. Análisis: F1501065

PADRE 08M19908
FRAM5694028588
JOCKO BESN

COMPATIBLE

MADRE 08M27684
ESPH3902638360
PACENCIA

COMPATIBLE

Lugo, 13 de Febrero de 2015

IMAGE 7

The farm abutted other cattle farms and there was the possibility that the BVD virus would return to pose a risk for the herd. As it was free from persistently infected cows, we made the decision to vaccinate all animals over 9 months with a new live vaccine that contains serotypes 1 and 2, BOVELA.

Those under 9 months were not vaccinated for economic reasons. The farmer was convinced that all these vaccines were useless and until that age the calves were isolated from other animals on this or the adjacent farms. They had contact with the animals on the farm during their lactation period, since they were raised with milk from their cows.

For the following 9 months, blood was drawn from all newborn calves, before taking the colostrum, to see if they presented the BVD antigen in order to prevent a persistently infected cow from entering the herd.

After this period, animals began to be born from cows who had already been vaccinated with Bovela. We stopped drawing blood from the newborn calves as the vaccine prevented the formation of persistently infected animals.

We are currently continuing to draw blood from a few sentinel heifers before being vaccinated and the results continue to be negative for the presence of P80 antibodies in blood. The herd's mortality is much lower, the number of calves born is higher, and the heifers are inseminated

Erreg. Zk./Nº Reg. 0.15.03524
 Sarriena/Entrada 14/07/2015
 Interea/Salida 14/07/2015

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IRTEERAKO TXOSTENA / INFORME DE SALIDA

JABEA/PROPIETARIO-ERREFERENTZIA/REFERENCIA:
 MOTAI/ESPECIE: BOVINO - ABELGORRIA
 LAGINKETA/MUESTREO: Cliente/Bezerakak
 LAGINA/MUESTRAS:
 11 Sangre entera / Odol osoa

MICROBIOLOGIA/INMUNOLOGIA

| ID.NEIKER | ID.CLIENTE | TIPO MUESTRA | ELISAng BVD | ELISAg BVD 8Dydo * |
|-----------|------------|---------------|-------------|--------------------|
| 001.00 | 3918 | Sangre entera | Neg | Neg |
| 002.00 | 4268 | Sangre entera | --- | POS |
| 003.00 | 4595 | Sangre entera | --- | POS |
| 004.00 | 8824 | Sangre entera | Neg | Neg |
| 005.00 | 4512 | Sangre entera | --- | POS |
| 006.00 | 4120 | Sangre entera | Neg | Neg |
| 007.00 | 4122 | Sangre entera | Neg | Neg |
| 008.00 | 8820 | Sangre entera | --- | POS |
| 009.00 | 8821 | Sangre entera | Neg | Neg |
| 010.00 | 4121 | Sangre entera | Neg | Neg |
| 011.00 | 4595 | Sangre entera | --- | POS |

ELISAng BVD: ELISA para la detección de antígenos del Virus ELISAg BVD 8Dydo: ELISAg anticuerpos BVD/80 días *
 POS: Positivo Neg: Negativo ---: Resultado al punto de corte. Identificar nueva muestra.

IMAGE 8

at younger ages as they reach the necessary size and weight earlier. The abortion and embryonic death rates are insignificant and very rare. When animals are introduced to the field for lactating cows, they have a better start to lactation, since they no longer suffer the acute episodes of viraemia, the immune system does not undergo immunosuppression caused by a viral infection or continuous viral reinfection and they better overcome the normal state of immunosuppression that lactating cows undergo during birth/postpartum.