Nortestosterone and casualty animals

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Northern Ireland, UK

National Surveillance Scheme
"Random"
No detention of carcases
Non-compliant: Investigate

Meat Inspection
All animals inspected
Ante & post mortem
Suspects detained
Non-compliant: Destroy meat
Investigate

Hormones and β-agonists in NI

Imprint Pellets
Hormones
β-Agonists

Low level of abuse or Smarter abusers?

Hormone testing in Northern Ireland

The far west of the European Union

The Wild West of the European Union??
17-α-19-Nortestosterone

Occurs naturally in
- pregnant cows (α)
- boars (β)
- stallions (β)
- sheep (α)

Another “difficult” compound
- boldenone
- zeranol
- testosterone
- oestradiol
- progesterone

Does not occur naturally in male cattle

No stolen animals: but very well-finished

2004
XEL-S

19-Nortestosterone decanoate  Trace
17ß-oestradiol  Major
17ß-oestradiol benzoate  Major
Testosterone propionate  Major
Trenbolone acetate  Trace

Laboratory findings

Anabolic preparations: none found
Injection sites  none found
Syringes  β-Nortestosterone decanoate
Urine from steers  No nortestosterone
High progesterone
**Hormones covered by NI testing schemes**

<table>
<thead>
<tr>
<th>HORMONE</th>
<th>HORMONE</th>
<th>HORMONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Boldenone</td>
<td>Methyltestosterone</td>
<td>Norethandrolone</td>
</tr>
<tr>
<td>β-Boldenone</td>
<td>D(-) Norgestrel</td>
<td>α-Nortestosterone</td>
</tr>
<tr>
<td>CLAD</td>
<td>β-Nortestosterone</td>
<td></td>
</tr>
<tr>
<td>Dienestrol</td>
<td>Progesterone</td>
<td>Stanozolol</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>Taleranol</td>
<td>β-Nortestosterone</td>
</tr>
<tr>
<td>Epitestosterone</td>
<td>Testosterone</td>
<td>Zearalenol</td>
</tr>
<tr>
<td>α-Estradiol</td>
<td></td>
<td>β-Zearalenol</td>
</tr>
<tr>
<td>β-Estradiol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethisterone</td>
<td>β-Zearalenol</td>
<td></td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>α-Zearalenone</td>
<td></td>
</tr>
<tr>
<td>Hexestrol</td>
<td></td>
<td>Zearalenone</td>
</tr>
<tr>
<td>16ß-Hydroxy stanozolol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medroxyprogesterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methenolone</td>
<td>Zeranol</td>
<td></td>
</tr>
</tbody>
</table>

**Proficiency Testing Schemes**

- α-Nortestosterone
- Last 6 years
  - 1 RIVM – CRL Ring Test
  - 8 Progetto Trieste Ring Tests
  - No false positives
  - No false negatives
  - All z-scores between –2 and +2
  - Analytical method under control

**Run time**

50 X 2.1 mm UPLC Column

31 analytes eluted in 5 minutes
System re-equilibrated and ready to inject again after 6 minutes

**As 2006 crisis developed...**

- Urine
  - Biosensor Screen
    - Compliant
    - Non-Compliant
      - LC (± MS/MS)
      - GC-MS/MS (heptafluorobutyric AA)

- d3-β-Nortestosterone
  - α-NT
  - β-NT

- d3-α-Nortestosterone
  - α-NT
  - β-NT
**OFES positive sample**

- **d₃-ß-Nortestosterone**
- **α-NT**
- **β-NT**

**GC-MS/MS**

**17α,19-Nortestosterone in bovine urine**

<table>
<thead>
<tr>
<th>Fortification Level</th>
<th>Mean Recovery</th>
<th>SD</th>
<th>ε</th>
<th>% Recovery</th>
<th>Within day CV</th>
<th>Between day CV</th>
<th>Intermediate CV</th>
<th>Precision CV</th>
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</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.56</td>
<td>0.06</td>
<td>10</td>
<td>107.6</td>
<td>5.5</td>
<td>5.0</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>1.08</td>
<td>0.09</td>
<td>10</td>
<td>107.9</td>
<td>6.9</td>
<td>5.6</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>1.69</td>
<td>0.13</td>
<td>21</td>
<td>112.4</td>
<td>6.6</td>
<td>5.0</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>2.28</td>
<td>0.16</td>
<td>21</td>
<td>113.1</td>
<td>5.3</td>
<td>5.5</td>
<td>7.7</td>
<td></td>
</tr>
</tbody>
</table>

- **CCα 0.30 µg/kg**
- **CCβ 0.59 µg/kg**

**2006 Emergency**

- **Meat Inspection Scheme**
- **10th March 2006**
- **α-Nortestosterone - 1.03 ppb**
- **β-Nortestosterone – Compliant**
- **Carcass condemned**
- **Subsequently found out: “casualty” animal**

**Casualty animal types**

- **FCI** Food Chain Information
  - Farmer declares animal injured

- **AMF** Ante-Mortem Finding
  - “walking wounded”
  - Injury discovered at Meat Plant

- **OFES** On-Farm Emergency Slaughter
  - Killed on farm, certified by Vet
  - Sent to Plant dead (< 3 hours)

**Test all casualties!!**

- Instruction went out from Ministry vets
- All Meat Plants in Northern Ireland
- “Sample all casualty animals”
- More non-compliant animals found
Sample splitting

Intense media/political interest
Allegations !!!
Interference with samples in transit
Interference with samples in laboratory
Sample splitting
  either in the lab (QA Unit)
  or in Meat Plant (Lab vet)
Renumber (blind)
Analyse - original sample screened positive

Re-testing in other labs

RI VM The Netherlands (n=11)
LABERCA France (n= 6)
Mixture of compliant and non-compliant
No contradictory results obtained
Finally “Lab testing is reliable!”
### Positives by casualty type

<table>
<thead>
<tr>
<th>Gender</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bull</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>9</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>16</td>
<td>38</td>
<td>52</td>
</tr>
</tbody>
</table>

All 72 positive animals removed from food chain
OFES animals predominate (86%)
Male (castrates) predominate (88%)

### Nortestosterone concentrations

**α-Nortestosterone**
*present in all 72 non-compliant samples*
*range CCα - 17.2 ppb*
*level similar to treated animals*

**β-Nortestosterone**
*present in only 5 non-compliant samples*
*range CCβ - 1.4 ppb*

### Clinical History - OFES only

<table>
<thead>
<tr>
<th>History</th>
<th>α-Nortestosterone Positive</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia/Down</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Back Injury</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Dislocation</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fracture</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>Hip Injury</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Lameness</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>Nerve Paralysis</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Down</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>66</strong></td>
<td><strong>128</strong></td>
</tr>
</tbody>
</table>

### Can NT use be out of control?

Do the farmers have a “history”?
Did the prevalence change?
What was found on farms?
Is “Dead on Arrival” relevant?
What about normal animals?
Does it happen elsewhere?
Have any follow-up animals been injured?
What other explanation is possible?

### Do the farmers have a “history”?

### Did the prevalence change?

<table>
<thead>
<tr>
<th>Weeks</th>
<th>% Samples testing positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-14</td>
<td>10</td>
</tr>
<tr>
<td>15-18</td>
<td>29</td>
</tr>
<tr>
<td>19-22</td>
<td>54</td>
</tr>
<tr>
<td>23-26</td>
<td>29</td>
</tr>
<tr>
<td>27-30</td>
<td>22</td>
</tr>
<tr>
<td>31-34</td>
<td>16</td>
</tr>
</tbody>
</table>
### On-farm investigations

**First cases**
- Police and Ministry vets
- Raid farm and house

**Later cases**
- Ministry vets
- Raid farm

*No nortestosterone detected*

### What is found in normal animals?

Other testing schemes

422 samples tested for nortestosterone

Three positives

1) **condemned emaciated**
2) **condemned TB reactor**
3) **under investigation**

1) & 2) not “normal”

3) at follow up, all urine negative
**Does it happen anywhere else?**

Irish Republic (OFES)
- Hormone problems in the past
  - 16 samples
  - 10 non-compliant for \(\alpha\)-NT

Great Britain (OFES & AMF)
- No known problem with hormones
  - 8 samples
  - 1 non-compliant for \(\alpha\)-NT

**Have any follow-up animals been injured?**

**Case 1 Casualty (AMF 06/04/06)**
- \(\alpha\)-Nortestosterone 1.5 ppb
- On farm visit 24/05/06
- 18 animals sampled
- All negative
- 1/18 fracture 28/06/06
- Retested
  - \(\alpha\)-Nortestosterone 1.88 ppb
  - \(\beta\)-Nortestosterone compliant

**Case 2 Casualty (OFES 30/06/06)**
- \(\alpha\)-Nortestosterone 1.5 ppb
- On farm visit 24/07/06
- 10 animals sampled
- All negative
- 1/10 fracture 07/08/06
- Retested
  - \(\alpha\)-Nortestosterone 3.41 ppb
  - \(\beta\)-Nortestosterone compliant

**Can NT use be out of control?**

- Farmer “history”? No
- Problem goes away? No
- On farm findings? Nothing
- Is “DoA” relevant? No
- Normal animals? Nothing obvious
- Just North Ireland? No
- Retested animals? Two positives
- Other explanations? Stress of the injury

**Stress**

- Biochemical link between stress & NT
- Difficult to model - can’t break a leg
- Simulated in laboratory
- Young bulls
- De-horn & castrate
- Collect urine & analyse
- No NT produced
- Investigations continue

**What do I need now?**

- **EU awareness of problem**
- **Debate on natural occurrence**
- **Sampling in other Member States**
- **Implementation of hair test**
- **Proof that it’s natural**
NORTESTOSTERONE IS NOT A NATURALLY OCCURRING COMPOUND IN MALE CATTLE.

D.G. Kennedy

SUMMARY

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INCOMING!