

## Estrogen-induced pancytopenia due to a Sertoli cell tumor in a cryptorchid Beauceron

*Oestrogeengeïnduceerde pancytopenie door een sertoliceltumor bij een cryptorche beauceron*

<sup>1</sup>H. De Bosschere, <sup>2</sup>C. Deprest

<sup>1</sup>Medisch Labo Bruyland – Afdeling Diergeneeskunde, Meiweg 1, B-8500 Kortrijk, Belgium

<sup>2</sup>Dierenartsenpraktijk Deprest, Verlovestraat 4, B-8755 Ruiselede, Belgium

### ABSTRACT

The present report describes the clinical history, radiography, ultrasonography, hematology, bone marrow sample, and histology of a case of estrogen-induced pancytopenia due to neoplastic change of cryptorchid testes. The anemia gradually improved after castration, several blood transfusions and treatment with nandrolonedecanoate.

### SAMENVATTING

Dit is een beschrijving van de voorgeschiedenis, radiografie, echografie, hematologie, beenmergpunctie en histologie van een oestrogeengeïnduceerde pancytopenie ten gevolge van een tumorale ontaarding van cryptorche testikels. Deze anemie verbeterde geleidelijk na castratie, verscheidene bloedtransfusies en nandrolonedecanoaat.

### INTRODUCTION

Pancytopenia is a medical condition in which there is a reduction in the numbers of red and white blood cells and of platelets due to damage to stem cells or to the bone marrow microenvironment leading to bone marrow failure. The frequently used term ‘aplastic anemia’ is confusing since all cell lines are affected, and not only the red blood cells. Bone marrow damage may be caused by infections, drugs, toxins, neoplasia, myelodysplasia, bone marrow necrosis, osteosclerosis, myelofibrosis or immune-mediated mechanisms, or it may be idiopathic (Harvey, 1997; Tilley and Smith, 2004; Villiers and Blackwood, 2005). In the acute form of the disease, the destruction of progenitor and dividing cells leads to leucopenia/neutropenia within 5 days and to thrombocytopenia within 8-10 days. Anemia develops more gradually due to the long red blood cell lifespan. Bone marrow aspiration reveals a mix of necrotic lysing cells, macrophages and stromal cells. Depending on the cause, the bone marrow may recover and be repopulated, usually within 3 weeks after the original marrow injury, or the disease may progress to the chronic form. In the chronic form, the stem cell damage is irreversible and the red marrow is replaced by fat, leading to neutropenia, thrombocytopenia and moderate to severe anemia (Villiers and Blackwood, 2005).

Estrogen-induced pancytopenia is only observed in dogs and ferrets (Crafts, 1948; Kociba and Caputo, 1981). The dog is very susceptible to the myelotoxic effects of estrogen when estrogen is administered in female dogs for urinary incontinence, mismating, estrus induction or pseudopregnancy, when it is administered

in male dogs for the management of prostatic hypertrophy or perianal adenoma, or when it is produced by a testicular tumor or ovarian granulosa cell tumor (Sontas *et al.*, 2009). The response of the bone marrow to estrogens varies from one dog to another, because some dogs may have fatal bone marrow suppression, whereas others have only mild to moderate damage (Crafts, 1948).

Clinical signs relate to thrombocytopenia (petechial hemorrhages, melena, etc.), neutropenia (pyrexia and sepsis) and anemia (lethargy and pallor).

The present case report describes the successful treatment of a paraneoplastic syndrome of estrogen-induced pancytopenia due to a Sertoli cell tumor in cryptorchid testes.

### CASE REPORT

#### Case history

A 9-year-old bilateral cryptorchid male Beauceron was presented in poor general condition. The owner was aware of the possible risks of cryptorchidism, but refused to castrate the dog. During the examination, the dog did not show any obvious signs of feminizing syndrome, such as gynecomastia or alopecia. However, based on the anamnesis of approximately one year prior to the time of presentation, the dog had then been making copulatory movements on the carpet. In the current anamnesis, however, there was no report of abnormal sexual behavior. Clinical examination revealed high fever (40.5°C) and increased heart beat (150 bpm). The dog had a lick granuloma on the hind leg and a large

**Table 1. Results of blood analysis of a 9-year-old bilateral cryptorchid Beauceron.**

Hematology	24/9	25/9	29/9	6/10	13/10	20/10	18/11	21/01	Units	References
Erythrocytes	2.90	1.86	2.2	1.61	1.66	1.56	3.54	5.94	x 10 <sup>12</sup> /l	5.5 – 7.5
Leucocytes	2.2	3.1	2.3	2.3	3.1	3.4	6.8	7.4	x 10 <sup>9</sup> /l	6.0 – 12.0
%Segmented neutrophils...	48.1	79.9	77.2	45.2	54.8	60.4	71.7	58.7		50 – 63
%Lymphocytes	47.7	14.6	14.6	45	36.7	30.2	23.9	32.9		13 – 30
%Monocytes	3.7	5.5	8.2	9.8	8.5	9.4	3.7	5.7		3 – 7
% Eosinophils	0.5	0	n.a.	n.a.	n.a.	n.a.	0.7	2.7		0 – 3
% Basophils	0.0	0	n.a.	n.a.	n.a.	n.a.	0	0		0 – 1
Hemoglobin	4.1	2.6	3.22	2.41	2.76	2.82	5	7.4	mmol/l	8.5 – 12.0
Hematocrit	210	138	140	104	112	110	299	416	ml/l	420 – 540
MCV	72	75	63.9	64.7	67.8	71	84	70	fl	67 – 80
MCH	14	14	18	15	15	16	14	12	fmol	13 – 16
MCHC	19	19	23	23	22	20	17	18	mmol/l	20 – 23
Platelets	1	5	3	4	12	6	22	133	x 10 <sup>3</sup> /μl	200 – 400
% Reticulocytes	0.5	0.7					2.4	5.8	%	< 2
# Reticulocytes	0.1	0.1					0.22	4.3	x 10 <sup>6</sup> /μ	
17-beta-estradiol	149								pg/ml	13 – 55

bite wound in the neck. The dog was treated conservatively with antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs). Nine days later there was only slight improvement in the dog's condition. The dog was anemic and showed syncope after jumping. Physical examination showed a temperature of 39.3°C, a systolic heart murmur grade 2/6 left apex due to the anemic status of the patient, and weak femoral pulses. Other examinations such as blood analysis, radiography and ultrasonography were carried out.

### Blood examination

The results of the hematology and follow-up analyses are summarized in Table 1. Initially, there was a moderate nonregenerative normocytic slightly hypochromic anemia, leucopenia/neutropenia and severe thrombocytopenia. The 17-beta-estradiol concentration was markedly increased at 149.2 pg/ml (normal values 13-55 pg/ml).

### Radiography

The x-ray of the thorax showed a normal heart with a VHS (vertebral heart score) < 10.5 and a slightly diminished lung vessel pattern. The x-ray of the abdomen showed an enlarged spleen with a caudal well circumscribed mass. The intestines were pushed dorsally and laterally by this mass situated on the midventral aspect of the caudal abdomen (Figure 1).

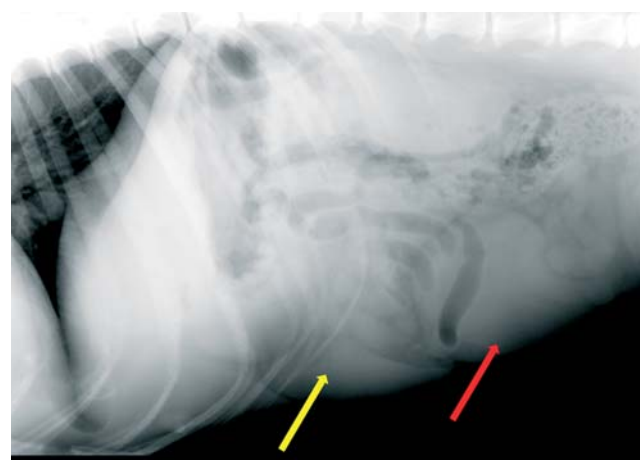
### Ultrasonography

On the ultrasonographic examination of the abdomen, the mass noticed on the x-ray consisted of two masses, both showing echogenic zones. The larger of the two masses was approximately 7 cm by 6 cm, while the other mass was smaller (Figure 2). These masses were suspected to be neoplastic transformations of the cryptorchid testes. Both testes were removed via a ven-

tral midline incision from the umbilicus to the pubis. Both neoplastic testicles were double ligated with Vicryl 2/0, transect and removed. The abdomen was closed using a standard procedure. Both testicles were histopathologically examined.

### Histopathology of the testes

The size of the testes was respectively, 3.5 cm in diameter and 11 cm by 7 cm (Figure 3). Hematoxylin eosin (HE) sections of the enlarged testis revealed a proliferation of Sertoli cells, spread over numerous tubuli, intermixed with a trabecular collagenous network. The neoplastic cells formed tubules of elongated cells in a parallel arrangement, with a large round to ovoid nucleus and a large amount of vacuolated or dense eosinophilic cytoplasm. The tumor consisted of well formed tubules that were lined by



**Figure 1.** The X-ray with the dog in right lateral recumbency shows an enlarged spleen (yellow arrow) and a well-circumscribed soft-tissue abdominal mass on the caudal midventral abdomen (red arrow) approximately twice the size of the kidneys. The intestines seem to be displaced dorsally and laterally of this mass.

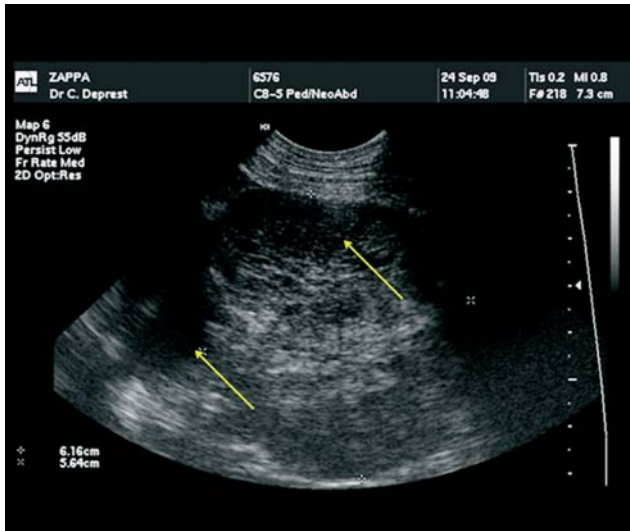


Figure 2. On ultrasonography, the mass observed on X-ray appears to be well circumscribed with irregular hypoechoic zones (yellow arrow), suggestive of fluid. The testicular parenchyma is mixed hypoechoic.



Figure 3. The left testicle changed into the largest tumor and measured 11 cm by 7 cm, the right testicle tumor measured 3.5 cm in diameter.

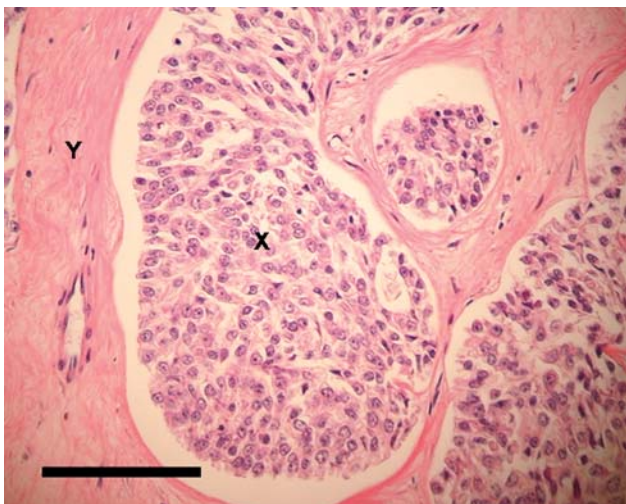


Figure 4. Neoplastic proliferation of Sertoli cells (X), spread over numerous tubuli, intermixed with a dense trabecular collagenous network (Y). The neoplastic cells formed tubules lined by multiple layers of elongated cells in a parallel arrangement, with a large round to ovoid nucleus and a large amount of eosinophilic cytoplasm. The cells are arranged perpendicularly to the basement membrane. HE staining - Bar = 100µm.

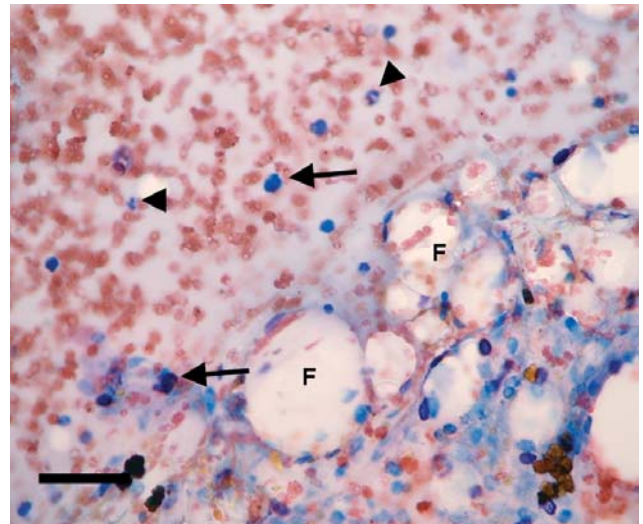


Figure 5. The bone marrow showed severe lack of erythroid, myeloid and megakaryocytic cell types. There were numerous mature erythrocytes, a moderate number of neutrophils (arrowheads) and a moderate number of large mononuclear cells (arrows). Presence of fat (F). Modified Wright stain - Bar = 50 µm

multiple layers of neoplastic Sertoli cells. These Sertoli cells were arranged perpendicularly to the basement membrane (Figure 4). The contralateral smaller atrophic testis showed severe fibrosis with a small number of tubular sections.

After surgery the anemia became worse (Table 1). Due to the nonregenerative nature of the anemia, a bone marrow aspiration was carried out for evaluation.

### Bone marrow cytology

The bone marrow showed severe lack of erythroid, myeloid and megakaryocytic cell types. There were numerous mature erythrocytes, a low number of neutrophils with some band neutrophils, and a low number of large mononuclear cells (Figure 5). Small amounts of fat tissue were also observed on the slides. These findings are consistent with estrogen-induced myelotoxicity.

### Treatment

A diagnosis of estrogen-induced pancytopenia due to a Sertoli cell tumor in a cryptorchid testis was made. After castration, the dog received 4 consecutive blood transfusions of 350 ml, each time at an interval of 4 to 6 days. The 2<sup>nd</sup> and 4<sup>th</sup> blood transfusions were followed by injections of Deca-Durabolin (200 mg IM - nandrolonedecanoate). Over time, the anemia gradually changed into a slightly regenerative macrocytic hypochromic anemia, and thrombocytopenia. Four months after the start of the treatment, the complete blood count was almost within reference levels (Table 1).

## DISCUSSION

Sertoli cell tumors of the testes arise from the supporting cells within the seminiferous tubuli. This is a common neoplasia in dogs, especially in dogs with cryptorchid testicles. Almost 50% of all canine Sertoli cell tumors arise in cryptorchid testes and the incidence of Sertoli cell tumors is more than 20 times higher in cryptorchid than in scrotal testes (Hayes and Pendergrass, 1976). Approximately 20 to 30% of dogs with Sertoli cell tumor manifest signs of hyperestrogenism, characterized by any combination of feminization, gynecomastia, atrophy of the contralateral testicle (as in the present case), squamous metaplasia within the prostate gland (often with accompanying suppurative prostatitis), alopecia and bone marrow atrophy (as in the present case) (McEntee, 1990). It has not been proven that estrogen is solely responsible for all these manifestations. Serum estrogen concentrations are not increased in some dogs with apparent hyperestrogenism associated with testicular Sertoli cell tumor (Grootenhuis *et al.*, 1990). However, in the present case the estrogen concentration was markedly increased. Estrogen-induced production of myelopoiesis-inhibitory factor by thymic stromal cells has been described (Grootenhuis *et al.*, 1990; Farris and Benjamin, 1993). The bone marrow suppressive effects accompanying Sertoli cell tumors, also known as aplastic anemia, can be so severe as to cause anemia, leukopenia and thrombocytopenia.

Aplastic anemia is characterized as a nonregenerative anemia with normal erythrocytic indices, neutropenia, and thrombocytopenia, and it suggests a multipotential stem cell disorder. Cytologic evaluation of the bone marrow of the present case confirmed bone marrow aplasia. Neutropenia from decreased cellular production usually has a minimal left shift (due to an increased demand for neutrophils and/or depletion of the bone marrow storage reserves of mature neutrophils).

Differential diagnosis of nonregenerative anemia includes: infections (canine parvovirus, Ehrlichia canis), drugs (estrogens, meloxicam, griseofulvin, chemotherapy, phenylbutazone, trimethoprim/sulphonamide), and idiopathic immune-mediated endogenous estrogen (Harvey, 1997; Villiers and Blackwood, 2005). Estrogen toxicosis is a common cause of myelosuppression in the dog. This condition may result from a paraneoplastic syndrome (Sertoli cell tumor that produces estrogen), as in the present case, or from the administration of an estrogen-based drug.

The clinical outcome of reported cases of estrogen toxicity has either been death or a long recovery period (Sontas *et al.*, 2009). Therapeutically, all the estrogen induced effects may disappear after removal of the underlying cause of the myelotoxicity, i.e. castration in the present case. Treatment also includes: correction of the anemia and thrombocytopenia, protection against infections, and stimulation of the remaining bone marrow (Hall, 1992). Untreated cases of estrogen induced myelotoxicity always have an unfavorable prognosis. Death occurs from complications of hemorrhage and infection.

However, in chronic cases, the stem cell damage may be irreversible and the red marrow is replaced by fat, leading to neutropenia, thrombocytopenia and moderate to severe anemia (Villiers and Blackwood, 2005).

The use of lithium appeared to be successful in inducing regeneration of the bone marrow in dogs (Hall, 1992). In humans, various drugs have been used successfully to treat aplastic anemia: corticosteroids, androgens, lithium carbonate and cyclophosphamide. Bone marrow transplantation is also another possibility in humans (Kjeldsberg *et al.*, 1989).

In the present case, Deca-Durabolin (nandrolonedecanoate) was used. This drug is an anabolic steroid and is useful in treating certain types of anemia, such as aplastic anemia and anemia due to chronic kidney failure. In certain cases where the bone marrow has stopped producing new erythrocytes, administration of anabolic steroids will stimulate this system again and bring the numbers of these cells back to normal levels. They also are known to stimulate the production of white blood cells and platelets, though to a lesser degree. In summary, the present dog showed a severe estrogen-induced pancytopenia due to neoplastic transformation of cryptorchid testes (paraneoplastic syndrome), which gradually improved to almost within reference ranges after castration, blood transfusions and administration of nandrolonedecanoate.

## ACKNOWLEDGEMENTS

We would like to thank the staff of Medisch Labo Bruyland and Labo Pathologie Yperman for their assistance.

## REFERENCES

- Crafts R. C. (1948). The effects of estrogens on the bone marrow of adult female dogs. *Blood* 3, 276–285.
- Farris G. M., Benjamin S. A. (1993). Inhibition of myelopoiesis by serum from dogs exposed to estrogen. *American Journal of Veterinary Research* 54, 1374–1379.
- Grootenhuis A. J., van Sluijs F. J., Klaij I. A., Steenbergen J., Tillerman M. A., Bevers M. M., Dieleman S. J., de Jong F. H. (1990). Inhibin, gonadotrophins and sex steroids in dogs with Sertoli cell tumors. *Journal of Endocrinology* 127, 235–242.
- Hall E. J. (1992). Use of lithium for treatment of estrogen-induced bone marrow hypoplasia in a dog. *Journal of the American Veterinary Medical Association* 200, 814–816.
- Harvey J. W. (1997). The erythrocyte. In: Kaneko J. J., Harvey J. W., Bruss M. L. (eds.). *Clinical Biochemistry of Domestic Animals*. 5<sup>th</sup> Ed., Academic Press, San Diego, pp. 166–167.
- Hayes H. M., Pendergrass T., W. (1976). Canine testicular tumors: epidemiologic features of 410 dogs. *International Journal of Cancer* 18, 482–487.
- Kjeldsberg C., Beutler E., Bell C., Hougie C., Foucar K., Savage R. (1989). Aplastic, hypoplastic and miscellaneous types of anemia. In: *Practical Diagnosis of Hematologic Disorders*. Revised edition, American Society of Clinical Pathologists, Chicago, pp.64–65.

Kociba G. J., Caputo C. A. (1981). Aplastic anemia associated with estrus in pet ferrets. *Journal of the American Veterinary Medical Association* 178, 1293-1294.

McEntee K. (1990). Scrotum, spermatic cord and testis: proliferative lesions. In: *Reproductive Pathology of Domestic Animals*. Academic Press Inc., San Diego, pp. 279-306.

Sontas H. B., Dokuzeylu B., Turna O., Ekici H. (2009). Estrogen-induced myelotoxicity in dogs: a review. *Canadian Veterinary Journal* 50, 1054 – 1058.

Tilley L. P., Smith F. W. K. (2004). Estrogen toxicity. In: Tilley L. P., Smith F. W. K. (eds.). *The 5-Minute Veterinary Consult: Canine and Feline*. 3<sup>rd</sup> Ed., Philadelphia, Lippincott Williams and Wilkins, pp. 430-431.

Villiers E., Blackwood L. (2005). Disorders of erythrocytes. In: *BSAVA Manual of Canine and Feline Clinical Pathology*. 2<sup>nd</sup> Ed., British Small Animal Veterinary Association, Gloucester, pp. 48.

## Uit het verleden

### SCHADEVERGOEDING EN BUREAUCRATIE ANNO 1839

Een van voornaamste onderdelen van de succesvolle bestrijding van de grote epidemieën onder het vee was en is nog steeds het opzetten door de overheid van een efficiënt systeem van reële schadevergoedingen voor gedwongen slachting van dieren en vernietiging van kadavers. Een beschrijving van hoe dit ontstond in de late 18<sup>de</sup> eeuw tijdens een buitengewoon virulente runderpestepidemie verscheen enkele jaren geleden in dit tijdschrift (De Herdt, R., Vlaams Diergeneeskundig Tijdschrift, jg. 67, 1998, p.199-208).

Wellicht vooral wanneer er geen ernstige bedreiging was, kon dat blijkbaar niet verlopen zonder complexe formaliteiten. Wat te denken van de hiernavolgende procedure die we putten uit een handboek voor gemeentesecretarissen uit het jaar 1839?

*Den ondergeteekenden ... landbouwer te ....., Arrondissement Administratief ....., Provincie..., neemt bij deze de nederige vrijheid het Gouvernement te verzoeken, hem eene schadevergoeding te willen vergunnen over het verlies eener melkkoe, dewelke van eene besmettelijke ziekte aengedaen zijnde, op bevel der Administratieve Overheid, den... 18 .. is moeten gedood en met huid en haer gedolven worden.*

*Hij verwacht dat zijn regtmatig verzoek zal ingewilligd zijn en staeft hetzelfde met volgende stukken:*

1. Verklaring van het Plaetselijk Bestuer wegens het kenbaer maken der ziekte,
2. Beshluit bevelende de afmaking,
3. Benoeming der experten,
4. Procesverbael van eeds aflegging,
5. Id. van schatting,
6. Id. wegens de afmaking,
7. Verklaring van bezit van volle gezondheid,
8. Extrakt uit de Rolle der Provinciale belasting, betrekkelijk het Veefonds over 183..,
9. Verklaring dat er in de belasting van het fonds voor den landbouw over 1830 is toegebracht geweest.

*..., den ..., 18..*

*'T IS DE GRATIE ENZ ...  
N...*

*De Gemeente besturen zullen zorgen dat de stukken vermeld onder nr. 8 en 9 van het hier vorenstaende borderel door den verliezer opgemaakt, nauwkeurig ingerigt zijnde aen den dossier gevoegd worden.*

*Bijaldien er vee bezweeke alvorens de noodige formaliteiten zijn kunnen in acht genomen worden, zal men niettemin om eene vergoeding te bekomen de hier voren aangewezen stukken opmaken en daerbij, zoo het mogelijk is, eene verklaring door eenen wettigen Veearts verleend, voegen.*

*Waer er voor de behandeling van het vee geenen erkenden Veearts geroepen geweest, doordien er geenen in min dan drie uren afstands der gemeente zich gevestigd vindt, alsdan moet het Gemeente Bestuer dit bepaeldelijk verklaren; zullende de afzonderlijke verklaring ten dien aengaende aen de andere daertoe opgemaakte stukken gevoegd zijn.*

Hier moet wel aan toegevoegd worden dat er ook een iets eenvoudiger procedure aangegeven werd. De vergoeding - in principe twee derden van de waarde - werd betaald uit een landbouwfonds dat gespijsd werd uit de *Bestiaelgelden*, een in veel streken toen al lang bestaande belasting op de dieren.

Er bestonden typeformulieren voor de in de tekst opgesomde verklaringen. Die van de eed van de schatters afgelegd in handen van burgemeester en schepenen luidde als volgt:

*Wij beloven de zieke koe waerop het aenkomt, regtzinniglijk te zullen prijzen, en ons geenszins door gunsten of geschenken te laten geleiden om dezelfde boven hare wezenlijke weerde te schatten.  
Zoo waerlijk helpe ons God Almagtig.*

Uit: Van Eeckhoutte, P.J., *Handleiding der gemeente secretarissen met alle slaggh van voorschriften voor het opmaken van bestierings akten*, Stock-Werbrouck, Roeselare, 1839, p. 227 -231.