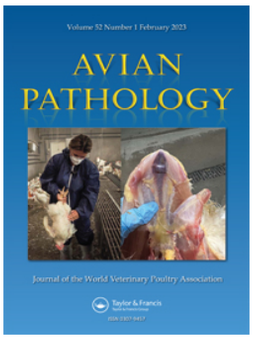

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ORIGINAL ARTICLE

Keel bone fractures in broiler breeders: is palpation a reliable diagnostic method?

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ABSTRACT

The aim of this study was to estimate the sensitivity and specificity of palpation relative to necropsy for detection of keel bone fractures (KBF) in broiler breeders. In addition, the study investigated observer reliability of palpation. Four commercial breeder flocks (Ross 308 $n = 2$, Ranger Gold $n = 1$, Hubbard JA 757, $n = 1$) were included in the study. A total of 400 hens and 60 roosters were examined after culling or slaughter, at end of lay (63 weeks). Keel bones were first palpated independently by two raters with a varying degree of experience in palpation of keel bones in laying hens. After palpation, a necropsy with investigation of the keel bone was conducted. The prevalence of KBF at end of lay for hens, as diagnosed by necropsy, varied from 14-58%, while only one in 60 assessed roosters had KBF. Palpation had poor sensitivity, with differences between raters ranging from 56% to 36% when hybrid is not considered. The specificity of palpation for all hybrids together was 85% and 88%, for the two raters, respectively. The two raters had moderate agreement, Gwet's agreement coefficient (95% confidence interval): 0.60 (0.52–0.69). In conclusion, palpation has poor diagnostic accuracy for detection of KBF in broiler breeders, and other methods, like necropsy, must be applied for reliable assessments. KBF appears to be a prevalent condition in different hybrids of broiler breeder hens, but not in roosters.

RESEARCH HIGHLIGHTS

- This is the first study to investigate palpation as a diagnostic method for detection of KBF in broiler breeders.
- Palpation has poor diagnostic accuracy for detection of KBF in broiler breeder hens with differences between raters.
- KBF can be a prevalent problem in broiler breeder hens of several hybrids, but not in roosters.

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Keel bone; keel bone fractures; broiler breeders; diagnostic method; palpation; necropsy

Introduction

Keel bone fractures (KBF) are likely the most prevalent health and welfare problem for commercial laying hens (Riber *et al.*, 2018; Toscano *et al.*, 2020; Thøfner *et al.*, 2021). KBF are characterized by callus formation, visible fracture lines and/or fragmented sections of the bone (Casey-Trott *et al.*, 2015; Thøfner *et al.*, 2020). Fractures to the keel bone appear to be a global problem, observed in layers in all types of commercial egg production systems (Riber *et al.*, 2018) and in different strains (Rufener & Makagon, 2020; Eusemann *et al.*, 2022). Several papers have reported alarmingly high prevalences of KBF in layers, regardless of the production system (Baur *et al.*, 2020; Thøfner *et al.*, 2021). Unfortunately, the literature regarding KBF in broiler breeders is very sparse. There are substantial knowledge gaps in terms of prevalence, diagnostic methods, and causative factors. A study by Gebhardt-Henrich *et al.* (2017) is one of the few published papers investigating the keel bones of broiler breeders. They found KBF in 25% of the investigated broiler breeder hens with access to perches, assessed at 45 weeks of age (Gebhardt-Henrich *et al.*, 2017).

The causes of KBF in laying hens are still debated in the scientific community and the causative factors are still not clear. Trauma due to collision within the barn was, for several years, the prevailing explanation (Sandilands *et al.*, 2009); however, this is no longer considered to be the major causative factor (Thøfner *et al.*, 2021). Several explanations have been suggested (Toscano *et al.*, 2020); and some studies have found an association between egg laying and KBF (Eusemann, Baulain *et al.*, 2018; Eusemann, Sharifi *et al.*, 2018; Eusemann *et al.*, 2020, 2022; Thøfner *et al.*, 2021) For instance, Eusemann, Baulain *et al.* (2018) and

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Eusemann, Sharifi *et al.* (2018) found that hens treated with deslorelin acetate, to inhibit egg laying, did not develop fractures to the keel bone. In addition, there is a positive association between KBF and early age for first egg, small birds, and estimated daily egg weight at onset of lay (Gebhardt-Henrich & Fröhlich, 2015; Thøfner *et al.*, 2021). The lack of KBF in roosters also indicates an association with egg-laying (Fleming *et al.*, 2004; Thøfner *et al.*, 2020; Kittelsen *et al.*, 2021). As broiler breeders are egg-layers, it could be speculated that KBF also occur in these birds. On the other hand, onset of lay is later in broiler breeders compared to laying hens and the breeders do not produce the same amount of eggs during the production phase compared to commercial layers (Waddell, 2005; Hubbard, 2015; Aviagen, 2016). In addition, broiler breeders are larger and with more breast muscles protecting the keel and might therefore be at a lower risk, since body size seems to be associated with KBF (Thøfner *et al.*, 2021).

There are several methods to investigate KBF in laying hens, and palpation has been the most common method (Casey-Trott *et al.*, 2015). This method is time efficient and can be done on-farm without having to cull the birds. However, palpation has been shown to underestimate the prevalence of KBF in laying hens (Tracy *et al.*, 2019; Thøfner *et al.*, 2021). Since broiler breeders have larger breast muscles covering the keel compared to laying hens, it can be hypothesized that palpation will be even less sensitive in the broiler breeders. More reliable methods are radiography and necropsy (Tracy *et al.*, 2019). However, radiographic equipment is expensive, the method is more time-consuming than palpation and may pose a health risk for the investigator. *Post mortem* examination is often considered the gold standard (Tracy *et al.*, 2019), the equipment is not expensive and it is not too time-consuming, but it requires the culling of birds and, thus, does not allow for longitudinal studies on the same birds.

The aim of this study was to estimate the diagnostic accuracy of palpation for detection of keel bone fractures in broiler breeders relative to *post mortem* examination. In addition, the study investigated the inter-rater reliability between the two observers performing the palpation.

Materials and methods

Study design

A total of four commercial broiler breeder flocks (Ross 308, $n = 2$, Ranger Gold $n = 1$, Hubbard JA 757, $n = 1$) were investigated between January and March 2022. The four flocks were selected from production lists based on the age of the birds. Within each flock, 100 hens and 20 roosters were selected after stunning, from different transport containers (when slaughtered at an abattoir, $n = 2$) or from different places within the barn (when culled with full-house gassing on farm, $n = 2$). The selection of birds was based on convenience sampling; at the slaughterhouse the staff collected 10 birds from each container, and, in the barn, birds were sampled during a transect walk after whole-house gassing, to represent different areas. Roosters were only examined in three of the four flocks, since the roosters had been slaughtered at an earlier time in one flock. Therefore, the study sample consisted of 400 hens and 60 roosters.

Birds and housing

Each flock consisted of approximately 7400 hens (range: 7316–7465) and 600 roosters (range: 503–754), kept in the same house. Both the roosters and the hens came from the same rearing facility; the roosters arrived at 17 weeks of age and the hens at 18 weeks of age. All houses were fully insulated with mechanical ventilation and concrete floor with wood shavings, nest boxes and perches on elevated slats (60 cm). The light regime included 13 h of darkness and lux during light periods varied from 10 to 30. The temperature in all four houses was 18°C. All flocks were managed after standardized practices according to the breeding companies and Norwegian regulation with regard to feed, water, ventilation, litter and lighting (KSL, 2020).

Keel bone examination

The keel bone examination, both palpation and necropsy, was performed *post mortem*. The birds were culled with CO_2 at 63 weeks of age, either as whole-house gassing on-farm or with multistage stunning, with gradual exposure to the CO_2 gas, followed by euthanasia due to bleeding after decapitation at the slaughterhouse. The keel bones of 100 hens and 20 roosters in each flock were examined for fractures. All birds were examined *post mortem* by palpation and necropsy, prior to *rigor mortis*.

Palpation

Keel bones were palpated independently by two assessors, and the bird was classified on a binary scale as having no fractures or at least one fracture. The raters had a varying degree of experience in palpation of keel bones in laying hens. One assessor was experienced and had participated in a 2-day Training School of the COST Action CA15224 "Identifying causes and solutions of keel bone damage in laying hens", in addition to over 500 live bird and 200 dead bird keel palpations in the field. The other assessor was moderately experienced, with approximately 100 keel palpations on dead laying hens. During palpation, the

assessors ran their fingers on the sagittal axis of the keel bone, the *crista sterni*, to feel for the presence or absence of fractures, evidenced by callus or palpable gaps of the bone (see Figure 1 for visualization of palpation method; this picture shows palpation of live birds; dead bird palpation was performed in the same way), as described by Tracy *et al.* (2019). The assessors were blinded to each other's scoring results.

Necropsy

External palpation was followed by necropsy by an experienced poultry veterinarian (the first author), including inspection of the visceral aspect of the keel bone. The keel bone was classified on a binary scale as having no fractures or at least one fracture. The dorsal aspect of the keel bone was inspected and KBF were defined as bones with visible or palpable callus formation (see Figure 1), visible fracture lines and/or fragmented sections of the bone. Additionally, during necropsy, fractures were classified according to the part of the keel in which the fracture was located (i.e. the keel was divided into three equal parts: cranial, middle and caudal part) along with the total number of fractures per anatomical site.

Statistical analysis

Descriptive statistics of the apparent prevalence of KBF among the necropsied birds was calculated based on the necropsy results. The diagnostic accuracy of palpation as a diagnostic method for detection of KBF was calculated separately for each rater and hybrid. The diagnostic accuracy of palpation was estimated relative to necropsy using a gold standard approach, i.e. assuming the reference method to have perfect accuracy.

Figure 1. (A) Palpation of the birds performed by running the fingers on the sagittal axis of the keel bone to fell for the presence or absence of fractures. The picture illustrates the technique performed on live birds, but in the current study it was performed *post mortem*. (B) Inspection of the visceral surface of the keel bone during necropsy. An old fracture with callus formation can be seen in the caudal zone of the keel bone.

Inter-rater agreement between the two raters was estimated using Gwet's agreement coefficient (AC) (Gwet, 2012) using the sample of 200 hens.

This study was performed under commercial settings. All keel bone examinations were performed *post mortem*. Therefore, approval by an ethics committee for bird experiments was not required according to Norwegian legislation (The Norwegian Ministry of Agriculture and Food, 2015).

Results

Prevalence of keel bone fractures

In total, 460 birds (400 hens and 60 roosters) from four flocks were examined for keel bone fractures, by both palpation and necropsy. The prevalence of KBF for the four flocks, as scored by necropsy, varied between 14% and 58% for hens in the four broiler breeder flocks (Table 1). One out of 60 roosters had a fracture; this was a single fracture in the caudal third of the keel bone. All observed fractures were located in the caudal third of the keel bone (see Figure 1). The number of fractures per hen per flock is presented in Table 2.

Diagnostic accuracy

The estimated sensitivity (Se) of palpation was 56% (95% CI: 47–65%) and 36% (95% CI: 28–45%), for Rater 1 and Rater 2, respectively, when all birds were grouped together. Sorted on hybrid, the Se (point estimate) ranged from 27-56% (Table 3). The Se was lowest for Ranger Gold in both raters: 54% (95% CI: 33–74%) for Rater 1, and 27% (95% CI: 11–48%) for Rater 2.

Compared to Se, the estimated specificity (Sp) of the two raters was overall higher and more similar between raters, 85% and 88% when the birds of all hybrids were grouped together. The largest discrepancy in Sp between raters was found for Ranger Gold where a range in Sp from 68–85% was found.

Inter-rater agreement

The estimated Gwet's AC was 0.60 (95% CI: 0.52–0.69) indicating moderate agreement between raters (Landis & Koch, 1977).

Table 1. Apparent prevalence of keel bone fractures based on necropsy among 400 hens and 60 roosters from four flocks of broiler breeders at 63 weeks of age.

Flock (*n*)	Hybrid	Hens		Roosters	
		n	Fractures n (%)	*n*	Fractures *n* (%)
Flock 1	Ross 308	100	54 (54)	20	1 (5)
Flock 2	Ross 308	100	58 (58)	0	– [a]
Flock 3	Ranger Gold	100	26 (26)	20	0 (0)
Flock 4	Hubbard JA757	100	14 (14)	20	0 (0)

[a]Not investigated.

Table 2. Number of keel bone fractures per hen per flock, $n = 100$ per flock

Flock	Hybrid	One fracture	Two fractures	Three fractures	Four fractures
Flock 1	Ross 308	46	7	1	–
Flock 2	Ross 308	43	11	3	1
Flock 3	Ranger Gold	24	2	–	–
Flock 4	Hubbard JA757	11	2	1	–

Discussion

The aim of this study was to investigate palpation as a diagnostic method for detection of keel bone fractures in broiler breeders, both hens and roosters. We estimated the sensitivity and specificity of palpation relative to necropsy assessment. In addition, the study investigated inter-rater reliability between the two raters performing the palpation. Overall, this study shows that palpation is not a reliable method in terms of detecting KBF in broiler breeder hens. Furthermore, the results indicate that KBF is a prevalent condition in broiler breeder hens. However, there are substantial differences between hybrids and flocks, ranging from 14% to 58% in this study.

The apparent prevalence of KBF at the end of lay, as diagnosed by necropsy, varied between 14% and 58% for hens in the four broiler breeder flocks. This is lower than the occurrence reported in commercial laying hens, where a prevalence of about 85–97% at end of lay has been reported (Rufener & Makagon, 2020). The reason for the lower prevalence is not clear, but it may be speculated that a later onset of lay, a lower egg production and an earlier age for end-of-lay (63 weeks, compared to 75–80 weeks), may contribute to the lower prevalence observed in the broiler breeders. The literature regarding KBF in broiler breeders is sparse. A study by Gebhardt-Henrich *et al.* (2017) examined keel bones of 80 Ross 308 broiler breeder hens, 45 weeks of age. They found KBF in 25% of the hens, assessed by palpation. This is notably lower than the results from the Ross 308 birds in the current study (54–58%). The birds in the study by Gebhardt-Henrich *et al.* (2017) were 18 weeks younger, which likely contributes to the different results. Another explanation for the difference between the two studies is the methodology used to assess the keel bone. Gebhardt-Heinrich *et al.* (2017) used palpation only to evaluate the keel bone of the broiler breeders. This method is known to underestimate the true prevalence of KBF in laying hens (Tracy *et al.*, 2019; Thøfner *et al.*, 2021). Our results support the assumption that the low Se of palpation will lead to a considerable underestimation of the prevalence of KBF in broiler breeders, similar to laying hens. The bias can be corrected by accounting for the imperfect accuracy when estimating true prevalence. However, this is not straightforward as the diagnostic accuracy of palpation is not constant across observers or hybrids. Additionally, it can be argued that necropsy is also an imperfect diagnostic method for detection of KBF. A previous study found histological evidence of fractures in some keels that were identified as non-fractured during necropsy (Scholz *et al.*, 2008). Estimating the diagnostic accuracy in the absence of a gold standard is possible using latent class analysis (LCA) (Hui & Walter, 1980). The design of the present study was not suitable for this approach, and, therefore, we present relative measures of Se and Sp. However, LCA could be considered in future studies regarding diagnostic test properties and true prevalence of keel bone fractures in laying hens or broiler breeders.

We found a moderate agreement between the two observers in the current study. The experience and training of the assessors influenced the accuracy of the palpation and Se was lower for the rater with least experience. This is in line with similar studies on laying hen palpation (Tracy *et al.*, 2019). However, regardless of training, the diagnostic accuracy of keel bone palpation in broiler breeders was low. There are several explanations for the low Se. First, the majority of the fractures were located on the dorsal side of the keel bone and this part of the bone is not accessible during palpation. Second, broiler breeders have larger breast muscles covering the keel bone, making it more difficult to palpate the sides of the keel bone crest and to access the dorsal plane of the caudal part of the keel.

There was a difference in the observed KBF prevalence between the broiler breeder hybrids, with fewest fractures in the Hubbard JA 757, followed by Gold and then Ross 308. However, statistical analysis of the effect of hybrid was not performed as there were too few flocks and substantial correlation between flock and hybrid, i.e. it was not possible to separate the flock effect from the effect of hybrid. Differences between genetic strains have previously been reported in laying hens (Heerkens *et al.*, 2016; Candelotto *et al.*, 2017; Eusemann, Baulain *et al.* 2018; Eusemann, Sharifi *et al.* 2018; Kittelsen *et al.*, 2021) Therefore, further studies are needed before we can make inference about the true prevalence of KBF in the different

Table 3. Diagnostic accuracy: sensitivity (Se) and specificity (Sp) with 95% confidence intervals (95% CI) of palpation for two raters across three different hybrids of broiler breeders, relative to necropsy.

Hybrid (*n*)	Rater 1				Rater 2			
	Se	95% CI	Sp	95% CI	Se	95% CI	Sp	95% CI
All (460)	56	47–65	85	80–89	36	28–45	88	84–92
Ross 308 (220)	55	45–65	87	79–93	38	29–48	85	76–92
Ranger Gold (120)	54	33–74	68	57–78	27	11–48	85	76–92
Hubbard JA757 (120)	71	41–92	98	93–100	36	12–65	94	88–98

meat-type hybrids. Such studies should include information on egg production, onset of lay and egg weight, since these are factors found to be linked with KBF in laying hens and can hopefully give some insight into the reason for the difference between the three breeder hybrids. There was also a considerable difference in KBF prevalence between roosters and hens. This is in line with results from laying hybrids (Fleming *et al.*, 2004; Kittelsen *et al.*, 2020) and supports the notion that KBF are primarily a health and welfare challenge for hens.

Conclusion

This is the first study to investigate the reliability of palpation to detect KBF in broiler breeders. The results from this study indicate that palpation has poor diagnostic accuracy for detection of KBF in broiler breeder hens with differences between raters. Furthermore, the study indicates that KBF can be a prevalent problem in broiler breeder hens.

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Disclosure statement

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