The reporting of animal welfare details in liver research: A review of studies describing bile duct ligation in mice (2011–2013)

To the Editor:

Studies involving animals play a key role in basic liver research and are most commonly carried out in laboratory mice. For example, bile duct ligation (BDL) is frequently carried out to model a range of hepatic pathologies including fibrosis and cholestasis [1]. When animals are used experimentally it is widely accepted that procedures should be refined to alleviate pain and distress [2]. Promoting laboratory animal welfare is not only of ethical value but of considerable scientific importance to minimize variability and improve validity of research involving animals [2–4].

Given the clear importance of refining experimental procedures, we conducted a literature review to assess the reporting of details related to mouse welfare in studies that involved BDL in mice. Although the BDL procedure is widely used in rats and has been well characterized for many years [5], it is increasingly carried out in mice because of the number of genetically altered mouse strains now available [1]. The search was performed in July 2014, using the search engine SCOPUS and search terms "mouse OR mice" and "bile duct ligation OR BDL" within keyword, title and abstract to identify relevant studies, published in English between 2011 and 2013. A paper was eligible for inclusion if it described mice undergoing experimental BDL and was available in electronic format from Newcastle University. Conference papers, letters and reviews were excluded. Papers (including supplementary data) were then carefully screened to identify details relating to animal welfare.

119 papers met our inclusion criteria (34 from 2011, 39 from 2012 and 46 from 2013); journals in which they were published are summarized in Table 1. Papers most commonly described mechanisms of cholestatic liver injury (66.4%) and therapeutic or toxic substances (17.6%). Transgenic mice were studied in 55.5% of the papers. The BDL procedure was carried out in North America (39%), Europe (27%) or Asia (24%). 10% of studies involved multiple institutions and the place where the animal work was carried out was not specified. 94% of papers made a statement related to ethical guidelines on the humane use of animals, with 86.6% of these studies referring to local ethical approval. The "Guide for the Care and Use of Laboratory Animals" [6] was the most frequently cited guideline relating to animal care (22.7% of papers).

Surgical and anaesthetic details were described in 57% of the papers, whereas 43% of the papers only referred to methods described in previous studies. Of the papers citing previously published methodologies, 26.7% referred to studies carried out in rats, which is problematic given the species differences (e.g. the gallbladder is present in mice and absent in rats). When methods were not described, we recorded anaesthetic and analgesic details from papers whose methodologies had been cited. Overall, only 3.4% of studies describing BDL in mice specified administration of a systemic analgesic agent (non-steroidal anti-inflammatory or opioid agent); administration of buprenorphine (n = 3) and fentanyl (n = 1) was reported in 2 papers from North America and in 2 from Europe. Administration of a systemic analgesic agent to mice undergoing BDL is considerably lower than has been previously reported for mice and rats, undergoing a range of surgical procedures (typically 20%) [4,7]. This is surprising given that BDL is a painful procedure in rodents, although pain can be alleviated through systemic analgesic administration [8]. Although it is possible that systemic analgesics were administered in these studies, but not reported (e.g. investigators may view analgesic administration as implicit in their statement relating to ethical guidelines), earlier work suggests that under-reporting of analgesic administration is relatively low [4]. Furthermore, the reporting of details in animal research should be comprehensive to allow experiments to be repeated [9]. Space restraints for reporting methodological details in some journals, particularly those with a high impact factor, may also be perceived as an obstacle for some authors however, an increasing number of journals allow authors to include supplementary data. Alternatively, papers which report animal welfare information in detail could be cited with any modifications to the published protocol clearly described. Analgesics may have been withheld if investigators had concerns about potential effects of the analgesics, however, uncontrolled post-surgical pain is a source of considerable experimental variability [4] and not a feature of cholestatic liver disease in most human patients.

Thirty percent of studies had no information concerning the anaesthetic protocol used. When the anaesthetic regimen was specified, the most common agents were: ketamine/xylazine (30%), isoflurane (29%), pentobarbital (18%) and ether (12%). Only 27.7% of studies described the administration of an anaesthetic agents with analgesic properties (ketamine, alfentanyl, tribromoethanol, tiletamine). Three of the anaesthetic regimens used give rise to potential animal welfare concerns: irritancy on induction (ether), narrow safety margin (pentobarbital) and unpredictable adverse effects (tribromoethanol) [3,4].

As bile duct ligation induces progressive disease and is associated with considerable morbidity and mortality [1], refinement of the BDL procedure should not just be limited to the peri-operative period and appropriate monitoring/supportive care is essential throughout the study [3,10]. Although 6.7% of studies specified saline injections and 5% described clinical monitoring, relatively few studies reported the provision of supportive care. A warm environment and soaked diet is generally beneficial to sick animals [3], but only 16% of studies reported the room temperature, which was not increased after surgery (18–21 °C [2.5%], 21–23 °C [13.5%]) and provision of a soaked diet was not reported. Experimental and humane end points are also particularly important in progressive disease studies where pain and distress are more likely to occur as disease progresses [11]. Although a small number of studies (less than 3%) continued for more than 6 weeks following BDL, when mice would have developed advanced disease, the majority (95%) specified clear experimental end points with 95% of end points at 4 weeks or earlier. Humane end points were referred to in one paper.

Table 1. Journals included in review.

Journal name	Number of papers included in review
Hepatology	22
Journal of Hepatology	14
Gastroenterology	9
American Journal of Physiology-Gastrointestinal and Liver Physiology	8
PLOS ONE	7
Liver International	6
The American Journal of Pathology	4
Laboratory Investigation	4
Biochemical and Biophysical Research Communications	4
Biochimica et Biophysica Acta	3
Free Radical Biology and Medicine	2
European Journal of Pharmacology	2
Journal of Biological Chemistry	2
Experimental and Toxicologic Pathology	2
Journals with one paper included in the review*	30

*American Journal of Physiology, Behavioural Brain Research, BMC Gastroenterology, Clinical and Experimental Medicine, European Radiology, Food and Chemical Toxicology, Gut, Gut Microbes, Journal of Clinical Investigation, Journal of Gastrointestinal Surgery, Journal of Hepato-Biliary-Pancreatic Sciences, Journal of Immunology, Journal of Toxicological Sciences, Journal of Leukocyte Biology, Journal of Nuclear Medicine, Journal of Pharmacology, Journal of Surgical Research, Molecular Nutrition and Food Research, Molecular Biology Reports, Molecular and Cellular Biology, Pathophysiology, Peptides, Pharmaceutical Biology, PLoS Genetics, Proceedings of the National Academy of Sciences of the United States of America Plus, Psychopharmacology, Surgery, Toxicological Sciences, Toxicology, Toxicology and Applied Pharmacology.

Given the clear importance of the humane treatment of laboratory animals with respect to both animal welfare and the translation of *in vivo* studies to human patients with liver disease, we would encourage investigators, carrying out research involving animals, to implement refinements and to report the measures taken to minimise pain and distress according to the ARRIVE guidelines [9]. Specific recommendations for animals that have undergone surgery include provision and reporting of an appropriate systemic analgesic, or the rationale if analgesics had been withheld. Finally, both the anaesthetic regimen and refinement details relating to the post-operative period (e.g. monitoring, nursing care and humane end points) should be specified.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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