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Short communication

Detection of *Babesia* species in questing *Ixodes ricinus* ticks in England and Wales

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ABSTRACT

Babesiosis, a disease in humans and animals is caused by piroplasms from the genus *Babesia* and is transmitted by ixodid ticks. Bovine babesiosis, commonly called redwater fever, is reported in cattle from many regions of the British Isles. The presence of *Babesia* in questing ticks in the United Kingdom (UK) and its potential impact on public and animal health has not been widely studied. Therefore, this study aimed to assess the presence of *Babesia* spp. in England and Wales using ticks collected over a six-year period. Questing *Ixodes ricinus* nymphs were collected at 20 recreational areas between 2014 and 2019 and screened for *Babesia*. Of 3912 nymphs tested, *Babesia* spp. were detected in 15, giving an overall prevalence of 0.38% [95%CI: 0.21–0.63%]. A number of *Babesia* species were identified including *B. venatorum* ($n = 9$), *B. divergens/capreoli* ($n = 5$) and *B. odocoilei*-like species ($n = 1$). Based on the low prevalence of *Babesia* detected in questing *I. ricinus* nymphs in the recreational areas studied, the likelihood of exposure to *Babesia*-infected ticks is lower compared to other pathogens more widely studied in the UK (e.g. *Borrelia burgdorferi* s.l.). However, localized areas of elevated risk may occur in pockets in England and Wales.

1. Introduction

Ixodid ticks can transmit a range of *Babesia* spp., which are piroplasms that infect red blood cells and, in some cases cause haemolytic anaemia or babesiosis (Kjemtrup and Conrad, 2000; Schnittger et al., 2012). Different species of *Babesia* have been associated with different reservoir hosts and vectors and are of animal and public health concern. In Europe, approximately 60 cases of human babesiosis have been reported (Hildebrandt et al., 2023; Yabsley and Shock, 2013), with the first reported case in 1957 in a Croatian farmer (Skrabalo and Deanovic, 1957). However, studies in Europe found 0.6–5.4% IgG antibodies for *Babesia* spp. in asymptomatic patients, so the number of cases is likely underestimated (Hunfeld et al., 2002; Rojko et al., 2008; Sonnleitner et al., 2014; Svensson et al., 2019). Severe babesiosis cases in humans can be fatal and are almost always associated with immunosuppressed or splenectomised patients (Hildebrandt et al., 2021). European cases of human babesiosis are most commonly attributed to *Babesia divergens,* including a recent case in the UK (now recovered) (Chan et al., 2021; Johnson et al., 2020) and it is transmitted by *Ixodes ricinus* ticks (Andersson et al., 2016; Gray and Ogden, 2021; Yabsley and Shock, 2013). *Babesia microti,* which is a parasite of small mammals, can be pathogenic to humans with eleven cases reported in Europe (Hildebrandt et al., 2023). It is likely transmitted by *I. ricinus* however, *Ixodes trianguliceps* and *Dermacentor reticulatus* ticks may be implicated as vectors, although more research is needed to confirm their role as vectors (Bown et al., 2008; Hodžić et al., 2017; Sands et al., 2022). *Babesia venatorum,* which has caused five human cases in Europe, is transmitted by *I. ricinus* and is a parasite of roe deer (*Capreolus capreolus*) (Bonnet et al., 2007, 2009). A recent detection of *B. venatorum* in sheep (*Ovis aries)* in Scotland suggests that they could act as reservoir hosts in the UK

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(Gray et al., 2019).

Babesiosis is also of veterinary concern as many species can affect domestic animals. For example, canine babesiosis can be caused by *Babesia canis, Babesia vogeli* and *Babesia gibsoni* and, while wildlife transmission hosts are unknown, their transmission is associated with two tick species; *Rhipicephalus sanguineus* sensu lato (*B. vogeli* and *B. gibsoni*) and *D. reticulatus* (*B. canis*) (Abdullah et al., 2018; de Marco et al., 2017; Solano-Gallego et al., 2016), the former being regularly imported in the UK via travelling dogs and the latter being established in parts of the UK (Hansford et al., 2023). Equine piroplasmosis is caused by *Babesia caballi* and is transmitted by *D. reticulatus,* which is present in the UK (Foldvari et al., 2016; Hansford et al., 2023). Finally, *B. divergens* causes redwater fever in cattle in parts of the UK and is transmitted by *I. ricinus* (Gray et al., 2021; McFadzean et al., 2023). A second less virulent *Babesia* spp., *Babesia major*, has been detected in the UK and is associated with transmission by *Haemaphysalis punctata* ticks (Altay et al., 2008; Liu et al., 2008; Silva et al., 2009; Uilenberg, 2006; Yabsley and Shock, 2013).

Three studies in the UK have tested adult ticks (*I. ricinus, Ixodes hexagonus, Ixodes canisuga* and *D. reticulatus*) collected from dogs (*Canis lupus familiaris)* and cats (*Felis catus*) and found that infection with *Babesia* spp. was very low (Abdullah et al., 2018; Davies et al., 2017; Smith et al., 2013). Other studies have found questing *H. punctata* and *D. reticulatus* ticks positive for *B. major* and *Babesia motasii* in the UK however, *D. reticulatus* has not been confirmed to be a vector yet (Phipps et al., 2022; Sands et al., 2022). To our knowledge, only two studies have looked at *Babesia* infection rates in questing *I. ricinus* ticks in the UK. They found 0.3% of ticks infected in southwest England (*B. divergens, Babesia capreoli* and *B. venatorum*) (Sands et al., 2022) and 0.2% of ticks infected in Scotland (*Babesia* from clade X including *B. venatorum* and *B. divergens*) (Olsthoorn et al., 2021). In this study, we screened questing *I. ricinus* nymphs collected across recreational areas in England and Wales between 2014 and 2019 for the presence of piroplasms.

2. Materials and methods

2.1. Tick collection

Questing nymphs were collected at 20 recreational areas in England (16 locations) and Wales (four locations) between 2014 and 2019 (see Cull et al. 2021 and Gandy et al. 2022 for more details). In total, 256 sampling points were surveyed (101 in woodlands, 89 in grassland, 42 in heathland, and 24 in woodland edge habitat). Nymphs were collected at each location once during the spring (April-June) by dragging a 1 m x 1 m cotton sheet over vegetation. Ticks were stored at −80 °C and identified to species level using morphological keys (Estrada-Peña et al., 2018; Hillyard, 1996).

2.2. Babesia spp. detection

DNA extracts that were originally used in previous studies (Cull et al., 2021; Gandy et al., 2022) were tested for *Babesia* spp. When possible, 50 questing *I. ricinus* nymphs were individually tested from each site for every year the site was surveyed. DNA lysates were obtained for every nymph using an ammonia-based method following (Hansford et al., 2015). Pan-piroplasm PCR and amplicon sequencing was carried out according to the procedure described in de Marco et al. (2017). In brief, a fragment of the *Babesia* 18S rRNA gene was amplified using primers Piro-A (5′-AATACCCAATCCTGACACAGGG-3′) and Piro-B (5′-TTAAATACGAATGCCCCCAAC-3′). Each reaction contained 12.5 μl of iTaq Universal Probes One-Step Kit reaction mix (Bio-Rad, Watford, UK), 1 μl of each primer (at 10 μM), 2 μl of DNA extract and nuclease-treated water in a final volume of 25 μl. Amplification was achieved using the following conditions; an initial denaturation step at 94 °C for 10 s followed by 45 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. Amplicons were separated in a 1.5% agarose gel (100 v / 60 min) impregnated with SYBR safe nucleic acid staining dye (Thermo Fisher Scientific, UK) and visualized under ultraviolet illumination. Samples of the correct size (approximately 400 base pairs) were sequenced using primers Piro-A and Piro-B at 1 pmol/ml using ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). Sequence data was edited and aligned using SeqMan Pro (DNAStar, Lasergene v15, Wisconsin, USA). DNA sequences generated by this project were submitted to NCBI GenBank (Supplementary Table S1). *Babesia* species were determined by BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Mapping

The map was produced using qGIS software (version 2.18.10) and confidence intervals were calculated using R software (R core teams, 2022).

3. Results

In total, 3912 questing *I. ricinus* nymphs were individually screened for the presence of *Babesia* spp. Piroplasms DNA was detected in 15 samples, giving an average infection rate of 0.38% [95%CI: 0.21–0.63%] (Table 1). No ticks were found to be infected with *Babesia* in Wales ($n = 480$ tested). In England, the infection rate was 1.35% [95% CI: 0.28–3.90] in central England, 0.46% [95%CI: 0.23–0.82] in southern England and 0.12% [95%CI: 0–0.67] in northern England (Fig. 1). Based on partial 18S rRNA sequence (GenBank accession numbers OR539582 to OR539596, Supplementary Table S1), we detected *B. venatorum* ($n = 9$), *B. divergens/capreoli* ($n = 5$) and a species closely related to *B. odocoilei* ($n = 1$) and referred to as *B. odocoilei*-like (Fig. 1). Fourteen of these positive samples came from ticks collected in woodlands and one (*B. divergens/capreoli)* from a nymph collected in a heathland.

4. Discussion

In this study, we tested questing *I. ricinus* nymphs collected across 20 recreational areas in England and Wales to assess potential human and veterinary risks for babesiosis. Of 3912 questing *I. ricinus* nymphs screened, 15 were found to be infected with piroplasms. This low prevalence of 0.38% is in line with two recent studies conducted in Scotland and England where researchers found a prevalence of *Babesia* spp. of 0.2–0.3% in questing ticks (Olsthoorn et al., 2021; Sands et al., 2022). Across Europe, studies have found infection rates for *Babesia* spp. in questing *Ixodes ricinus* nymphs ranging, on average, from 0 to 2.3% (Azagi et al., 2021; Casati et al., 2006; Cotté et al., 2010; Hamsikova et al., 2016; McKiernan et al., 2022; Radzijevskaja et al., 2008). Two studies found higher infection rates in questing nymphs with 5.2% in Slovenia ($n = 135$) and 11.1% in Poland ($n = 234$) (Cotté et al., 2010; Duh et al., 2001; Skotarczak and Cichocka, 2001) whilst one study found an unusually high prevalence of 49.0% in unfed ticks in Austria (Blaschitz et al., 2008). Other tick species can also transmit piroplasms; in southern England and Wales, between 0 and 7.5% of questing *D. reticulatus* were found infected with *Babesia* spp. (de Marco et al., 2017; Sands et al., 2022), with one location associated with a disease outbreak in dogs that had 82% of questing adult ticks ($n = 17$ tested) infected with *Babesia canis* (de Marco et al., 2017). *Haemaphysalis punctata* is another vector native to the UK and one study found 1.3% of questing ticks infected with *B. motasi* and *B. major* ($n = 302$ tested) (Phipps et al., 2022).

The *Babesia* species identified; *B. venatorum* ($n = 9$), *B. divergens/capreoli* ($n = 5$) and *B. odocoilei*-like species ($n = 1$) were based on high sequence identity within a partial fragment of the 18S rRNA gene. This approach can differentiate *Babesia* spp. although *B. divergens* and *B. capreoli* share a high level of sequence identity and have been grouped together to reflect this. All of these species are known to circulate in the

Table 1
Nymphal infection prevalence (NIP) of *Babesia* spp. (%) in questing *Ixodes ricinus* nymphs at 20 locations across England and Wales collected between 2014 and 2019. Figures in square brackets represent 95% confidence intervals.

Locations	NIP [95%CI] (+ve/tested)	*Babesia* species	Locations	NIP [95%CI] (+ve/tested)	*Babesia* species
Northern England	**0.1% [0–0.7] (1/825)**		**Southern England**	**0.4% [0.2–0.8] (11/2385)**	
Forest of Bowland	0.3% [0–1.9] (1/299)	*B. divergens/capreoli*	Blackdown Hills	0.7% [0–4.1] (1/134)	*B. venatorum*
Lake District	0% (0/397)		Swinley forest	0.7% [0–2.4] (2/297)	*B. venatorum* *B. divergens/ capreoli*
North York Moors	0% (0/129)		Cranborne Chase	1.0% [0.1–3.6] (2/200)	*B. venatorum* *B. divergens/ capreoli*
Central England	**1.4% [0.3–3.9] (3/222)**		Dartmoor	0% (0/244)	
Cotswolds	1.7% [0.4–5.0] (3/172)	*B. venatorum*	Exmoor	0.7% [0–2.4] (2/294)	*B. venatorum* *B. odocoilei*-like
Thetford forest	0% (0/50)		Mendip Hills	0% (0/68)	
Wales	**0% (0/480)**		New Forest	0.3% [0–1.9] (1/299)	*B. divergens/ capreoli*
Clwydian range	0% (0/49)		Quantock Hills	0% (0/204)	
Gower	0% (0/14)		Richmond Park	0% (0/300)	
Pembrokeshire coast	0% (0/158)		South Downs	0.7% [0–2.4] (2/298)	*B. venatorum*
Snowdonia	0% (0/259)		Surrey Hills	2.1% [0–11.3] (1/47)	*B. divergens/ capreoli*

UK (Johnson et al., 2021; Olsthoorn et al., 2021; Sands et al., 2022) but this is the first time that *B. odocoilei*-like species has been detected in questing ticks in the UK. Whilst *B. divergens* and *B. venatorum* have been identified as the causative agent for babesiosis in humans with 40 cases reported in Europe (35 attributed to *B. divergens* and five to *B. venatorum*) (Hildebrandt et al., 2021, 2023), *B. odocoilei* has only be implicated in two human cases in Canada so far (Scott et al., 2021).

In this study, 14 out of the 15 nymphs that tested positive for piroplasms were collected in woodlands where deer are known to occur. This is in line with the known ecology of *Babesia* as *B. venatorum, B. capreoli* and *B. odocoilei*-like species can be maintained by wild cervids (Andersson et al., 2016; Azagi et al., 2021; Scott et al., 2021). One nymph collected in a heathland in the New Forest National park was positive for *B. divergens/B. capreoli* and came from an area where free ranging cattle graze. Thus, it is more likely to be infected with *B. divergens*, which is maintained in cattle however, we were not able to confirm the species (Andersson et al., 2016). Further work should be conducted on the amplification of parts of the 18S rDNA and cytochrome c oxidase subunit I (COI) gene to distinguish between *B. capreoli* and *B. divergens* as suggested by previous studies (Azagi et al., 2021; Malandrin et al., 2010). Transmission of piroplasms within the ticks can be both transovarial and transstadial, which could lead to presence of infected ticks in regions where transmission hosts do not occur, as a result of infected larvae being dropped by birds (Hildebrandt et al., 2021).

In terms of public health, about 60 cases of human babesiosis have been reported in Europe so far (Hildebrandt et al., 2023). However, this figure is likely to underestimate the number of people infected due to a combination of most infections remaining asymptomatic, misdiagnosis and underreporting. Indeed, several studies in Europe reported between 0.6 and 5.4% IgG antibodies for *Babesia* spp. in asymptomatic patients (2.5% in Sweden, 2.8% in Slovenia, 0.6–5.4% in Germany) (Hunfeld et al., 2002; Rojko et al., 2008; Sonnleitner et al., 2014; Svensson et al., 2019). Furthermore, studies detected 16.3% and 39.2% IgG antibodies for *Babesia* spp. in Sweden and Spain in patients positive for *Borrelia burgdorferi* sensu lato (Montero et al., 2023; Svensson et al., 2019) and between 9 and 39.7% in patients with a recent history of tick bite and with symptoms of tick-borne disease in Belgium (Lempereur et al., 2015). In the UK, only two cases of UK-acquired human babesiosis have been diagnosed so far (Chan et al., 2021; Entrican et al., 1979). Low disease incidence and low prevalence in the tick population suggests the overall human health risk posed by *Babesia* spp. in the UK is low.

Regarding disease risk in animals, although redwater fever is detected in a number of regions of the British Isles, only 251 cases were reported in the UK between 2002 and 2020 (APHA, 2020). However, mild infection with recovery may not be identified and severe cases can be successfully managed with imidocarb dipropionate if treated promptly, so the causative agent may not be tested for. This means that reported cases represent an underestimate of the disease burden in the UK and the economic burden to the livestock industry is also likely to be underestimated (Fanelli, 2021). The UK is considered to be free of canine babesiosis and rare cases that occur usually associated with recent travel overseas (de Marco et al., 2017). However, a recent cluster of canine babesiosis cases in England concerning dogs that did not travel abroad was followed by field surveys and 82% of *D. reticulatus* ticks that were tested ($n = 17$) from a nearby location tested positive for *B. canis*. This highlights the need for greater surveillance to better understand and fill in knowledge gaps of the dynamic interaction between ticks, and their pathogen and host (de Marco et al., 2017).

5. Conclusion

In conclusion, this study highlights the low prevalence of piroplasms in questing *I. ricinus* ticks in the UK, collected from recreational areas. Further evidence of the circulation *Babesia* spp. is provided, along with the first evidence of *B. odocoilei*-like species in questing *I. ricinus* in the UK. The human health risk of *Babesia* spp. circulating in the UK could be further defined with sero-surveillance studies. We recommend further surveys of ticks collected from livestock premises with red water fever, to identify local drivers of elevated *Babesia* spp. prevalence in ticks as well as continuing to communicate about tick awareness.

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CRediT authorship contribution statement

Sara Gandy: Data curation, Formal analysis, Investigation, Visualization, Writing – review & editing, Writing – original draft. **Jolyon Medlock:** Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Benjamin Cull:** Investigation, Data curation, Methodology, Writing – review & editing. **Rob Smith:** Writing – review & editing. **Zoë Gibney:** Writing – review & editing. **Sanam Sewgobind:** Investigation, Writing – review & editing. **Insiyah Parekh:** Investigation, Writing – review & editing. **Sophie Harding:** Investigation, Writing – review & editing. **Nicholas Johnson:** Investigation, Writing – original draft, Writing – review & editing. **Kayleigh Hansford:** Conceptualization, Data curation, Writing – original draft,

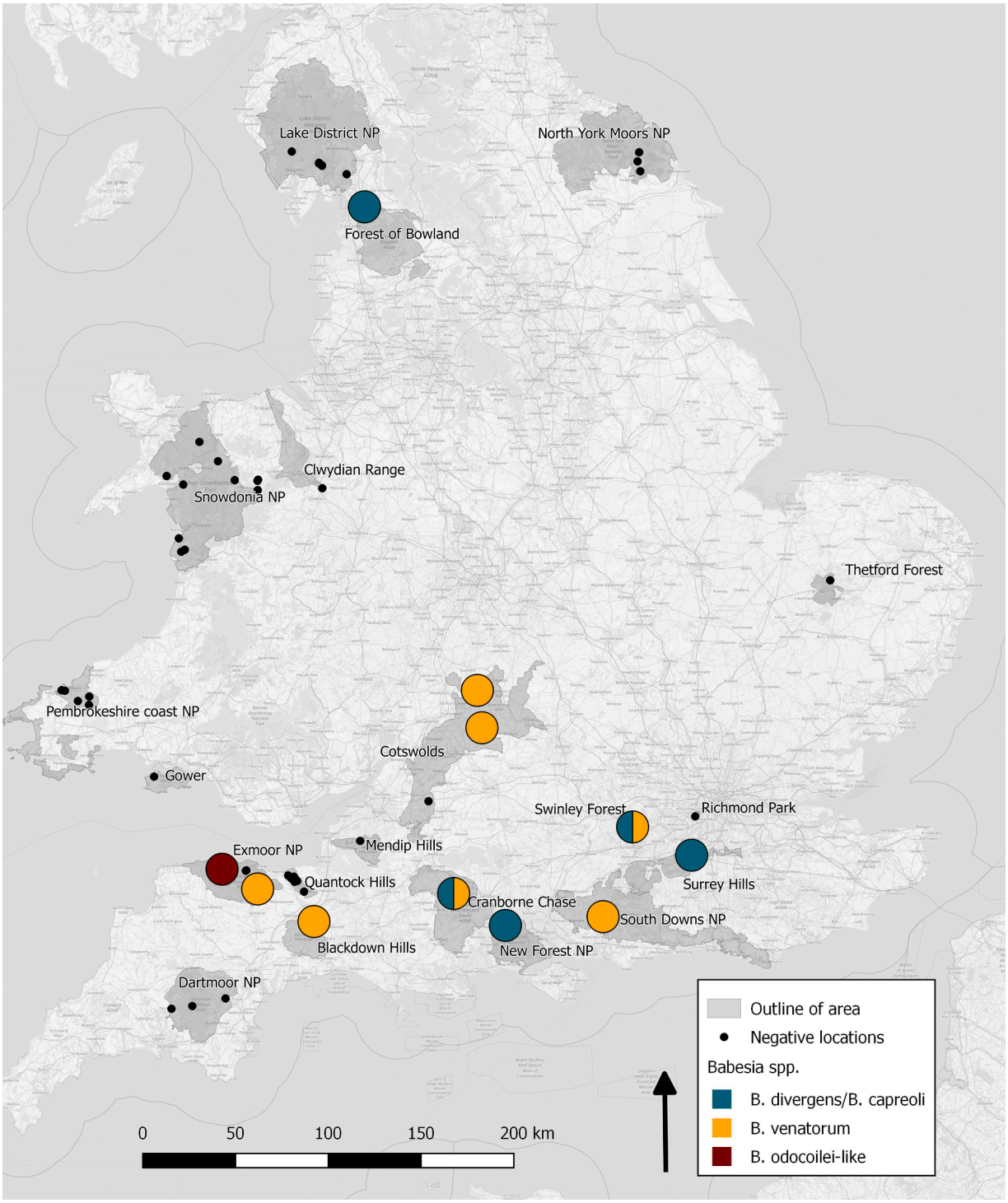

Fig. 1. Map showing the twenty locations where tick collection took place. Black circles symbolise sites where ticks were tested and *Babesia* was not detected and colored circles represent sites where *Babesia* spp. was detected (*B. divergens/capreoli* in blue, *B. venatorum* in orange and *B. odocoilei*-like in red).

Writing – review & editing.

Declaration of Competing Interest

The authors report no declaration of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102291.

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