0:00:06.4 Sarah Crespi: Welcome to the Science Podcast for January 22nd, 2021. I'm Sarah Crespi. Each week we feature the most interesting news and research published in Science and the sister journals. First up this week online news editor, David Grimm, shares a new estimate for the number of research rodents in US labs. You should have your guess ready. Next staff writer, Jennifer Couzin-Frankel, talks about a new hypothesis for the cause of IBS, also known as irritable bowel syndrome. Could it be a local gut-based allergy to certain foods? Finally, researcher Taline Kazandjian discusses the evolution of venom spitting in snakes. Why did this method of self-defense arise three different times in cobras?

0:00:52.7 SC: First up this week we have online news editor, David Grimm. We're gonna talk about How many rodents that's rats and mice are used in US biomedical research each year. Hi, Dave.

0:01:04.7 David Grimm: Hey, Sarah.

0:01:05.4 SC: When I first saw this on the line up, I immediately sent you a guess for how many rodents and mice might be used by researchers every year. I was way off.

0:01:15.5 DG: What was your guess, Sarah?

0:01:15.6 SC: It was 8 million.

0:01:16.5 DG: Okay.

0:01:16.7 SC: Okay, so everyone listening, you guess your own guess. I was way off, that's one clue. So Dave, according to the study that we're gonna be talking about today, how many rats and mice are used or are housed for biomedical research each year in the US?

0:01:29.9 DG: So according to the study, it's 111 million.

0:01:33.0 SC: So I was way off. Were you surprised by this number?

0:01:35.7 DG: Kind of. I mean, the author behind the study, whose name is Larry Carbone, he's at the University of California, San Francisco or actually just left there, had written a book about 15 years ago where he made a guesstimate that was 80 to 100 million. So I'd heard a very high figure before, but that figure's a lot higher than what other groups have estimated. The estimate tends to be around 15 to 20 million.

0:02:01.6 SC: Why is this number mysterious? Why are people guessing or estimating how many of these animals are used in research? This isn't something that we track?

0:02:09.6 DG: Right. So there's something called the Animal Welfare Act, which is a federal law that's been around for more than 50 years actually. And basically what it allows is the United States
Department of Agriculture, the USDA, to go to universities, private companies, any facility that has animals that are covered by that act, the USDA goes in and inspects those animals for welfare and counts them. Now the problem is that there are only a limited number of the animals that are covered by that act, and that includes dogs, cats, rabbits, monkeys. But mice and rats, and birds for the most part have always been excluded or mostly been excluded from that act. And that means that the only agency that would go from institution to institution counting these animals is not counting mice and rats. So, nobody actually has any idea how many of these animals are being used in US Facilities.

0:03:06.3 SC: How does this value though, this 110 million or maybe the low value of 25 million, compare with those other lab animal populations that you're talking about. Rabbits, cats, dogs.

0:03:16.4 DG: It's vastly more, so no matter whose number you believe, everybody seems to agree that lab mice and rats make up at least 93% of all of the mammals used in bio-medical research. So the total number of all those other animals I was talking about, these animals that are covered by the Animal Welfare Act, in the whole United States is about 780,000.

0:03:38.8 SC: How did the author of this piece, this paper, come up with the 110 value?

0:03:43.5 DG: Well, what these other groups have done is kind of extrapolated. They said, What is Europe do and can we extrapolate that to the US or they were making guesstimates. So Carbone decided that he would try to do a more scientific and rigorous approach, so he basically did public records request. And he looked at 16 of the top institutions in the country that receive national institutes of health funding. These institutions when they do get inspected by another organization called AAALAC, they do have to report their mouse and rat numbers to AAALAC. Now, AAALAC keeps those numbers confidential, which is why nobody knows what those numbers are, but Carbone basically filed a public records request, he emailed a few places, and he said, How many mice and rats are you reporting to AAALAC?

0:04:25.5 DG: And so he was able to get a number for these 16 institutions, which was about 5.5 million mice and rats and then he compared it to the number of animals at these institutions that have these Animal Welfare Act animals, which is about 39,000 animals. And that give them a ratio of 99.3% of all of the mammals used at these 16 institutions are mice and rats. And when he extrapolates that nationwide to the more than 900 scientific institutions out there, he gets a figure of about 111 million.

0:04:57.2 SC: So he did a different kind of math. So instead of taking Europe's numbers and multiplying by how many researchers we have here or how much funding we have, he looked at institutions in the US, the ratio of the non-rodent animals to rodent animals and then extrapolated.

0:05:12.8 DG: Exactly.

0:05:13.4 SC: So we don't know which is correct.

0:05:14.7 DG: Regardless of how you slice it, it's still an extrapolation. This is an extrapolation...
based on actual US data, so that's what makes it potentially more powerful than some of these other extrapolations. But there's still a lot of potential problems with the methodology here.

0:05:30.2 SC: Now, the reason we're talking about this, the reason there are people arguing about this, is this number is kind of a political football. This number is being used by different groups to advocate for different things. Can you give us a breakdown?

0:05:43.4 DG: Right. So animal welfare groups, especially groups that have argued that mice and rats should be covered by the Animal Welfare Act, this number is very powerful for them, 'cause they can say, Look, there's 100 million mammals in US labs that are not getting this federal protection and that's an outrage. Meanwhile, advocates for the biomedical community can say, well, that number's way off, it's really closer to 20 million. And they also make the argument that even though mice and rats are not covered by the Animal Welfare Act, universities have what are called institutional animal care and use committees, which do look after the welfare of mice and rats, and also AAALAC itself when it does its inspections, does consider them, the welfare of these rodents.

0:06:23.1 DG: And so, they're saying that no matter what the number is, and they still believe the number is much lower, because they think this extrapolation was kind of unfair that these top funded institutions don't necessarily represent the 900-plus institutions that are out there, so you can't really make this extrapolation. But even if you could, they say these animals are still well cared for, and therefore regardless of what the number is, it doesn't really matter in terms of their welfare in scientific labs.

0:06:49.2 SC: Well, are we ever gonna get a true count of this population, do you think, Dave?

0:06:53.0 DG: So what Carbone saying is that, regardless of whether you believe his number or not, he's really helping to start the conversation. His belief is that there are so many of these animals that they deserved to be counted, and that somebody like him shouldn't have to go through all the rigmarole, filing all these public records requests to get this information. That this should be public and transparent information, just like it is for cats, dogs, monkeys, and other animals.

0:07:15.0 SC: Okay, thanks so much, Dave.

0:07:16.3 DG: Thanks Sarah.

0:07:17.7 SC: David Grimm is the online news editor for Science. You can find a link to this story at sciencemag.org/podcasts. Stick around for my interview with Jennifer Couzin-Frankel about the role of the gut's immune system in IBS.

[music]

0:07:38.5 SC: Now we have staff writer, Jennifer Couzin-Frankel. She wrote a story on a potential cause for IBS, also known as irritable bowel syndrome. There's a possibility it might have something to do with allergies localized to the gut. Let's get into it. Hi Jennifer.
Jennifer Couzin-Frankel: Hi, thanks for having me.

SC: Sure, let's start with IBS. This is a common ailment, but not one that we understand all that well. What do we know about this?

JC: Yeah, so this has long been a very mysterious condition, and a very frustrating and painful condition for the many, many people who suffer from it. It's marked by symptoms mainly pain, cramping, diarrhea, constipation, often associated with eating. The treatment basically involves managing the symptoms. It's often diagnosed by just ruling out everything else. So as you can imagine, it's not easy for the people who suffer from it, because we don't have a whole lot that's concrete to really offer them.

SC: One of the first clues you talk about in your story for what's going on here was this idea that in IBS patients, there's something different about the immune system in the gut, or how immune molecules are working in the gut. How so?

JC: Yeah, so I should say, first of all, that different groups have reported different findings and IBS is also very variable, so it's probably the case that there's not one cause that explains it for everybody. But like you say, there was a group in Italy that roughly 15-ish years ago, was looking at biopsies of intestinal tissue from people with IBS, and found that essentially the immune system looked different than in healthy people. These immune cells called mast cells, which are sort of an alert system and tend to alert or react when there is an outside invader, like a pathogen of some sort, were kind of reacting, even though these patients didn't have any outside Invader. And when the mast cells react, what they do is they release chemicals like histamines and other chemicals, and that appeared to be happening in these patients.

SC: And is that where pain might be coming from, the fact that they're turning on certain immune reactions?

JC: Yeah, so that's a very reasonable theory, and that was certainly what this original group proposed. They also found that nerve cells in the gut were in very close proximity to these mast cells. And so when the mast cells were releasing their chemicals, the nerve cells would kind of react to that, start to fire excessively and that could then be interpreted as a pain signal. So that was sort of a theory that was proposed a while back that could potentially help explain pain, but there were still certain things that it didn't explain, that we didn't understand.

SC: The next piece of the puzzle you talk about was basically seeing if this could be tied to allergy. Can you talk about how that worked?

JC: So really the question was, if this is happening, why is this happening? And what is causing it? We certainly know that people with IBS, when they eat, they can experience pain and cramping and discomfort. That's well known, experienced by a lot of people. And so what this new research did was the group behind the research theorized that maybe when you eat, you're having some kind of localized, just in the gut, immune reaction of some sort to proteins in food, and that's what they decided to study.
0:11:15.0 SC: In order to study how this might be linked to allergy, researchers first looked at mice, and they basically gave them IBS.

0:11:24.2 JC: I should back up and just say that there are a number of different causes of IBS. One of the triggers is thought to be intestinal infections. It could be food poisoning, it could be a really mild infection, and in a subset of patients, someone has an infection like that, and then they go on to develop IBS and we don't really know why, but that's been seen in a lot of people. So this group looked at that particular trigger for IBS and then they tried to mimic it in the mice and really understand what was going on.

0:11:50.8 SC: How do they do that?

0:11:52.5 JC: So they took mice and they gave them basically, a gut infection, and then at the same time as they were infected, the researchers fed the mice egg white protein. So many, many foods have proteins in them called antigens, and people who have food allergies, like regular food allergies react to the antigens in foods. So they fed them the food antigens, the egg white antigens, and then the mice recovered from the infection, just as a human being would, and then they fed them the egg white protein again. And this time the mice essentially reacted with what appeared to be pain, while eating and they measured that by looking at stomach muscle contractions.

0:12:32.8 SC: And then were they able to look and see if the immune system was involved? Particularly the mast cells we've been talking about.

0:12:41.1 JC: In the gut of the mice, the egg white protein was anchoring itself to antibodies that are called IgE antibodies and IgE antibodies are bound to mast cells, the immune cells that this earlier group had been looking at. And in the mice, as we see in people with allergies, when you have that binding happen, the mast cells become activated and release their chemicals, and then that sets off the whole reaction we were talking about before.

0:13:08.4 SC: This is like a food allergy then, but not global?

0:13:13.6 JC: The sort of chain reaction that they saw in the mice, you eat the antigen, the protein, the antigen binds to IgE, which then activates mast cells, which then release the chemical, that's what happens in a food allergy. And the chemical release is what causes all these symptoms we associate with food allergy, like rashes and sometimes difficulty breathing and symptoms that can be all over the body. In someone with food allergies, you can do blood tests and you can see IgE that's specific to certain antigens in the blood, and that's in part on how we diagnose a food allergy.

0:13:48.0 SC: Right. So you can take someone's blood, give that blood a peanut and the blood will tell you it's allergic to peanuts. [chuckle]

0:13:54.4 JC: Sort of. You're kind of looking for the IgE in the blood. Exactly, but you can diagnose it with a blood test because it's what's called systemic. It's all over the body. Now, in these mice, blood tests did not show essentially a food allergy. These mice didn't have a food allergy to
egg white protein.

0:14:12.3 SC: According to a blood test?

0:14:14.2 JC: Yes, according to a blood test. So if they were people coming in for a blood test for an egg white allergy, they didn't have one, but they had what looked kind of like an egg white allergy, but just in their gut. It was like a localized allergy, and it was just their gut that was reacting to the protein.

0:14:30.1 SC: So what happens next? We have this link in mice, can we connect it to people?

0:14:36.4 JC: So what this group did to try and test that was they recruited 12 people with IBS and eight people who were healthy. They tested them for four common food allergies, to cow's milk, gluten, wheat, and soy. These are all pretty common allergies in people, and all of those patients were negative. They did not have food allergies to those particular substances. Then what they did was... Now this part doesn't sound so pleasant, but it was really valuable, is they injected these potential allergens rectally, so they were getting it into the same system as in the mice, and every one of the volunteers with IBS had a localized reaction pretty much like the mice did, to at least one of those antigens, one of the four that were tested and some had reactions to more than one. And then in the control group of eight healthy people, there were two people who did have a borderline gut reaction to one of the antigens. But the suspicion is that some people who aren't affected by IBS might still have mild reactions and their guts can kinda tolerate them. These healthy people didn't have any IBS symptoms, so this is preliminary, but it was interesting that it did kind of match up with the mice.

0:15:46.3 SC: We know that there are more than one way for IBS to come about, it's not always about infection. Is that something that they're considering and following up on this research?

0:15:56.2 JC: Yes, so in the mice, they were really just looking at this origin with an infection, and there are definitely other ways that IBS can start. We also don't even always know why it starts, so one of the other common triggers of IBS is stress. So the same group is now interested in exploring in mice whether stress can induce this same kind of what looks like a local allergic reaction in the gut, just as we saw with the intestinal infection model. Does that translate to another type of IBS?

0:16:29.5 SC: One thing I was curious about this is they looked at multiple things that people are allergic to in food. You talked about cow's milk or gluten. Is the idea here that any one of these things would cause pain in IBS patient, or you might at some point, be able to narrow down which one is causing this problem in the gut, this local allergic reaction.

0:16:48.5 JC: I think we don't know whether we're talking about specific foods and different people may be reacting to different foods, or whether it's broader. I will say that a lot of people with IBS believe that they have allergies, because they do react more to certain foods, but then of course, they get a food allergy test and they're often not shown to be allergic. So it would be interesting if this is helping explain that. One person I spoke with thought that if we're able to determine this in patients, it could help encourage people to go on certain diets that restrict foods that sometimes are
really tough to restrict, because they're in so much.

0:17:29.4 SC: And this could also open up further down the line possible treatments instead of just managing the symptoms of IBS.

0:17:36.6 JC: Yes. So I think if in at least some patients, this kind of immune cascade is behind at least the pain and cramping that we see, there may be ways to target it more directly. Instead of just managing a symptom, there might be immune treatments that could be used to really get at the mechanism.

0:17:57.6 SC: That's great. Alright, thank you so much, Jennifer.

0:18:00.3 JC: Thank you.

0:18:01.2 SC: Jennifer Couzin-Frankel is a staff writer for Science. You can find a link to the article we discussed at sciencemag.org/podcasts. Stay tuned for an interview with Taline Kazandjian about venom spitting Cobras.

[music]

0:18:19.3 SC: My five-year-old daughter is pretty into snakes right now. I recently got the low down on the best starter snakes, corn snake, and how she wants garter snakes next, because they can live together in a group. Okay, maybe there are live-in snakes on the horizon for me, but at no point will they be cobras. Cobras are highly venomous and some of them even spit that venom, Taline Kazandjian and colleagues, wrote about the evolution of the spitting behavior in three different cobras this week in Science. Hi, Taline.

0:18:53.1 Taline Kazandjian: Hello.

0:18:54.3 SC: You work at a snake bite research center. I'm guessing you have some venomous guys there?

0:19:01.6 TK: We have a lot. Most of our stock is from the areas that we work most closely with, so it would be sub-Saharan Africa and Asia where we can. So we've got a lot of cobras, mambas, we've got a close relative of the Russell's viper, a lot of saw-scaled vipers. So yeah, so we've got a lot of venomous on our hands.

0:19:20.0 SC: Is it a different procedure when you're handling a venomous snake versus handling a venomous snake that spits that venom?

0:19:26.5 TK: Yes, I don't directly handle the snakes myself. I have been in the room while they have been handled, and when you're working with spitting snake, you have to wear a visor, because if you get even a small amount of that in your eye, it's really gonna hurt and it can cause some temporary eye damage.
0:19:43.1 SC: I know that most snakes use venom to subdue or kill their prey. Is that why the cobras are spitting venom?

0:19:52.7 TK: No. When venom is spat and it hits the eye, you don't get systemically envenomed. It literally just causes some damage and pain and irritation to the eye. They're actually using this venom defensively to ward off attackers.

0:20:03.7 SC: You knew spitting was for defense going into this work. So what exactly were you trying to find out about cobra venom spitting?

0:20:10.7 TK: Well, we wanted to see whether this defensive use of venom resulted in changes in the underlying venom composition, to see if their venom perhaps contains toxins that are more useful for defensive purposes. And one of the reasons we also wanted to look at this is because we've noticed that in actual biting cases, spitting cobras can have very different symptoms to non-spitting cobras. So we thought that as well suggested that there might be something going on with their venom that's slightly different from their non-spitting counterparts.

0:20:40.1 SC: So what did you find out about their venoms?

0:20:43.2 TK: Just looking at the proteins that are in the venom themselves, we actually found that spitting cobras have a lot more of these toxins called Phospholipase A2s, than non-spitting cobras. Phospholipase A2s are very tricky toxins. They have a lot of different functions.

0:21:00.3 SC: What did you see that was functionally different between the venoms that are spat and the ones that aren't spat.

0:21:06.1 TK: One of the big uses of defensive venoms in animals is causing pain. Pain is a very useful at deterring an aggressor. If you're in pain, you're very likely to be distracted by that pain and not by whatever it is in front of you. So we wanted to test if spitting cobra venoms could induce pain more than non-spitting cobra venoms. Now, pain is pretty difficult to measure yet alone test. It's not really that ethical. So what some of our colleagues did was they tested spitting cobra venoms and non-spitting cobra venoms on these isolated trigeminal mouse neurons, which are the neurons that innervate your face, and are responsible for sensations in your face. And they found that while all cobra venoms activate these neurons, spitting cobras venoms activate them far more. And we thought that this would be because of these interesting toxins, these phospholipase A2s, but it turns out that there was actually a different toxin responsible. Something called cytotoxins which are found in all cobra venoms.

0:22:11.4 TK: But when you add Phospholipase A2s to these cytotoxins, it activates these neurons way more than just cytotoxins on their own. So basically these spitting cobras are taking advantage of this toxin that's already in a lot of cobra venoms, this cytotoxin, and they're making it much more efficient by increasing the abundance of these phospholipase A2s in their venoms, which is particularly useful when you're spraying a venom and you're not entirely sure how much of that venom gonna hit the target. You want each drop to be as potent as possible.
SC: If you're using a venom to kill your prey or to stun your prey, you don't necessarily need it to be a painful experience, but if you're using it as a defense, you're gonna want it to be very painful to interact with the spit.

TK: There's some incapacitative potential of causing pain in prey, but it's gonna be far less effective than just knocking it out or causing such damage to the limbs that it cannot move. Pain is far more useful as a defense than any kind of offense.

SC: So it's interesting that not only did this venom, the spitting venom change in order to become more defensive, but it also did this multiple times across different lineages of cobras.

TK: Yeah, spitting has actually evolved three times in this quite small group of related snakes, it's evolved twice in the genus of true cobras called Naja, once in the African Cobras and once in the Asian Cobras, and it's also evolved once in this African mono-specific genus called Hemachatus. It's quite an important defense that it's evolved three times.

SC: We talked about this as a defense, but what about the kind of notorious hood of a cobra? That's a defense mechanism too, right?

TK: The hooding is a very characteristic defense of cobras, and it's been shown in previous research that this hooding is associated with the cytotoxicity of the venom. And we actually think that this combination of behavior of the hooding defense in raising your body off of the ground with the molecular pre-adaptation of having a cytotoxic venom that can cause pain in your eye, pre-adapts cobras to evolve spitting, because it doesn't take too much to increase the effectiveness of their venoms by just adding a little bit of PLA2. And it just takes a small amount of modification to the fangs to be able to force your venom through as a spray.

SC: You looked into a number of possible predators that might have brought about this spitting defense, and you threw away things that trample, like a water buffalo and things that strike from the sky like a bird of prey. And what are we left with?

TK: Bipedal hominids or basically: human ancestors. If you look at modern day primates, and even monkeys as an extension, a lot of them can recognize snakes from sight and some can even distinguish those which are venomous. In the wild, you have primates that can group together and preemptively mob these snakes and kill them before they become any more of a perceived threat. A lot of the times when these primates mob snakes, they can use tools as projectiles or as a long distance threat. When you are looking at quadrupedal primates, this is less of an issue because they don't have the capacity to attack from a distance as much as a bipedal hominid would.

TK: But when you suddenly have this primate which is able to walk upright and able to throw things at a distance at you, and will do so just when they perceive that you are a threat, suddenly it becomes a very dangerous aggressor, and it kind of facilitates the need for a long distance defense.
0:25:56.8 SC: So it's a throwing war.

[laughter]

0:25:58.7 TK: Yeah. Except snakes sadly don't have hands, they've got to use what they have, which is fangs and venom.

0:26:05.8 SC: When you look at the timeline of hominin evolution and where they were living and where these snakes were living, when they developed spitting, did some of that line up?

0:26:16.1 TK: It would help to have more precise stating of divergence times, but the separation of hominids from Pan, which is chimpanzees and Bonobos, coincides with the emergence of spitting in African Cobras, and the migration of hominids to Asia coincides with the emergence of spitting in Asian cobras. So while the timing isn't exactly precise, there is some compelling evidence there, and there's also been fossil evidence of spitting cobras found at a human site in Africa in the Cradle of humanity.

0:26:51.2 SC: Really interesting. Alright, thank you so much, Taline.

0:26:54.9 TK: Oh, thank you for having me.

0:26:56.0 SC: Taline Kazandjian is a post-doctoral research associate at the Center for snake bite research and interventions, at the Liverpool School of Tropical Medicine. You can find a link to the research we've discussed at sciencemag.org/podcasts.

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0:27:10.9 SC: And that concludes this edition of the Science Podcast. If you have any comments or suggestions for the show, write to us at sciencepodcast@aaas.org. You can listen to the show on the Science website at sciencemag.org/podcasts. On the site, you'll find links to the research and news discussed in the episode, and of course you can subscribe anywhere you get your podcasts. The show was edited and produced by Sarah Crespi with production help from Podigy, Meagan Cantwell and Joel Goldberg. Transcripts are by Scribie. Jeffrey Cook composed the music. On behalf of Science Magazine and its publisher AAAS, thanks for joining us.